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SHORT COMMUNICATION

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Sequence analysis, gene expression, and chromosomal assignment of mouse *Borg4* gene and its human orthologue

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Abstract The Borg (binder of Rho GTPases) family proteins interact with CDC42 and TC10 in a guanosine triphosphate (GTP)-dependent manner. We have isolated a full-length cDNA of the mouse *Borg4* gene, which is a member of this family. Sequence analysis revealed that this gene encoded a putative 349-amino acid protein. By reverse transcription — coupled polymerase chain reaction (RT-PCR) analysis, we observed that *Borg4* was expressed ubiquitously in adult tissues. Additionally, we determined the entire cDNA sequence of the putative human *Borg4* orthologue. By fluorescence in situ hybridization, mouse *Borg4* and the putative human orthologue have been assigned to mouse chromosome 11E and human chromosome 17q24–25, which has been described as syntenic to the mouse region.

Key words Cell motility · Binder of Rho GTPase · CDC42 TC10 · *Borg4* · Full-length cDNA · Fluorescence in situ hybridization

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Introduction

Cell motility and morphogenesis are considerably complex phenomena, and the molecular mechanisms are still under investigation. Because an extremely large number of genes collaborate and there is cross-talk between them in these phenomena, it is essential to completely identify the unknown genes involved in the processes. The Rho family of guanosine triphosphatases (GTPases) is known to play important roles in cytoskeletal modeling (Aelst and D'Souza-Schorey 1997: Kjoller and Hal 1999), and, interestingly, it is reported that a member of the Rho protein family, named TC10, promotes nerve elongation in neuronal cells (Tanabe et al. 2000). Recently, a new protein family, named Borg, which interacts with Cdc42 or TC10, has been identified (Joberty et al. 1999). The proteins of this family have been shown to share the CRIB (Cdc42, Rac interactive binding) domain and specific domains named BH (Borg homology) 1, 2, and 3. The CRIB domain is an amino acid motif (consensus sequence: I-S-X-P-$(X)_{2-4}$-F-X-H-X-X-H-V-G) which defines candidates for effector proteins of Cdc42 and Rac GTPases in a GTP-dependent manner (Berbelo et al. 1995). The ectopic expression of Borg1, 2, and 3 induces the loss of actin stress fibers, suggesting that the Borgs inhibit the Rho function. The Borg family, Borg1 to Borg5 (Borg5 was previously known as MSE55) (Bahou et al. 1992) cDNAs were isolated using a two-hybrid screen and expressed sequence tags (EST) database and were revealed to contain several highly conserved BH1, 2, and 3 domains, as well as CRIB. As for Borg4, however, only CRIB and BH1 could be demonstrated, because of the partial cDNA isolates (Joberty et al. 1999).

Here we report the cloning of the full-length mouse *Borg4* and its human orthologue cDNAs. *Borg4* shares, in common with the other Borg family members, highly conserved CRIB, BH1, and BH3 domains, and a less well conserved BH2 domain. We further showed that *Borg4* was expressed ubiquitously at low levels in adult mouse tissues, and mapped mouse *Borg4* to mouse chromosome 11E and the human orthologue to human chromosome 17q24–25 by fluorescence in situ hybridization (FISH).

Materials and methods

DNA sequencing

The mouse *Borg4* cDNA clone (MNCb-0220; DDBJ/EMBL/GenBank accession number AB035088; the 5′-part of the sequence was deposited as accession number AU035226) and the putative human orthologue clone (KAIA1777; accession number AB042237) were derived from a mouse brain and a human adult ileum mucosa cDNA library made by the oligo-capping method (Suzuki et al. 1997; Suzuki et al. 2000). The method allowed us to replace the cap structure of mRNA with synthetic oligonucleotides (5′-oligo), which could then serve as the sequence tag for the mRNA start site. Using the oligo-capped mRNAs and oligo-dT primer, we were able to amplify full-length cDNAs at a high frequency. The insert was sequenced on an ABI 310 automated sequencer (Perkin-Elmer, Norwalk, CT, USA) by the primer walking method.

