Biochemical and Biophysical Research Communications **282,** 116–123 (2001)
doi:10.1006/bbrc.2001.4551, available online at http://www.idealibrary.com on IDEAL®

Molecular Cloning and Characterization of *MFRP*, a Novel Gene Encoding a Membrane-Type Frizzled-Related Protein

Masaru Katoh[1]

Genetics and Cell Biology Section, Genetics Division, National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan

Received February 15, 2001

The Frizzled-type cysteine-rich domain (CRD) is a binding motif for soluble-type glycoprotein WNTs, which play key roles in embryogenesis and carcinogenesis. Here, we have cloned and characterized a novel gene *MFRP*, encoding a type II transmembrane protein with CRD. In addition to CRD, two tandem-repeats containing the Cubilin domain ~ the MFRP domain were present in the extracellular region of MFRP. Although MFRP was homologous to Corin, FZDs, and SFRPs in CRD, amino-acid identities between CRD in MFRP and CRDs in these molecules were less than 40%. The *MFRP* gene on 11q23 consisted of at least 13 exons. The 4.0-kb *MFRP* was not detected by Northern blot analysis in normal tissues other than adult and fetal brain. The *MFRP* mRNA was undetectable in seven gastric cancer cell lines, seven brain tumor cell lines, and other eight tumor cell lines. Regional distribution of the *MFRP* mRNA in human brain was further investigated, and *MFRP* was found to be expressed strongly in medulla oblongata, and weakly in hippocampus and corpus callosum. Thus, MFRP with CRD might play key roles in medulla oblongata as a regulator of the WNT signaling pathway.

Key Words: **WNT; cysteine-rich domain; medulla oblongata; 11q23.**

The extracellular Frizzled-type cysteine-rich domain (CRD) is a binding motif for glycoprotein WNTs. At least 19 WNT genes exist in the human genome (Katoh, unpublished data), and WNTs play key roles in cell fate determination and malignant transformation (1, 2). WNT signals are transduced through cell-surface WNT receptors to the following intracellular signaling pathways: the c-Jun N-terminal kinase (JNK) pathway (3), the Ca^{2+}-releasing pathway (4), or the ß-catenin – TCF pathway (5). WNT receptors are seven-transmembrane receptors with CDR encoded by members of the *FZD* gene family (6–11).

In search of a novel gene homologous to CRD of FZD3, human genome sequence AP001557 was found to contain exons encoding a novel CRD. Here, overlapping cDNAs derived from the novel gene was isolated, and the novel gene was found to encode a type II transmembrane protein with CRD. Thus, the novel gene was designated *MFRP* (Membrane-type Frizzled-related protein). CRD of MFRP was homologous to those of FZDs (6–11), Corin (12), and SFRPs (13–15); however, amino-acid identities in CRD between MFRP and other CRD molecules were less than 40%. The 4.0-kb *MFRP* mRNA was not detected in various normal tissues other than adult and fetal brain by Northern blot analyses. Within adult brain, *MFRP* was strongly expressed in medulla oblongata. In addition to cDNA structure and expression profile, gene structure and chromosomal localization of *MFRP* will also be described in this manuscript.

MATERIALS AND METHODS

Identification of human genome fragment encoding a novel CRD protein by using the Tblastn program. Human genome sequence corresponding to a novel gene encoding a CRD protein was searched for with the BLAST search program (http://www.ncbi.nlm.nih.gov) as described previously (16). Amino-acid sequence of CRD in the WNT receptor FZD3 (11) was used as a query sequence for the Tblastn program, in which the query amino-acid sequence was compared with the human genome draft sequence translated in six frames, namely three frames in the sense direction and in the antisense direction.

Exon prediction in the genome draft sequence around the CRD exons by using the Tblastx program. Candidate exons around the novel CRD gene fragment might encode a protein motif homologous

The nucleotide sequence data of *MFRP* will appear in the DDBJ/EMBL/GenBank Databases under the Accession No. AB055505.

Abbreviations used: CRD, Frizzled-type cysteine-rich domain; CUB, Cubilin domain; MFD, MFRP domain; EST, expressed sequence tag.

[1] To whom correspondence and requests for reprints should be addressed. Fax: +81-3-3541-2685. E-mail: mkatoh@ncc.go.jp.

0006-291X/01 $35.00

TABLE 1

Oligonucleotide Primers

Primer	Orientation	Nucleotide sequence	Nucleotide positions
PM-07	Sense	ACTCCAGAAAGCCATGCAGAG	118–138 of *MFRP*
PM-08	Anti-sense	TCAGGGCTGGGCACAAGCTTC	1887–1867 of *MFRP*
PM-21	Sense	CAGCCCAAGAACTGGTCTAGC	1–21 of *MFRP*
PM-22	Anti-sense	ATGACATCTGAGAAGTCCTTCATGG	170–146 of *MFRP*
PM-31	Sense	ACGGCAGTGATGAGACCAATTG	1007–1028 of *MFRP*
PM-32	Anti-sense	CACAAGCTTCCAGGTCAGCTG	1876–1856 of *MFRP*
PM-34	Anti-sense	TGGGAAATAACTCGTGGTGTCG	2519–2498 of *MFRP*
BACT01	Sense	GACTACCTCATGAAGATCCT	631–651 of β-actin
BACT02	Anti-sense	GCGGATGTCCACGTCACACT	943–924 of β-actin

to known proteins. Based on this hypothesis, the nucleotide sequences of the 5′- or 3′-flanking regions of the novel CRD gene fragment were used a query sequence for the Tblastx program, in which the query nucleotide sequence translated in six frames were compared with the protein sequence data base.

