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Alternative Splicing of the *WNT-2B/WNT-13* Gene

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Secreted glycoprotein WNTs play important roles in carcinogenesis and development. We have previously reported molecular cloning of *WNT-2B/WNT-13*. Here, we have isolated a novel *WNT-2B* isoform (*WNT-2B2*), in addition to the original *WNT-2B* isoform (*WNT-2B1*). *WNT-2B1* and *WNT-2B2* are completely different in the 5′-UTR and in the N-terminal part of the coding region. The N-terminal hydrophobic domain is contained in WNT-2B1, but not in WNT-2B2. WNT-2B1 and WNT-2B2 share the WNT-core domain, and show 87.0% amino-acid identity. We have determined the structure of the *WNT-2B* gene. The *WNT-2B1* mRNA consists of exons 1, 2, and 4–7, while the *WNT-2B2* mRNA consists of exons 3–7. *WNT-2B2* was expressed in fetal brain, fetal lung, fetal kidney, caudate nucleus, testis, glioblastoma cell lines A172, SW1783, gastric cancer cell lines MKN28, MKN74, and cervical cancer cell line HeLa S3. *WNT-2B1* expression level was relatively higher in fetal brain and fetal lung than in other tissues or cell lines expressing *WNT-2B2*. These results indicate that the *WNT-2B1* and *WNT-2B2* mRNAs are transcribed due to alternative splicing with distinct expression profile. This is the first report on the *WNT* isoforms derived from the same gene due to alternative splicing.

Key Words: **WNT; gastric cancer; glioblastoma; fetal lung; caudate nucleus.**

The *WNT* genes, encoding secreted glycoproteins with conserved 22 cysteine residues, are involved in carcinogenesis and in fetal development (1, 2). WNTs bind to seven-transmembrane receptors with the N-terminal cysteine-rich domain encoded by the *Frizzled* (*FZD*) genes (3–9). The WNT signal is transduced through FZD to the β-catenin–TCF pathway or to the JNK pathway (10, 11).

The nucleotide sequence data of *WNT-2B1 and WNT-2B2 cDNAs* will appear in the DDBJ/EMBL/GenBank databases under Accession Nos. AB045116 and AB045117, respectively.

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The *Wnt-1, Wnt-3,* and *Wnt-10b* genes were identified as proto-oncogenes activated by proviral insertion in mouse mammary tumors (12–14). The *WNT-16* gene was identified as a proto-oncogene activated by the E2A-Pbx1 chimeric transcription factor caused by the t(1;19) translocation in pre-B acute lymphoblastoid leukemia (15). The *WNT-2* gene was isolated as a Wnt-1-related gene during isolation of the cystic fibrosis gene located on human chromosome 7 (16). *WNT-2B/WNT-13, Wnt-3a, Wnt-4, Wnt-5a, Wnt-5b, Wnt-6, Wnt-7a, Wnt-7b,* and *WNT-8B* were isolated by PCR with degenerate primers based on the homology among the *WNT* gene family (1, 17–20).

Human *WNT-2B* (1) and mouse *Wnt-2b* (21) are very homologous; however, they are completely different in the 5′-UTR and in the N-terminal part of the coding region. In this manuscript, we have isolated two types of the human *WNT-2B* cDNAs: *WNT-2B1* (the original human *WNT-2B* type), and *WNT-2B2* (the mouse *Wnt-2b* type). By isolating the human *WNT-2B* genomic clones, we have demonstrated that the *WNT-2B1* and *WNT-2B2* mRNAs are transcribed due to alternative splicing. The differential expression pattern of two *WNT-2B* isoforms in normal tissues as well as in cancer cell lines will also be described. This is the first report on the *WNT* isoforms derived from the same gene due to alternative splicing.

MATERIALS AND METHODS

Cell lines and poly (A)$^+$ RNA extraction. Poly(A)$^+$ RNAs were extracted from the following human cancer cell lines with the FastTrack 2.0 Kit (Invitrogen): DBTRG-05MG, T98G, U-373MG, SW1088, SW1783, A-172, and HS683 (brain tumor), HeLa S3 (cervical cancer), MKN28, and MKN74 (gastric cancer).

Northern blot analyses. Two micrograms of poly(A)$^+$ RNA extracted from indicated sources were separated by 1.0% agarose gels containing 17.9% formaldehyde in 1 × MOPS buffer, and were transferred onto nitrocellulose filters, and then were fixed by baking at 80°C for 2 h in a vacuum oven. Northern blot filters were hybridized with a [α-^{32}P] dCTP-labeled probe at 68°C for 1 h in QuikHyb solution (Stratagene). Filters were washed in 2 × SSC buffer and 0.1% SDS at room temperature for 15 min twice, in 0.1 × SSC buffer and 0.1% SDS at 60°C for 30 min, and then were exposed to XAR-5 film (Kodak).