Reverse transcription — coupled polymerase chain reaction (RT-PCR)

Primers used for RT-PCR were designed to amplify the 362-bp fragment (bases 2691–3052 of *Borg4* cDNA). The primers were 5′-GGGGTGTGAGCTACGCTTT-3′ and 5′-CCCAGCACAGGAAAGTAATGA-3′. The templates of the mouse total mRNA were purchased from OriGene (Rockville, MD, USA) and treated with RNase-free DNase-I (Takara, Kyoto, Japan) before amplification. One microliter of total mRNA was amplified, using the One Step RNA PCR Kit (Takara). Temperature and time schedules were: 40 cycles of 94°C for 30s, 60°C for 30s, and 72°C for 90s. PCR products were separated on 1.5% agarose gel with a 100-bp ladder DNA marker (GIBCO-BRL, Gaithersburg, MD, USA).

Chromosomal mapping by fluorescence in situ hybridization (FISH)

FISH was performed as described previously (Hirai et al. 1996). After overnight hybridization with biotin-labeled *Borg4* cDNA, the slides were washed in 50% formamide/2 × standard saline citrate (SSC) at 42°C for 15min, followed by a wash in 1 × SSC at room temperature for 15min. The slides were then blocked with 4% bovine serum albumin/4 × SSC at 37°C for 30min. Signal amplification was achieved using rabbit anti-biotin (ENZO, New York, NY, USA), fluorescein-labeled goat anti-rabbit IgG (ENZO), and Cy2-labeled donkey anti-goat IgG (Amersham Pharmacia, Uppsala, Sweden). The chromosomes were counterstained with propidium iodide. Slides were examined with a fluorescence microscope (BX60; Olympus, Tokyo, Japan) equipped with an IB filter. For mouse, the same metaphase spreads were also viewed with a UV filter for G-band analysis.

Results and discussion

We have isolated full-length cDNA clones from a mouse brain cDNA library constructed by Sugano et al., using the oligo-capping method, as described previously (Suzuki et al. 1997). Among a number of complete nucleotide (nt) sequences of cDNA clones sequenced, we found the clone MNCb-0220, which completely matched the partial mouse *Borg4* nucleotide sequence (accession number AF165114). The insert length of the cDNA clone is 3420bp and the deduced amino acid has 349 residues (corresponding to nt 691–1737), with a predicted mass of 37.9kDa and a hypothetical pI of 5.20. Compared with the other family member proteins, the mouse *Borg4* protein was shown to contained highly conserved CRIB, BH1, and BH3 domains, and a less conserved BH2 domain (Fig. 1A).

By further database searching, we found a candidate cDNA sequence for the human *Borg4* homologue deposited in GenBank as CEP4 (cdc42 effector protein 4; accession number AF099664). However, the CEP4 nucleotide sequence in the database was incomplete in the untranslated region; therefore, we searched for a full-length *Borg4* cDNA clone in a full-length enriched cDNA library from human adult ileum mucosa (Suzuki et al. 2000) and found the clone KAIA1777. The insert length of KAIA1777 was 2732bp and the coding sequence shared 80% identity with that of mouse *Borg4*. The deduced amino acid sequence of human *Borg4*, as designated here, and mouse *Borg4* are aligned in Fig. 1B. The sequences of the N-terminal region are well conserved, whereas those of the C-terminal flanking region vary greatly (total identity, 77%). All three Borg homology domains are highly conserved in the two proteins, suggesting the functional significance of these domains (Fig. 1B, shown by rectangles). In the 5′-untranslated region (UTR), only the 70-bp sequence juxtaposing the translation start site showed high homology, but it was reduced in the upstream region, suggesting that this 70-bp segment possessed some functional element. The 3′-UTR of KAIA1777, however, was quite dissimilar to mouse *Borg4* and contained 173bp of the medium reiteration frequency repeat (MER53). To confirm that this dissimilarity between mouse/human *Borg4* was not due to the chimeric clones, polymerase chain reaction (PCR) analysis was performed, using a primer set derived from two regions with different similarity scores. The effective amplification of the DNA fragment from the human genomic boundary region allowed us to suppose that the discordance might be responsible for alternative exon or polyA signal choices, and affect the transcriptional regulation.