Cell lines and poly(A)$^+$ RNA. Poly (A)$^+$ RNAs of human fetal brain, lung, liver, and kidney (Clontech Laboratories) were purchased. Poly(A)$^+$ RNAs were extracted from human brain tumor cell lines, DBTRG-05MG, T98G, U-373MG, SW1088, SW1783, A-172, and HS683, and from gastric cancer cell lines OKAJIMA, TMK1, MKN7, MKN28, MKN45, MKN74, and KATO-III with the Fast-Track RNA extraction kit (Invitrogen) as described previously (16)

cDNA-PCR and nucleotide sequence analyses. Forty nanograms of poly(A)$^+$ RNA was used as a template of one-step cDNA-PCR with the One-Step RT-PCR kit (Qiagen) as described previously (16). After reverse-transcription reaction at 50°C for 30 min with a mixture of Omniscript and Sensiscript reverse transcriptase, HotStar-Taq DNA polymerase was activated by heating at 95°C for 15 min. Then, the *MFRP* cDNA was amplified by 33 cycles of PCR (94°C for 0.5 min, 60°C for 0.5 min, 72°C for 2 min), and the *ß-actin* cDNA was amplified by 24 cycles of PCR, followed by final extension at 72°C for 10 min. cDNA-PCR products were purified with QIAEX II gel extraction kit, and ligated into the TA cloning vector pCR2.1 (Invitrogen) for the subsequent nucleotide sequence analyses with ABI310 Sequencer (PE Applied Biosystems). Nucleotide sequences of oligonucleotide primers are listed in Table 1.

Northern blot analyses. MTN Northern blot filters (Clontech Laboratories) containing two μg of poly(A)$^+$ RNA for each lane were hybridized with a [α-^{32}P] dCTP-labeled MFRS probe at 68°C for 1 h in QuikHyb solution (Stratagene). Filters were washed in 2 × SSC buffer with 0.1% SDS at room temperature for 15 min twice, and in 0.1 × SSC buffer with 0.1% SDS at 60°C for 30 min. Then, filters were exposed to the Imaging plate (Fuji) for the image analysis in the Storm system (Molecular Dynamics) as described previously (17). The MFRS probe corresponds to the nucleotide position 1007–1876 of the *MFRP* cDNA.

RESULTS

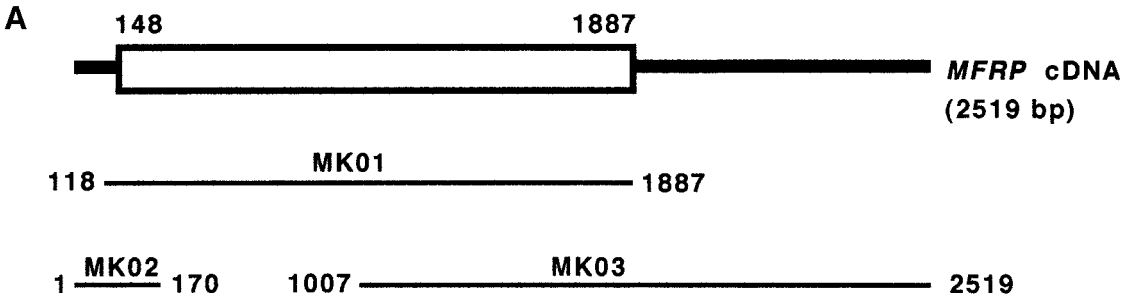
Isolation of MK01 ~ MK03 cDNAs

A novel CRD gene on the human genome draft sequence was searched for with the Tblastn program by using CRD of FZD3 (codon 28–127) (12) as a query sequence. The human genome sequence AP001557 was found to contain a novel gene fragment encoding a CRD protein. CRD of the novel gene showed 36% amino-acid identity with that of FZD3. Then, putative exons homologous to the known protein motifs in the genome draft sequence around the CRD exons were searched for with the Tblastx program. Although no putative exon was identified in the 3′-flanking region, two genome fragments in the 5′-flanking region of the CRD exons were found to be able to encode polypeptide homologous to the Cubilin domain (CUB) present in the intrinsic factor-vitamin B12 receptor, Cubilin (18, 19). These results suggested that the putative novel gene might encode a polypeptide with two CUBs and one CRD.