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TABLE 1

List of PCR Primers

Primer	Nucleotide sequence	Nucleotide position
PW2B1-01	AACTGAAGGATCCTTGAGGACG	174–195 of *WNT-2B1*
PW2B1-02	CCCACTGAACGCATGATGTCTG	353–332 of *WNT-2B1*
PW2B2-01	CGGGAGTCTTCGGGGAGCTATGCTGAGACC	1–30 of *WNT-2B2*
PW2B2-02	TCAGGTCTGGTCCAGCCACTC	1195–1175 of *WNT-2B2*
PW2B2-03	CCCGCCTAGGTCTTGCCTGC	129–148 of *WNT-2B2*
PW2B2-04	CCCACTGAACGCATGATGTCTG	309–288 of *WNT-2B2*
BACT-02	GCGGATGTCCACGTCACACT	943–924 of *β-actin*
BACT-03	CCACTGGCATCGTGATGGAC	516–535 of *β-actin*

cDNA library screening. Human fetal lung cDNA library in λgt10 (CLONTECH) was screened with the human *WNT-2B/WNT-13* cDNA fragment WTGC3 (1) as previously described (22). After secondary screening, phage DNAs were purified with Lambda Midi Kit (QIAGEN). The cDNA insert was excised from phage vector λgt10 with *EcoRI* digestion, and was subcloned into pUC118 plasmid vector (TaKaRa). Plasmid DNAs were purified by Plasmid Mini Kit (QIAGEN), and aliquots were used for nucleotide sequence analyses with ABI310 Sequencer (PE Applied Biosystems).

Genomic DNA library screening. Human placental genomic DNA library in EMBL3 SP6/T7 (CLONTECH) was screened with the *WNT-2B1* cDNA as previously described (22). After secondary screening, phage DNAs were purified with Lambda Midi Kit (QIAGEN) for sequence analyses.

cDNA-PCR. mRNA levels of *WNT-2B* isoforms in each tissue and each cancer cell line were compared by multiplex PCR with OneStep RT-PCR Kit (QIAGEN). Fifty nanograms of poly(A)$^+$ RNAs was used as a template of one-step cDNA-PCR. After reverse-transcription reaction at 50°C for 30 min with Omniscript Reverse Transcriptase and Sensiscript Reverse Transcriptase, HotStarTaq DNA Polymerase was activated by heating at 95°C for 15 min, and then cDNAs were amplified by 31 cycles of PCR (0.5 min at 94°C, 0.5 min at 60°C, and 2 min at 72°C), followed by final extension at 72°C for 10 min. Nucleotide sequences of PCR primers are listed in Table 1. Multiplex RT-PCR with a pair of *WNT-2B* primers (1.0 μM each) and a pair of *β-actin* primers (67 nM each) amplified the *WNT-2B* cDNA fragment together with the *β-actin* cDNA fragment as an internal control. cDNA-PCR products were purified with QIAEX Kit (QIAGEN), and were ligated to the TA-cloning vector pCR2.1 (Invitrogen). Plasmid DNAs were extracted with Plasmid Mini Kit (QIAGEN) for nucleotide sequencing analysis.

RESULTS

Existence of WNT-2B1 mRNA in Normal Human Tissue

The original human *WNT-2B* (*WNT-2B1*) cDNA isolated from gastric cancer mRNAs (1) and the mouse *Wnt-2b* cDNA isolated from embryo mRNAs (21) are very homologous except for the 5′-UTR and the N-terminal part of the coding region (Fig. 1A). This divergence might be due to (i) species specificity, (ii) alternative splicing, (iii) genetic alteration in gastric cancer, or (iv) cloning artifact. At first, we tried to confirm the existence of the *WNT-2B1* mRNA in normal human tissue.

We have previously reported that the *WNT-2B* mRNA is expressed in human fetal lung (1). Thus, the human fetal lung cDNA library (CLONTECH) was screened with the human *WNT-2B* cDNA fragment WTGC3, which was previously isolated from human gastric cancer mRNAs. One clone was isolated out of 1.0×10^6 clones, and was designated the *WNT-2B1* cDNA. Nucleotide sequence analysis of the *WNT-2B1* cDNA revealed that the N-terminal part of the coding region was not homologous to the mouse *Wnt-2b* cDNA at all, but was identical to the previously reported human *WNT-2B* cDNA.

This result indicates that the *WNT-2B1* mRNA exists in normal human tissues, and that the divergence between the original human *WNT-2B* cDNA and the mouse *Wnt-2b* cDNA in the N-terminal part of the coding region is not due to genetic alteration in gastric cancer or cloning artifact, but to species specificity or alternative splicing.

WNT-2B1 mRNA Consists of Six Exons

To obtain a clue for the further explanation of divergence between the human *WNT-2B1* and the mouse *Wnt-2b* in the N-terminal part of the coding region, we tried to determine the exon–intron boundaries of the human *WNT-2B* gene.

The human placental genomic DNA library (CLONTECH) was screened with the *WNT-2B1* cDNA probe, and 11 clones were isolated out of 7.0×10^5 clones. The *WNT-2B* genomic clones were sequenced with primers corresponding to the nucleotide sequence of the *WNT-2B1* cDNA (Table 2). Comparison between

FIG. 1. Amino acid sequence and hydrophobicity analyses of WNT-2B1 and WNT-2B2. (A) Amino-acid comparison among human WNT-2B1, WNT-2B2, and mouse Wnt-2b. Amino acids are numbered on the right. Conserved amino acids among human WNT-2B1, WNT-2B2, and mouse Wnt-2b are indicated by asterisk below the alignment. (B) Kyte & Doolittle hydrophobicity analysis on WNT-2B1. The N-terminal hydrophobic domain corresponding to the typical signal peptide is indicated by SP. (C) Kyte & Doolittle hydrophobicity analysis on WNT-2B2. WNT-2B2 lacks the N-terminal hydrophobic domain.