It is reported that *Borg* family genes are expressed in distinct patterns (Bahou et al. 1992; Joberty et al. 1999). However, Joberty et al. failed to show the expression pattern of *Borg4* and suggested that it may be expressed only in fetal tissues. Because we had isolated the mouse *Borg4* full-length cDNA from an adult mouse brain cDNA library, we performed northern blot analysis, using the cDNA as a probe to confirm its expression. But no signal for the *Borg4* transcripts was observed in any tissues (data not shown).

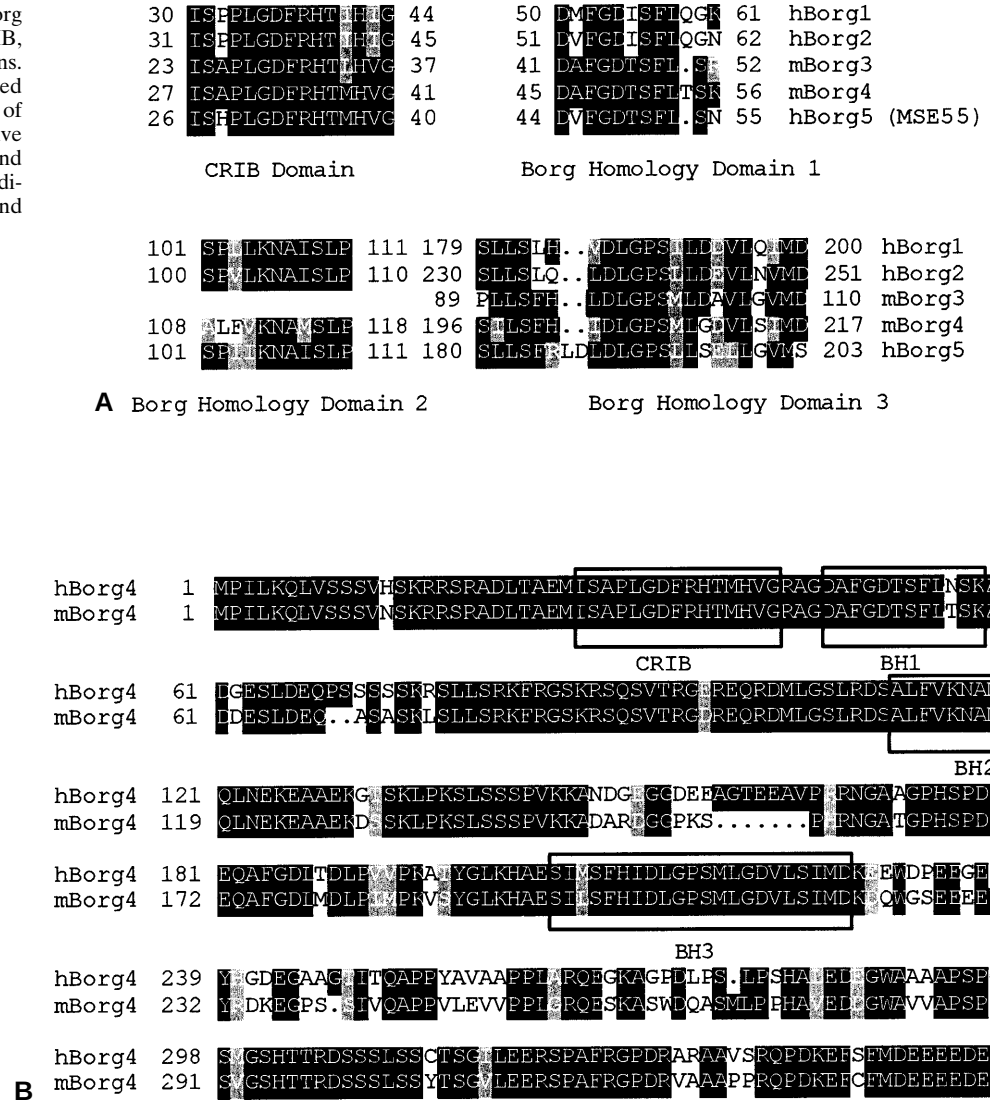

Fig. 1. **A** Alignment of the Borg family proteins for the CRIB, BH1, BH2, and BH3 domains. *Borg3* lacks the conserved BH2 domain. **B** Alignment of KAIA1777 protein (putative human *Borg4* homologue) and mouse *Borg4*. Identities are indicated by *black background*, and similar residues are *shadowed*

Subsequently, we used an RT-PCR method to assay its expression. The result showed that *Borg4* was expressed ubiquitously in adult tissues (Fig. 2), unlike the non-uniformly expressed other family proteins. Considering the result of northern blot analysis, mouse *Borg4* may be expressed at quite low levels.

Although a working draft of the human genome sequence has been available in the NCBI database, we found no genomic sequence preferably matched to the KAIA1777 and MNCb-0220 clones. To determine the chromosomal localization of *Borg4*, FISH was performed, using the MNCb-0220 and KAIA1777 