In the 5′-flanking region of the putative first CUB exon, several sense primers were designed based on the genome sequence just up-stream of the nucleotide sequence resembling the Kozak's consensus sequence for initiation of translation. In the 3′-flanking region of the CRD exons, several anti-sense primers were designed every hundred bases. cDNA-PCR with many combinations of sense and anti-sense primers were performed, and the 1770-bp MK01 cDNA was isolated by cDNA-PCR with PM-07 and PM-08 primers from poly(A)$^+$ RNA of human fetal brain, but not from those of human fetal lung, liver, and kidney (data not shown). Nucleotide sequence analyses revealed that the MK01 cDNA contained a 1740-bp ORF (Fig. 1A).

Then, sense primer PM-21 was designed based on the genome sequence upstream of the PM-07 primer, and anti-sense primer PM-22 was designed based on the nucleotide sequence of MK01 cDNA to determine the structure of the 5′-UTR. The MK02 cDNA was isolated by one-step cDNA-PCR with PM-21 and PM-22 primers from poly(A)+ RNA of human fetal brain, but not from those of human fetal lung, liver, and kidney (Fig. 1A). Three in-flame stop codons and the Kozak's consensus-like sequence followed by the initiator methionine were identified in the MK02 cDNA.

Next, sense primer PM-31 was designed based on the nucleotide sequence of MK01 cDNA, and anti-sense PM-34 was designed based on the genome sequence downstream of the PM-08 primer. The MK03 cDNA, isolated by cDNA-PCR with PM-31 and PM-34 prim-



B

```
MKDFSDVILCMEATESSKTEFCNPAFEPESGPPCPPPVFPEDASYSVPAPWHGRRPRGLR  60

PDCRFSWLCVLLLSSLLLLLLGLLVAIILAQLQAAPPSGASHSPLPAGGLTTTTTTPTIT 120

                          ----------CUB1----------
TSQAAGTPKGQQESGVSPSPQSTCGGLLSGPRGFFSSPNYPDPYPPNTHCVWHIQVATDH 180

-------                   ==========
AIQLKIEALSIESVASCLFDRLELSPEPEGPLLRVCGRVPPPTLNTNASHLLVVFVSDSS 240

============MFD1=======================================
VEGFGFHAWYQAMAPGRGSCAHDEFRCDQLICLLPDSVCDGFANCADGSDETNCSAKFSG 300

----------CUB2----------
CGGNLTGLQGTFSTPSYLQQYPHQLLCTWHISVPAGHSIELQFHNFSLEAQDECKFDYVE 360

                  ==========MFD2===
VYETSSSGAFSLLGRFCGAEPPPHLVSSHHELAVLFRTDHGISSGGFSATYLAFNATENP 420

==========
CGPSELSCQAGGCKGVQWMCDMWRDCTDGSDDNCSGPLFPPPELACEPVQVEMCLGLSYN 480

TTAFPNIWVGMITQEEVVEVLSGYKSLTSLPCYQHFRRLLCGLLVPRCTPLGSVLPPCRS 540

VCQEAEHQCQSGLALLGTPWPFNCNRLPEAADLEACAQP                      579
```

C

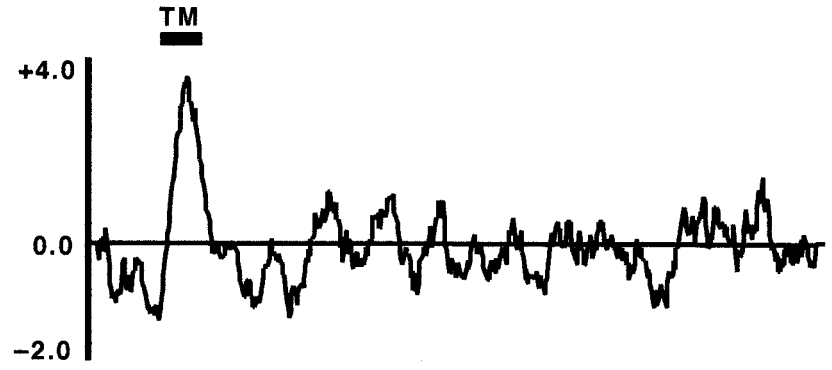
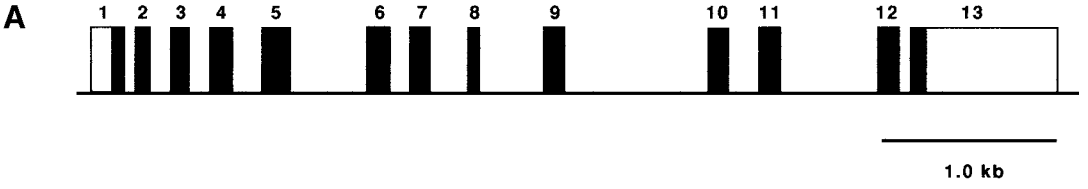


FIG. 1. Structure of the *MFRP* cDNA and deduced amino-acid sequence of MFRP. (A) Schematic presentation of *MFRP* cDNAs. ORF and UTRs are depicted as an open box and solid bars, respectively. Overlapping *MFRP* cDNAs (MK01, MK02, and MK03) are also indicated by solid bars. (B) Deduced amino-acid sequence of MFRP. Amino acids are numbered on the right. Transmembrane domain (bold overline), Cubilin domains (CUB1 and CUB2) (overline), MFRP domains (MFD1 and MFD2) (double overline), and Cysteine-rich domain (CRD) (open box) are indicated. (C) Kyte-Doolittle hydrophobicity analysis on MFRP polypeptide. A transmembrane domain is indicated by a bold bar. MFRP is a type II transmembrane protein with CRD.