A

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WNT-2B1   MLDGLGV--VAISIFGIQLKTEGSLRTAVPGIPTQSAFNKCLQR----------------YIGAL   47
WNT-2B2   MLRPGGAEEAAQLPLRRASAPVPVPSPAAPDGSRASARLGLACLLLLLLLTLPARVDTSWWYIGAL   66
Wnt-2b    MLKLQGEDEAAQLAPRRARVPVPR--PTAPDVSPSSARLGLACLLLLLLLTLPARVDTSWWYIGAL   64
          **   *    *                    *     **                          *****

WNT-2B1   GARVICDNIPGLVSRQRQLCQRYPDIMRSVGEGAREWIRECQHQFRHHRWNCTTLDRDHTVFGRVM  113
WNT-2B2   GARVICDNIPGLVSRQRQLCQRYPDIMRSVGEGAREWIRECQHQFRHHRWNCTTLDRDHTVFGRVM  132
Wnt-2b    GARVICDNIPGLVSRQRQLCQRYPDIMRSVGEGAREWIRECQHQFRHHRWNCTTLDRDHTVFGRAM  130
          ************************************************************** *

WNT-2B1   LRSSREAAFVYAISSAGVVHAITRACSQGELSVCSCDPYTRGRHHDQRGDFDWGGCSDNIHYGVRF  179
WNT-2B2   LRSSREAAFVYAISSAGVVHAITRACSQGELSVCSCDPYTRGRHHDQRGDFDWGGCSDNIHYGVRF  198
Wnt-2b    LRSSREAAFVYAISSAGVVHAITRACSQGELSVCSCDPYTRGRHHDQRGDFDWGGCSDNIHYGVRF  196
          ************************************************************

WNT-2B1   AKAFVDAKEKRLKDARALMNLHNNRCGRTAVRRFLKLECKCHGVSGSCTLRTCWRALSDFRRTGDY  245
WNT-2B2   AKAFVDAKEKRLKDARALMNLHNNRCGRTAVRRFLKLECKCHGVSGSCTLRTCWRALSDFRRTGDY  264
Wnt-2b    AKAFVDAKEKRLKDARALMNLHNNRCGRTAVRRFLKLECKCHGVSGSCTLRTCWRALSDFRRTGDY  262
          ************************************************************

WNT-2B1   LRRRYDGAVQVMATQDGANFTAARQGYRRATRTDLVYFDNSPDYCVLDKAAGSLGTAGRVCSKTSK  311
WNT-2B2   LRRRYDGAVQVMATQDGANFTAARQGYRRATRTDLVYFDNSPDYCVLDKAAGSLGTAGRVCSKTSK  330
Wnt-2b    LRRRYDGAVQVTATQDGANFTAARQGYRHATRTDLVYFDNSPDYCVLDKASGSLGTAGRVCSKTSK  328
          ********** ********** ********** ********** **********

WNT-2B1   GTDGCEIMCCGRGYDTTRVTRVTQCECKFHWCCAVRCKECRNTVDVHTCKAPKKAEWLDQT        372
WNT-2B2   GTDGCEIMCCGRGYDTTRVTRVTQCECKFHWCCAVRCKECRNTVDVHTCKAPKKAEWLDQT        391
Wnt-2b    GTDGCEIMCCGRGYDTTRVTRVTQCECKFHWCCAVRCKECRNTVDVHTCKAPKKAEWLDQT        389
          ************************************************************
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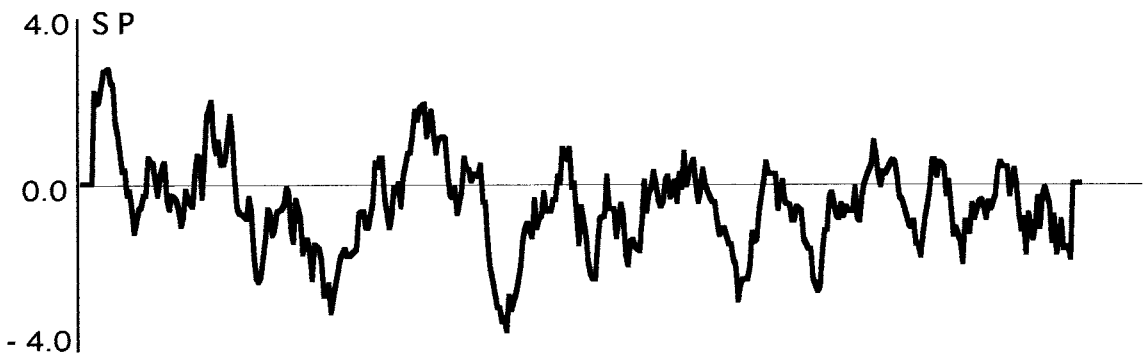
B