clones as probes. For KAIA1777, we detected FISH signals on human chromosome 17q24–25 (Fig. 3A), and this result was confirmed by PCR analysis of human and rodent somatic cell hybrid panels (data not shown). With MNCb-0220, specific signals were observed in the mouse chromosome 11E region (Fig. 3B, C). To confirm the chromosome identification, a painting probe of mouse chromosome 11 was hybridized to the same metaphase spreads after signal detection (data not shown). Human chromosome 17q24–25 and mouse chromosome 11E have been described as syntenic regions (Carver and Stubbs 1997). Despite some dissimilarity in the nucleotide sequences, the chromosomal localization supports the idea that CEP4 is the orthologue of mouse *Borg4*.

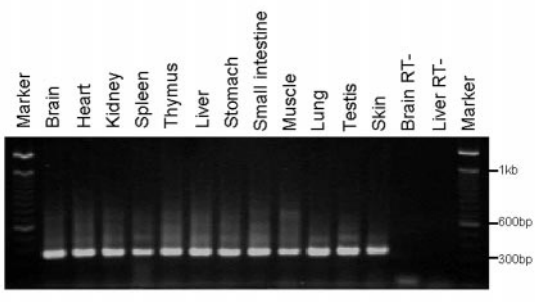

Fig. 2. Tissue distribution analysis of mouse *Borg4* transcript, using reverse transcription-coupled polymerase chain reaction (RT-PCR). The 12 tissues examined and the controls (— reverse transcriptase *RT*) are indicated *above each lane*

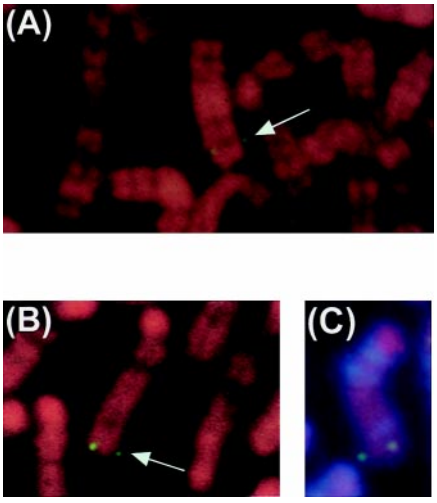

Fig. 3A–C. Chromosomal mapping of human (KAIA1777) and mouse (MNCb-0220) *Borg4* genes by fluorescence in situ hybridization (FISH). **A** Doublet FISH signals (*arrow*) are observed on human chromosome 17q24–25. **B** Metaphase, showing doublet on mouse chromosome 11 at region 11E (*arrow*). **C** Another metaphase is viewed with a UV filter for G-band analysis

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