FIG. 2. Domain structure of MFRP. (A) Schematic presentation of MFRP polypeptide. (B) Alignment of CUB1 and CUB2 in MFRP. (C) Alignment of MFD1 and MFD2 in MFRP. (D) Alignment of CRD in MFRP with CRDs in Corin and FZDs. Amino-acid identity in CRD are shown on the right. Conserved amino-acid residues are shown below alignments.



B

Exon No.	Exon size	Sequence around exon – intron boundaries			Domain in MFRP
1	> 201 bp	5'-UTR	·····	AGCAAG gtaagg	
2	103 bp	t t ccag ACCGAG	·····	GGCATG gtaacc	
3	114 bp	ccgcag GTCGGC	·····	TGGCCC gtaagt	Transmembrane domain
4	156 bp	ct ccag AGCTGC	·····	AGTCCA gtgagt	Transmembrane domain
5	214 bp	ct gcag CCTGTG	·····	CCTCAG gtaggt	Cubilin domain 1 (CUB1)
6	131 bp	t t gcag GGTTTG	·····	GGCGCG gtgagt	MFRP domain 1 (MFD1)
7	126 bp	at ccag GGAGCT	·····	TCTCGG gtacgg	MFRP domain 1 (MFD1)
8	77 bp	ct acag GGTGTG	·····	CAACTG gtaagc	Cubilin domain 2 (CUB2)
9	149 bp	ccccag CTCTGC	·····	GGGCAG gtacag	Cubilin domain 2 (CUB2)
10	131 bp	gggcag GTTCTG	·····	CGGAGA gtaggt	MFRP domain 2 (MFD2)
11	132 bp	ct gcag ACCCCT	·····	CCCCAG gtgaga	MFRP domain 2 (MFD2)
12	128 bp	ct gcag AGCTGG	·····	TACAAG gtct t c	Cysteine-rich domain (CRD)
13	> 857 bp	ccgcag AGCCTG	·····	3'-UTR	Cysteine-rich domain (CRD)

FIG. 3. Structure of the *MFRP* gene. (A) Schematic presentation of the *MFRP* gene. Exons corresponding to ORF are indicated by closed boxes, and exons corresponding to UTRs by open boxes. The *MFRP* gene consists of at least 13 exons. (B) Exon–intron boundaries of the *MFRP* gene. Exon sequences are shown large caps, while intron sequences by small caps. Size and encoding domain of each exon are also shown.

ers, corresponded to a part of ORF and the 632-bp 3′-UTR (Fig. 1A).

By combining nucleotide sequences of the MK01, MK02, and MK03 cDNAs isolated in this study, the novel cDNA were found to consist of the 147-bp 5′-UTR, the 1740-bp ORF, and the 632-bp 3′-UTR (Fig. 1A).

Deduced Amino Acid Sequence of MFRP

The novel gene was predicted to encode a polypeptide of 579 amino-acid residues (Fig. 1B). A hydrophobic region (codon 70–100) was identified by using the Kyte-Doolittle hydrophobicity analysis. This result indicated that the polypeptide encoded by the novel gene was a type II transmembrane protein (Fig. 1C). Based on the designation of *SFRP* (Secreted-type Frizzled-related protein), the novel gene, encoding a transmembrane-type polypeptide with a CRD, was designated *MFRP* (Membrane-type Frizzled-related protein).

The N-terminal cytoplasmic region (codon 1–69) of MFRP was much shorter than its extracellular region (codon 101–579) with seven N-glycosylation sites. Two tandem-repeats of CUB ~ the MFRP domain (MFD), and the C-terminal CRD were present in the extracellular region of MFRP (Fig. 2A).

CUB1 (codon 144–192) and CUB2 (codon 301–349) showed 45% amino-acid identity (Fig. 2B), and MFD1 (codon 216–294) and MFD2 (codon 377–454) showed 35% amino-acid identity (Fig. 2C). CRD of MFRP (codon 466–564) was homologous to CRDs of Corin, FZDs, and SFRPs. Corin is a type II transmembrane protein with 2 CRDs, FZDs are seven-transmembrane receptors with a CRD, and SFPRs are soluble-type polypeptide with a CRD. Amino-acid identities between CRD of MFRP and CRDs of these molecules were less than 40% (Fig. 2D).

Genome Structure and Chromosomal Localization of the MFRP Gene

The Blastn program was used to compare the nucleotide sequence of the *MFRP* cDNA with the human genome draft sequence. The 2519-bp *MFRP* cDNA was split into 13 exons in the human genome sequence AP001557 on human chromosome 11q23 region (Fig. 3A). These results indicate that the *MFRP* gene, consisting of at least 13 exons, are located on the human chromosome 11q23 region.

Consensus sequences of splice donor and acceptor sites (20) were found in the exon-intron boundaries of the *MFRP* gene (Fig. 3B). CUB1 was encoded by a single exon, while CUB2 was split by an intron. MFD1, MFD2, and CRD were also encoded by two exons (Fig. 3B).