C

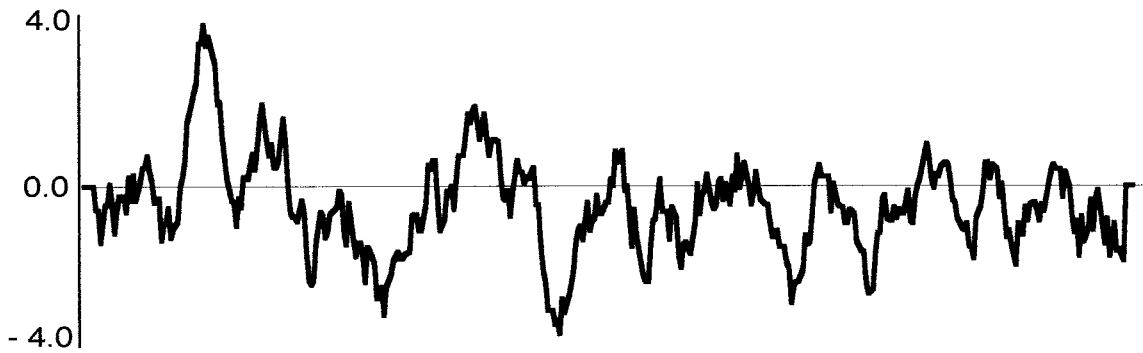


TABLE 2

List of Sequence Primers

Primer	Nucleotide sequence	Nucleotide position
PW13G-01	ACATGTTGGATGGCCTTG	119–136 of *WNT-2B1*
PW13G-02	TGAACGCTGACTGTGTAG	229–212 of *WNT-2B1*
PW13G-03	TGAAGGATCCTTGAGGAC	177–195 of *WNT-2B1*
PW13G-04	AACGCTGGCACAGCTG	328–313 of *WNT-2B1*
PW13G-05	GTACATTGGGGCACTG	246–261 of *WNT-2B1*
PW13G-06	TGGATGTTGTCACTGCAG	641–624 of *WNT-2B1*
PW13G-07	AGCCAGGGTGAACTGAGT	538–555 of *WNT-2B1*
PW13G-08	CACGGCGATAGCCTTG	943–928 of *WNT-2B1*
PW13G-09	AAGTGCCATGGCGTGAG	775–791 of *WNT-2B1*
PW13G-10	ATTCCTTGCACCGTACAG	1171–1154 of *WNT-2B1*
PW13G-11	TGTCTGCAGCAAGACATC	1032–1049 of *WNT-2B1*
PW13G-13	GACACGTCCTGGTGGT	DTSWW + gt
PW13G-14	TCCCGTGAGCAGCCAGTC	Intron 3

the nucleotide sequences of the *WNT-2B1* cDNA and the *WNT-2B* genomic clones revealed that the *WNT-2B1* mRNA is split into six parts in the *WNT-2B* genomic clones (Fig. 2A). The consensus sequences for splice donor and acceptor sites (23) were found around the exon–intron boundaries of the *WNT-2B* gene (Fig. 2C). These results indicate that the *WNT-2B1* mRNA consists of six exons.

Coincidence of Divergence Junction and Splice Junction

Although the first and second exons corresponding to the 5′-UTR and the N-terminal part of the coding region of the *WNT-2B1* mRNA were not homologous to the mouse *Wnt-2b* mRNA at all, the exons that followed were highly homologous. Interestingly, the junction of divergence between the human *WNT-2B1* mRNA and the mouse *Wnt-2b* mRNA was consistent with the splicing junction in the human *WNT-2B1* mRNA. These results strongly suggest that the divergence in the N-terminal part of the coding region would be due to alternative splicing.

Identification of a Novel Exon in the Human WNT-2B Gene

By sequencing analysis of the *WNT-2B* genomic clones with a primer corresponding to the mouse *Wnt-2b* cDNA, we next searched for a novel exon of the mouse *Wnt-2b* type within the human *WNT-2B* gene. The amino-acid sequence encoded by the putative mouse-*Wnt-2b* type exon might be as follows: MLKLQG-EDEAAQLAPRRARVPVPRPTAPDVSPSSARLGLA-CLLLLLLLTLPARVDTSWW. The sequence primer PW13G-13 (5′-GACACGTCCTGGTGgt-3′) was synthesized depending on the nucleotide sequence corresponding to DTSWW + gt (the consensus sequence of splice donor site). Sequencing of the W13G-11 genomic clone with the PW13G-13 primer showed the nucleotide sequence of the following intron in the human *WNT-2B* gene (Fig. 2B).

Primer PW13G-14 (5′-TCCCGTGAGCAGCCAGTC-3′) was synthesized depending on the nucleotide sequence of the intron identified above (Fig. 2B). Sequencing of the W13G-11 genomic clone with the PW13G-14 primer showed the existence of a novel exon, which apparently contains the 5′-UTR and the N-terminal part of the coding region homologous to the mouse *Wnt-2b* mRNA (Fig. 2B). Deduced amino-acid sequence of the novel exon in the human *WNT-2B* gene and that of the corresponding region in the mouse Wnt-2b polypeptide showed 74% amino-acid identity.

Alternative Splicing of the WNT-2B Gene

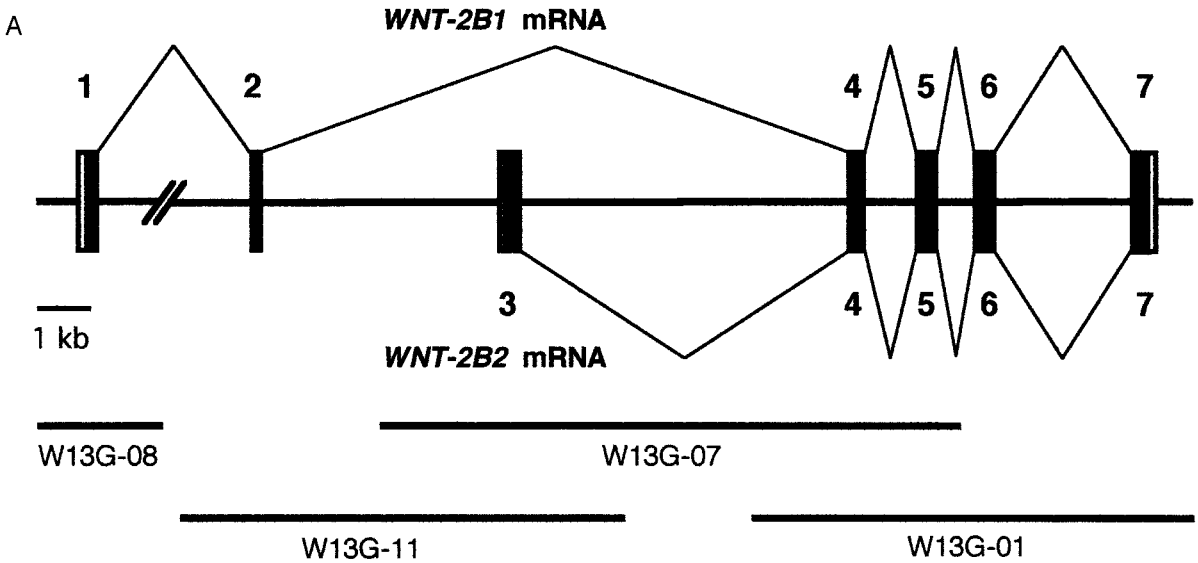
Existence of the *WNT-2B2* mRNA containing the novel exon was confirmed by cDNA-PCR with the PW2B2-01 primer corresponding to the novel exon and the PW2B2-02 primer corresponding to the last exon. cDNA-PCR with the PW2B2-01 and the PW2B2-02 primers amplified a PCR product of 1195-bp in length, which was designated the *WNT-2B2* cDNA. Sequence analyses indicated that the *WNT-2B2* cDNA consists of the novel exon identified above, and the exons that follow and are conserved between *WNT-2B1* and *WNT-2B2* (Fig. 2).

The W13G-11 genomic clone contains both the second exon for the *WNT-2B1* and the novel exon specific to the *WNT-2B2* mRNA, while the W13G-07 genomic clone contains both the novel exon specific to the *WNT-2B2* mRNA and the exons that follow and are conserved between *WNT-2B1* and *WNT-2B2* (Fig. 2A). These results indicate that the novel exon specific to the *WNT-2B2* mRNA locates between the second and third exons for the *WNT-2B1* mRNA gene. Thus, the novel exon specific to the *WNT-2B2* mRNA was designated exon 3 of the *WNT-2B*.

The *WNT-2B1* mRNA consists of exons 1, 2, and 4–7, while the *WNT-2B2* mRNA consists of exons 3–7. The *WNT-2B1* mRNA and the *WNT-2B2* mRNA are transcribed due to alternative splicing (Fig. 2A).

FIG. 2. Alternative splicing of the human *WNT-2B* gene. (A) Schematic representation of the human *WNT-2B* gene. Exons and introns are indicated by boxes and bars, respectively. The coding region is indicated by closed box, while UTR by open box. The *WNT-2B* genomic clones are shown by bars below the scheme of the *WNT-2B* gene. *WNT-2B1* mRNA consists of exons 1, 2, 4–7. *WNT-2B2* mRNA consists of exons 3–7. (B) Partial nucleotide sequence of exon 3 and intron 3. Predicted amino-acid sequence is shown below the nucleotide sequence. Primers W13G-13 and W13G-14, which were used for the identification of exon 3 in the *WNT-2B* genomic clone are also shown. (C) Nucleotide sequence around the exon-intron boundaries in the *WNT-2B* gene. Nucleotide sequence of the *WNT-2B* gene in exon is indicated by capital letters, while that in intron is indicated by small letters.

A



B

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CGGGAGTCTTCGGGGAGCTATGCTGAGACCGGGTGGTGCGGAGGAAGCTGCGCAGCTCCCGCTTCGGCGCGC
                    M  L  R  P  G  G  A  E  E  A  A  Q  L  P  L  R  R  A

CAGCGCCCCGGTCCCTGTGCCGTCGCCCGCGGCCCCCGACGGCTCCCGGGCTTCGGCCCGCCTAGGTCTTGC
  S  A  P  V  P  V  P  S  P  A  A  P  D  G  S  R  A  S  A  R  L  G  L  A

                                                    PW13G-13
                                                   ---------------->
CTGCCTTCTGCTCCTGCTGCTGCTGACGCTGCCGGCCCGCGTAGACACGTCCTGGTGgtaagtgtggctctc
  C  L  L  L  L  L  L  L  T  L  P  A  R  V  D  T  S  W  W