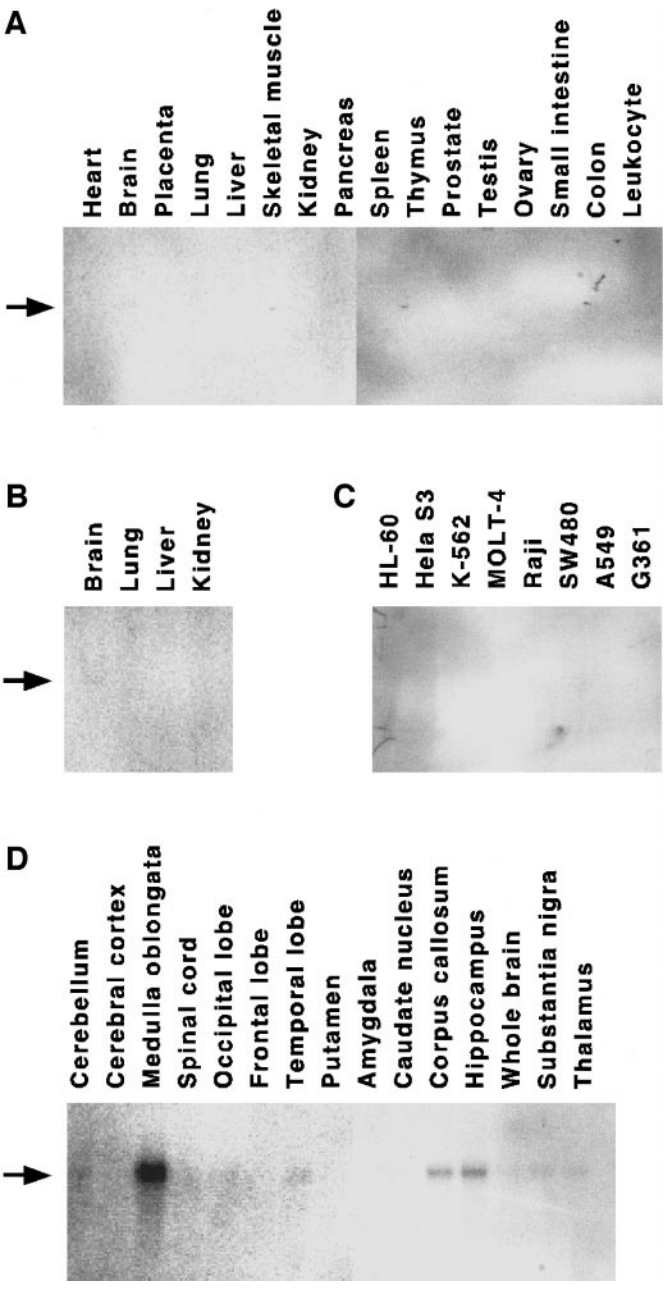


FIG. 4. Northern blot analyses on *MFRP*. (A) Adult human tissues. (B) Fetal human tissues. (C) Human cancer cell lines. (D) Human brain parts. Multiple Tissue Northern filters (Clontech Laboratories) containing 2 μg of poly(A)$^+$ RNAs for each lane were hybridized with the MFRS probe, corresponding to the nucleotide position 1007–1876 of the *MFRP* cDNA. In normal human tissues, the 4.0-kb *MFRP* mRNA was detected faintly in adult and fetal brain. *MFRP* was not detected in human cancer cell lines HL-60, HeLa S3, K-562, MOLT-4, Raji, SW480, A539, and G361. Within adult brain, *MFRP* was expressed highly in medulla oblongata, and weakly in hippocampus and corpus callosum.

Expression Profile of MFRP

The MFRS probe (nucleotide position 1007–1876 of the *MFRP* cDNA) was constructed by PCR with PM-31