                              PW13G-14
                             <----------------
aggctgggcgggtgaggcgcttggtaggagaggccggaggcgcctggagggactggctgctcacgggaccag
```

C

Exon No.	Exon Size (bp)	Sequence around the Exon - Intron Junction	
1	> 174	5'-UTR ·····	CTAAAA gtaagt
2	71	gaccag ACTGAA ·····	GCAAAG gtacat
3	> 201	5'-UTR ·····	CTGGTG gtaagt
4	221	ttgcag GTACAT ·····	TCAGAA gtaaga
5	278	ctctag GTAGCC ·····	CGCACG gtcagt
6	265	ccccag GCTGTG ·····	CTGCAG gtgagt
7	> 277	acccag GTTCCC ·····	3'-UTR

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WNT-2B1     CQHQFRHHRWNCTTLDRDHTV-------FGRVMLRSSREAAFVYAISSAGVVHAITRACSQGELSVCSC
WNT-2B2     CQHQFRHHRWNCTTLDRDHTV-------FGRVMLRSSREAAFVYAISSAGVVHAITRACSQGELSVCSC
WNT-1       CKWQFRNRRWNCPTAPGPHL--------FGKIVNRGCRETAFIFAITSAGVTHSVARSCSEGSIESCTC
WNT-2       CQHQFRQHRWNCNTLDRDHSL-------FGRVLLRSSRESAFVYAISSAGVVFAITRACSQGEVKSCSC
WNT-5A      CQYQFRHRRWNCSTVDNTSV--------FGRVMQIGSRETAFTYAVSAAGVVNAMSRACREGELSTCGC
WNT-7A      CQFQFRNGRWNCSALGERTV--------FGKELKVGSREAAFTYAIIAAGVAHAITAACTQGNLSDCGC
WNT-8B      CKYQFAWDRWNCPERALQLSS-------HGGLRSA-NRETAFVHAISSAGVMYTLTRNCSLGDFDNCGC
WNT-10B     CQHQLRDQRWNCSALEGGGRLPH-----HSAILKRGFRESAFSFSMLAAGVMHAVATACSLGKLVSCGC
WNT-11      CRRAFADMRWNCSSIELAPN--------YLLDLERGTRESAFVYALSAATISHAIARACTSGDLPGCSC
WNT-16      CGSQFRHERWNCMITAAATTAPMGASPLFGYELSSGTKETAFIYAVMAAGLVHSVTRSCSAGNMTECSC
Consensus   C-------RWNC-------------------------E-AF------A---------C--G----C-C

WNT-2B1     DPYTRGRHHDQRGD---------------------------------FDWGGCSDNIHYGVRFAKAFVD
WNT-2B2     DPYTRGRHHDQRGD---------------------------------FDWGGCSDNIHYGVRFAKAFVD
WNT-1       DYRRRGPGGPD-----------------------------------WHWGGCSDNIDFGRLFGREFVD
WNT-2       DPKKMGSAKDSKGI---------------------------------FDWGGCSDNIDYGIKFARAFVD
WNT-5A      SRAARPKDLPRD-----------------------------------WLWGGCGDNIDYGYRFAKEFVD
WNT-7A      DKEKQGQYHRDEG-----------------------------------WKWGGCSADIRYGIGFAKVFVD
WNT-8B      DDSRNGQLGGQG-----------------------------------WLWGGCSDNVGFGEAISKQFVD
WNT-10B     GWKGSGEQDRLRAKLLQLQALSRGKSFPHSLPSPGPGSSPSPGPQDTWEWGGCNHDMDFGEKFSRDFLD
WNT-11      GPVPGEPPGPG-------------------------------------NRWGRCADNLSYGLLMGAKFSD
WNT-16      DTTLQNGGSASEG-----------------------------------WHWGGCSDDVQYGMWFSRKFLD
Consensus   ---------------------------------------------WG-C------G------F-D

WNT-2B1     AKEKRLKDA-------RALMNLHNNRCGRTAVRRFLKLECKCHGVSGSCTLRTCWRALSDFRRTGDYLR
WNT-2B2     AKEKRLKDA-------RALMNLHNNRCGRTAVRRFLKLECKCHGVSGSCTLRTCWRALSDFRRTGDYLR
WNT-1       SGEKGRDL--------RFLMNLHNNEAGRTTVFSEMRQECKCHGMSGSCTVRTCWMRLPTLRAVGDVLR
WNT-2       AKERKGKDA-------RALMNLHNNRAGRKAVKRFLKQECKCHGVSGSCTLRTCWLAMADFRKTGDYLW
WNT-5A      ARERERIHAKGSYESARILMNLHNNEAGRRTVYNLADVACKCHGVSGSCSLKTCWLQLADFRKVGDALK
WNT-7A      AREIKQNA--------RTLMNLHNNEAGRKILEENMKLECKCHGVSGSCTTKTCWTTLPQFRELGYVLK
WNT-8B      ALETGQDA--------RAAMNLHNNEAGRKAVKGTMKRTCKCHGVSGSCTTQTCWLQLPEFREVGAHLK
WNT-10B     SREAPRDI--------QARMRIHNNRVGRQVVTENLKRKCKCHGTSGSCQFKTCWRAAPEFRAVGAALR
WNT-11      APMKVKKTGSQA----NKLMRLHNSEVGRQALRASLEMKCKCHGVSGSCSIRTCWKGLQELQDVAADLK
WNT-16      FPIGNTTGKENKV---LLAMNLHNNEAGRQAVAKLMSVDCRCHGVSGSCAVKTCWKTMSSFEKIGHLLK
Consensus   ------------------M--HN---GR----------C-CHG-SGSC---TCW-----------L-

WNT-2B1     RRYDGAVQVMATQDGANFTAARQGYRRATRT---------DLVYFDNSPDYCVLDKAAGSLGTAGRVCS
WNT-2B2     RRYDGAVQVMATQDGANFTAARQGYRRATRT---------DLVYFDNSPDYCVLDKAAGSLGTAGRVCS
WNT-1       DRFDGASRVLYGNRGSNRASRAELLRLEPEDPAHKPPSPHDLVYFEKSPNFCTYSGRLGTAGTAGRACN
WNT-2       RKYNGAIQVVMNQDGTGFTVANERFKKPTKN---------DLVYFENSPDYCIRDREAGSLGTAGRVCN
WNT-5A      EKYDSAAAMRLNSRGKLVQVNSRFNSPTTQ----------DLVYIDPSPDYCVRNESTGSLGTQGRLCN
WNT-7A      DKYNEAVHVEPVRASRNKRPTFLKIKKPLSYRKPMDT---DLVYIEKSPNYCEEDPVTGSVGTQGRACN
WNT-8B      EKYHAALKVDLLQGAGNSAAARGAIADTFRSISTR-----ELVHLEDSPDYCLENKTLGLLGTEGRECL
WNT-10B     ERLGRAIFIDTHNRNSGAFQPRLRPRRLSG----------ELVYFEKSPDFCERDPTMGSPGTRGRACN
WNT-11      TRYLSATKVVHRPMGTRKHLVPKDLDIRPVKDW-------ELVYLQSSPDFCMKNEKVGSHGTQDRQCN
WNT-16      DKYENSIQISDKTKRKMRRREKDQRKIPIHKD--------DLLYVNKSPNYCVEDKKLGIPGTQGRECN
Consensus   ----------------------------------------L-----SP--C------G--GT--R-C-

WNT-2B1     KTSKGT-----DGCEIMC--CGRGYDTTRVTRVTQCECKFHWCCAVRCKECRNTVDVHTC
WNT-2B2     KTSKGT-----DGCEIMC--CGRGYDTTRVTRVTQCECKFHWCCAVRCKECRNTVDVHTC  (100 %)
WNT-1       SSSPAL-----DGCELLC--CGRGHRTRTQRVTERCNCTFHWCCHVSCRNCTHTRVLHEC  (46.9%)
WNT-2       LTSRGM-----DSCEVMC--CGRGYDTSHVTRMTKCGCKFHWCCAVRCQDCLEALDVHTC  (72.5%)
WNT-5A      KTSEGM-----DGCELMC--CGRGYDQFKTVQTERCHCKFHWCCYVKCKKCTEIVDQFVC  (53.5%)
WNT-7A      KTAPQA-----SGCDLMC--CGRGYNTHQYARVWQCNCKFQWCCYVKCNKCSERTEMYTC  (49.8%)
WNT-8B      RRGRALGRWELRSCRRLCGDCGLAVEERRAETVSSCNCKFHWCCAVRCEQCRRRVTKYFC  (43.2%)
WNT-10B     KTSRLL-----DGCGSLC--CGRGHNVLRQTRVERCHCRFHWCCYVLCDECKVTEWVNVC  (43.2%)
WNT-11      KTSNGS-----DSCDLMC--CGRGYNPYTDRVVERCHCKYHWCCYVTCRRCERTVERYVC  (40.7%)
WNT-16      RTSEGA-----DGCNLLC--CGRGYNTHVVRHVERCECKFIWCCYVRCRRCESMTDVHTC  (43.2%)
Consensus   -------------C---C--CG-------------C-C---WCC-V-C--C--------C
```