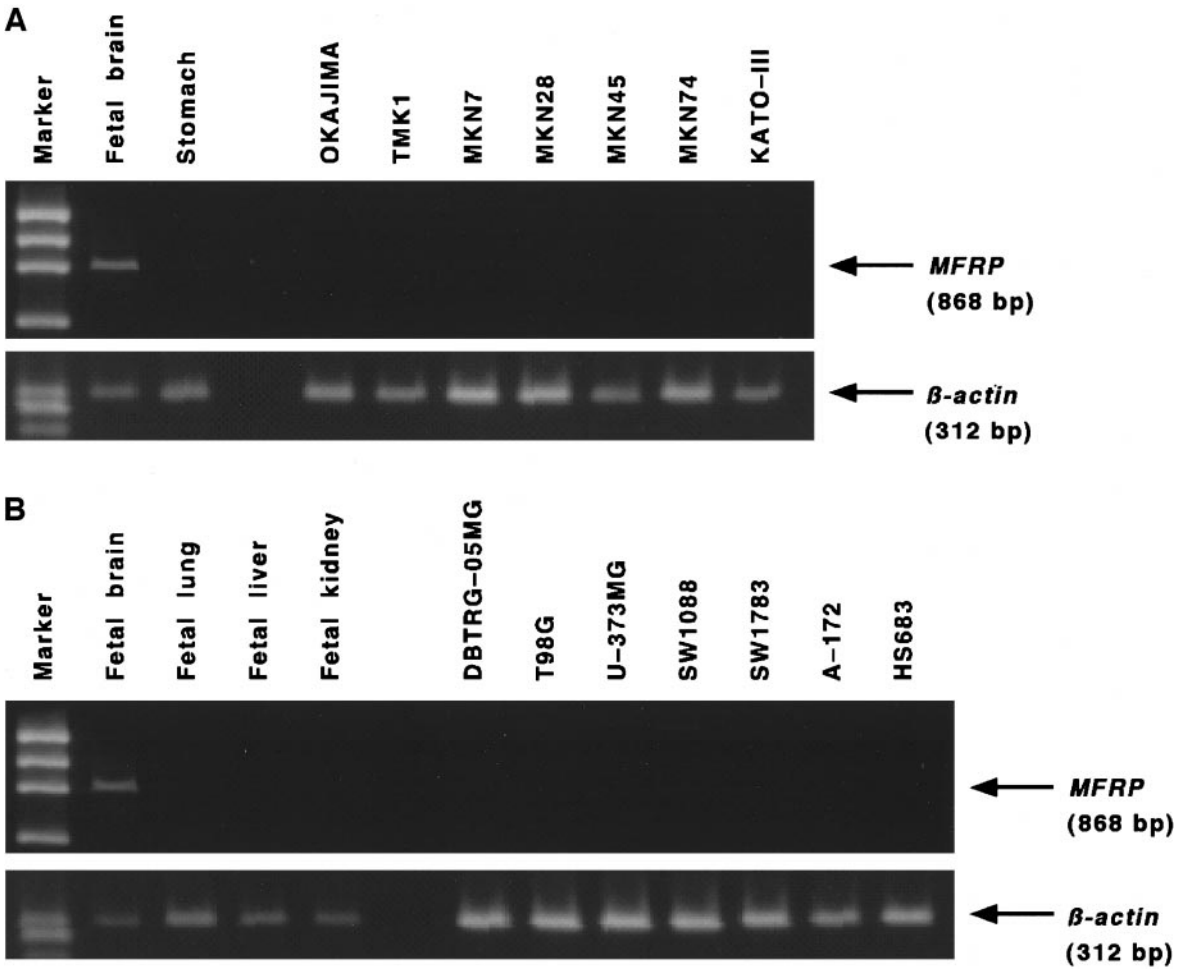


FIG. 5. cDNA-PCR analyses on *MFRP*. (A) Gastric cancer cell lines. (B) Brain tumor cell lines. Forty nanograms of poly $(A)^+$ RNA was used as a template of one-step cDNA-PCR with the One-Step RT-PCR Kit (Qiagen) as described previously (16). The 868-bp *MFRP* cDNA fragment was amplified by 33 cycles of cDNA-PCR with PM-31 and PM-32 primers, and the 312-bp *ß-actin* cDNA fragment was amplified by 24 cycles of cDNA-PCR with BACT01 and BACT02 primers. The *MFRP* mRNA was detected in human fetal brain (positive control), but not in seven gastric cancer cell lines and in seven brain tumor cell lines.

and PM-32 primers. As the MFRS probe was not significantly homologous to any genes or any expressed sequence tags (ESTs) in the DDBJ/EMBL/GenBank Data Bases, the MFRS probe was expected to be a *MFRP* specific probe. In fact, Northern blot analyses revealed that the MFRS probe hybridized to a single band of 4.0-kb in size (Fig. 4). *MFRP* was not detected in various normal tissues, except faint expression in adult brain (Fig. 1A) and fetal brain (Fig. 1B).

The *MFRP* mRNA was not detected by Northern blot analysis in human cancer cell lines HL-60, HeLa S3, K-562, MOLT-4, Raji, SW480, A539, and G361 by Northern blot analysis (Fig. 4C). The *MFRP* mRNA was detected by one-step cDNA-PCR with PM-31 and PM-32 primers in fetal brain, but not in seven gastric cancer cell lines and seven brain tumor cell lines (Fig. 5).

As the *MFRP* mRNA was faintly expressed in adult brain, regional distribution of the *MFRP* mRNA in human brain was further investigated by Northern blot analysis. The 4.0-kb *MFRP* mRNA was expressed weakly in hippocampus and corpus callosum, and was expressed strongly in medulla oblongata (Fig. 4D).

DISCUSSION

The *MFRP* gene was cloned and characterized in this study. *MFRP* was found to encode a type II transmembrane protein of 579 amino-acid residues, which consisted of the N-terminal cytoplasmic region, the transmembrane domain, and the extracellular region with two tandem-repeats of CUB ~ MFD as well as the C-terminal CRD (Fig. 1).

CRD of MFRP matched to the following consensus sequence of CRD (11): C – X(7) – C – X(4) – YN – X – T – X(2) – PN – X(6 or 7) – Q – X(17) – C – X(8) – C – X(4) – P – X – C – X(9 or 10) – C – X(10) – WP – X(2 or 4) – C. CRD of MFRP was homologous to those of Corin, FZDs, and SFRPs; however, amino-acid identity be-

tween CRD of MFRP and those of other CRD molecules were less than 40%. Although the role of Corin in the WNT signaling pathway remains to be elucidated, FZDs are seven-transmembrane-type WNT receptors, and SFRPs are secreted-type WNT antagonists. These results suggest that MFRP with a CRD might be also implicated in the WNT signaling pathway, just like FZDs and SFRPs.

The 4.0-kb *MFRP* mRNA was not detected in normal human tissues, except faint expression in adult and fetal brain (Figs. 4A and 4B). The *MFRP* cDNA was isolated from poly(A)$^+$ RNA of fetal brain, but not from those of fetal lung, liver, and kidney (Fig. 5B). These results suggest that *MFRP* might be a neural tissue-specific mRNA.

The *MFRP* gene was located on human chromosome 11q23, which region is frequently rearranged or deleted in several types of human tumor, including acute leukemia, neuroblastoma, melanoma, lung cancer, breast cancer, cervical cancer, and colorectal cancer (21–27). Expression of the *MFRP* mRNA was not detected in 22 tumor cell lines derived from various tissues (Figs. 4C and 5). *MFRP* expression might be repressed in most cell lines derived from nonneural tissues probably due to tissue specific transcriptional mechanism as mentioned above; however, loss of *MFRP* expression in some tumors derived from neural tissues might be in part due to deletion of the *MFRP* gene or hypermethylation in the *MFRP* promoter. Thus, we should next search expression profile of the *MFRP* mRNA as well as genetic alterations of the *MFRP* gene in brain tumors other than glioblastomas as well as in neuroblastomas.

Regional localization of the *MFRP* mRNA in human brain was further investigated by Northern blot analysis, and the *MFRP* mRNA was found to be strongly expressed in medulla oblongata (Fig. 3D). As *MFRP* encoded a transmembrane protein with CRD, it is reasonable to speculate that CRD of MFRP might also have the binding capacity to WNTs. There are three possibilities for the interaction between MFRP and WNTs: (i) MFRP might function as another class of cell-surface WNT receptor; (ii) MFRP might function as a coreceptor for WNTs to facilitate the association between WNTs and FZDs; (iii) MFRP might function as a transmembrane-type WNT-antagonist. In any case, *MFRP* would play key roles in medulla oblongata as a regulator of the WNT signaling pathway.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for the Specific Research Area from the Ministry of Education, Science, and Culture of Japan (to M.K.).

REFERENCES

1. Moon, R. T., Brown, J. D., and Torres, M. (1997) *WNTs* modulates cell fate and behavior during vertebrate development. *Trends Genet.* **13,** 157–162.
2. Peifer, M., and Polakis, P. (2000) Wnt signaling in oncogenesis and enbryogenesis – a link outside the nucleus. *Science* **287,** 1606–1609.
3. Boutros, M., Paricio, N., Strutt, D. I., and Mlodzik, M. (1998) Dishevelled activates JNK and discriminates between JNK pathway in planar polarity and wingless signaling. *Cell* **94,** 109–118.
4. Kuhl, M., Sheldahl, L. C., Malbon, C. C., and Moon, R. T. (2000) Ca^{2+}/calmodulin-dependent protein kinase II is stimulated by Wnt and Frizzled homologs and promotes ventral cell fates in *Xenopus*. *J. Biol. Chem.* **275,** 12701–12711.
5. Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destree, O., and Clevers, H. (1996) XTcf-3 transcription factor mediates ß-catenin-induced axis formation in *Xenopus* embryos. *Cell* **86,** 391–399.
6. Wang, Y., Macke, J. P., Abella, B. S., Andreasson, K., Worley, P., Gilbert, D. J., Copeland, N. G., Jenkins, N. A., and Nathans, J. (1996) A large family of putative transmembrane receptors homologous to the product of the *Drosophila* tissue polarity gene *frizzled*. *J. Biol. Chem.* **271,** 4468–4476.
7. Wang, Y. K., Samos, C. H., Peoples, R., Perez-Jurado, L. A., Nusse, R., and Francke, U. (1997) A novel human homologue of the *Drosophila frizzled* wnt receptor gene binds wingless protein and is in the Williams syndrome deletion at 7q11.23. *Hum. Mol. Genet.* **6,** 465–472.
8. Sagara, N., Toda, G., Hirai, M., Terada, M., and Katoh, M. (1998) Molecular cloning, differential expression, and chromosomal localization of human *Frizzled-1*, *Frizzled-2*, and *Frizzled-7*. *Biochem. Biophys. Res. Commun.* **252,** 117–122.
9. Koike, J., Takagi, A., Miwa, T., Hirai, M., Terada, M., and Katoh, M. (1999) Molecular cloning of *Frizzled-10*, a novel member of the *Frizzled* gene family. *Biochem. Biophys. Res. Commun.* **262,** 39–43, doi:10.1006/bbrc.1999.1161.
10. Kirikoshi, H., Sagara, N., Koike, J., Tanaka, K., Sekihara, H., Hirai, M., and Katoh, M. (1999) Molecular cloning and characterization of human *Frizzled-4* on chromosome 11q14-q21. *Biochem. Biophys. Res. Commun.* **264,** 955–961, doi:10.1006/bbrc.1999.1612.
11. Kirikoshi, H., Koike, J., Sagara, N., Saitoh, T., Tokuhara, M., Tanaka, K., Sekihara, H., Hirai, M., and Katoh, M. (2000) Molecular cloning and genomic structure of human *Frizzled-3* at chromosome 8p21. *Biochem. Biophys. Res. Commun.* **271,** 8–14, doi:10.1006/bbrc.2000.2578.
12. Yan, W., Sheng, N., Seto, M., Morser, J., and Wu, Q. (1999) Corin: A mosaic transmembrane serine protease encoded by a novel cDNA from human heart. *J. Biol. Chem.* **274,** 14926–14935.
13. Rattner, A., Hsieh, J. C., Smallwood, P. M., Gilbert, D. J., Copeland, N. G., Jenkins, N. A., and Nathans, J. (1997) A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *Proc. Natl. Acad. Sci. USA* **94,** 2859–2863.
14. Finch, P. W., He, X., Kelley, M. J., Uren, A., Schaudies, R. P., Popescu, N. C., Rudikoff, S., Aaronson, S. A., Varmus, H. E., and Rubin, J. S. (1997) Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. *Proc. Natl. Acad. Sci. USA* **94,** 6770–6775.
15. Chang, J. T., Esumi, N., Moore, K., Li, Y., Zhang, S., Chew, C., Goodman, B., Rattner, A., Moody, S., Stetten, G., Campochiaro,