FIG. 3. Comparison among the members of the human WNT family in the WNT-core domain. Partial amino-acid identity with WNT-2B1 in the WNT-core domain is shown in the parentheses after the amino-acid sequence. Conserved amino-acid sequence among the human WNT family is shown below the alignment.

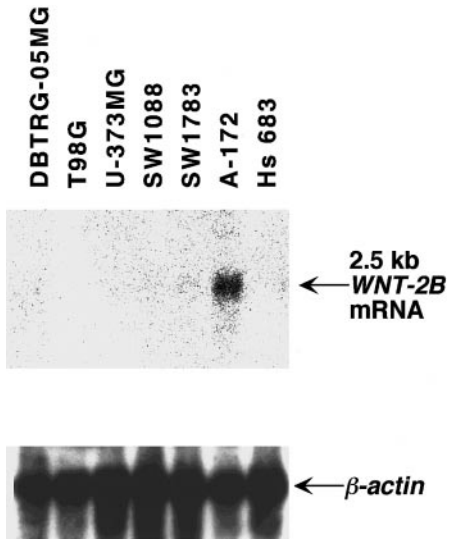

FIG. 4. Expression of *WNT-2B* mRNA among cell lines derived from human brain tumor. Northern blot filters containing 2 μg of poly(A)$^+$ RNA extracted from brain tumor cell lines were hybridized with the *WNT-2B* cDNA probe as described previously (1). The *WNT-2B* mRNA of 2.5-kb in size is indicated with arrow.

Amino-Acid Sequence of WNT-2B1 and WNT-2B2

Although WNT-2B1 and WNT-2B2 are identical in the WNT-core domain, WNT-2B1 and WNT-2B2 are completely divergent in the N-terminal region. Total amino-acid identity between WNT-2B1 and WNT-2B2 is 87.0%. Partial amino-acid identity between WNT-2B1 and WNT-2B2 in the N-terminal region is 16%.

WNT-2B1 is a polypeptide of 372 amino-acid residues with the N-terminal hydrophobic domain, while WNT-2B2 is a polypeptide of 391 amino-acid residues lacking the N-terminal hydrophobic domain (Fig. 1).

WNT-2B1 and WNT-2B2 share the WNT-core domain with conserved 22 cysteine residues. In the WNT-core domain, WNT-2B isoforms are most homologous to WNT-2 (72.5% amino-acid identity) among members of the human WNT family (Fig. 3).