P. A. and, Zack, D. J. (1999) Cloning and characterization of a secreted frizzled-related protein that is expressed by the retinal pigment epithelium. *Hum. Mol. Genet.* **8,** 575–583.

16. Katoh, M., Kirikoshi, H., Saitoh, T., Sagara, N., and Koike, J. (2000) Alternative splicing of the *WNT-2B/WNT-13* gene. *Biochem. Biophys. Res. Commun.* **275,** 209–216, doi:10.1006/bbrc.2000.3252.
17. Sagara, N., and Katoh, M. (2000) Mitomycin C resistance induced by *TCF-3* overexpression in gastric cancer cell line MKN28 is associated with *DT-diaphorase* down-regulation. *Cancer Res.* **60,** 5959–5962.
18. Bork, P., and Beckmann, G. (1993) The CUB domain. A widespread module in developmentally regulated proteins. *J. Mol. Biol.* **231,** 539–545.
19. Kozyraki, R., Kristiansen, M., Silahtaroglu, A., Hansen, C., Jacobsen, C., Tommerup, N., Verroust, P. J., and Moestrup, S. K. (1998) The human intrinsic factor–vitamin B12 receptor, cubilin: Molecular characterization and chromosomal mapping of the gene to 10p within the autosomal recessive megaloblastic anemia (MGA1) region. *Blood* **91,** 3593–3600.
20. Padgett, R. A., Grabowski, P. J., Konarska, M. M., Seiler, S., and Sharp, P. A. (1986) Splicing of messenger RNA precursors. *Annu. Rev. Biochem.* **55,** 1119–1150.
21. Gauwerky, C. E., and Croce, C. M. (1993) Chromosomal translocations in leukemia. *Semin. Cancer Biol.* **4,** 333–340.
22. Maris, J. M., and Matthay, K. K. (1999) Molecular biology of neuroblastoma. *J. Clin. Oncol.* **17,** 2264–2279.
23. Herbst, R. A., Mommert, S., Casper, U., Podewski, E. K., Kiehl, P., Kapp, A., and Weiss, J. (2000) 11q23 allelic loss is associated with regional lymph node metastasis in melanoma. *Clin. Cancer Res.* **6,** 3222–3227.
24. Murakami, Y., Nobukuni, T., Tamura, K., Maruyama, T., Sekiya, T., Arai, Y., Gomyou, H., Tanigami, A., Ohki, M., Cabin, D., Frischmeyer, P., Hunt, P., and Reeves, R. H. (1998) Localization of tumor suppressor activity important in nonsmall cell lung carcinoma on chromosome 11q. *Proc. Natl. Acad. Sci. USA* **95,** 8153–8158.
25. Tomlinson, I. P., Strickland, J. E., Lee, A. S., Bromley, L., Evans, M. F., Morton, J., McGee, J. O. (1995) Loss of heterozygosity on chromosome 11 q in breast cancer. *J. Clin. Pathol.* **48,** 424–428.
26. Lazo, P. A. (1999) The molecular genetics of cervical carcinoma. *Br. J. Cancer* **80,** 2008–2018.
27. Lee, A. S., Seo, Y. C., Chang, A., Tohari, S., Eu, K. W., Seow-Choen, F., and McGee, J. O. (2000) Detailed deletion mapping at chromosome 11q23 in colorectal carcinoma. *Br. J. Cancer* **83,** 750–755.