Differential Expression of WNT2B1 and WNT2B2 mRNAs

We have previously reported mRNA expression of *WNT-2B* in normal human tissues including caudate nucleus, testis, fetal brain, fetal lung, and fetal kidney, and also in human cancer cell lines including HeLa S3 (cervical cancer), MKN28 and MKN74 (gastric cancer) by Northern blot analyses (1). In this study, we have investigated the *WNT-2B* mRNA expression among human cell lines derived from brain tumor by Northern blot analysis, and detected a large amount of the *WNT-2B* mRNA in A-172 (glioblastoma), and a very small amount in SW1783 (glioblastoma) (Fig. 4).

Differential expression of the *WNT-2B1* and *WNT-2B2* mRNAs in the above mentioned normal tissues and cancer cell lines was investigated by two sets of multiplex RT-PCR: (i) with a pair of *WNT-2B1* primers and a pair of *β-actin* primers, or (ii) with a pair of *WNT-2B2* primers and a pair of *β-actin* primers (Fig. 5). *WNT-2B2* was expressed in fetal brain, fetal lung, fetal kidney, caudate nucleus, testis, glioblastoma cell lines A172, SW1783, gastric cancer cell lines MKN28, MKN74, and cervical cancer cell line HeLa S3. *WNT-2B1* expression level was relatively higher in fetal brain and fetal lung than in other tissues or cell lines expressing *WNT-2B2* (Fig. 5). These results indicate that the *WNT-2B1* and *WNT-2B2* mRNAs are transcribed due to alternative splicing with distinct expression profile.

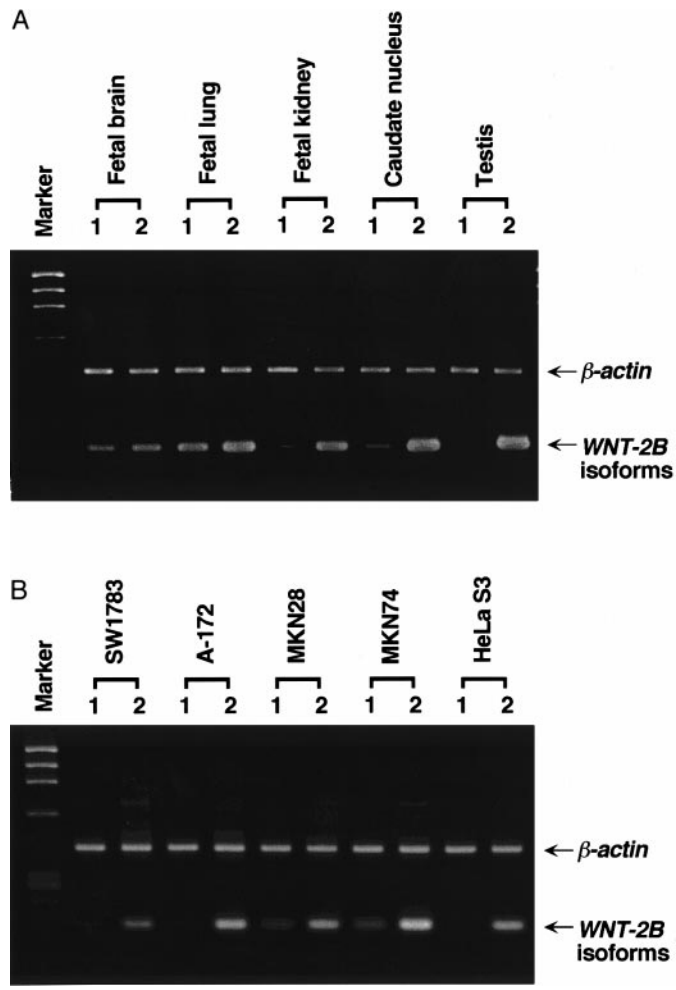

FIG. 5. Differential expression of *WNT-2B1* and *WNT-2B2*. (A) Normal tissues. (B) Cancer cell lines. Each tissue or cell line was analyzed by two sets of multiplex RT-PCR (Lane 1 and Lane 2). Lane 1, multiplex RT-PCR with 1.0 μM each of WNT-2B1 primers (PW2B1-01, PW2B1-02) and 67 nM each of *β-actin* primers (BACT-02, BACT-03) amplifies the *WNT-2B1* cDNA fragment (180 bp) together with the *β-actin* cDNA fragment (428 bp). Lane 2, multiplex RT-PCR with 1.0 μM each of WNT-2B2 primers (PW2B1-03, PW2B1-04) and 67 nM each of *β-actin* primers (BACT-02, BACT-03) amplifies the *WNT-2B2* cDNA fragment (181 bp) together with the *β-actin* cDNA fragment (428 bp).

DISCUSSION

Two types of the human *WNT-2B* cDNA, *WNT-2B1* (the original human *WNT-2B* type), and *WNT-2B2* (the mouse *Wnt-2b* type), were isolated from the human fetal lung cDNA library and human caudate nucleus mRNA, respectively. WNT-2B1 and WNT-2B2 are almost identical except for a completely distinct N-terminal region (Fig. 1A). WNT-2B1 contains the N-terminal hydrophobic domain (Fig. 1B), while WNT-2B2 lacks the N-terminal hydrophobic domain (Fig. 1C).

The human *WNT-2B* genomic clones were isolated to elucidate how the WNT-2B isoforms are transcribed. Comparison between the nucleotide sequences of the *WNT-2B* cDNAs and the *WNT-2B* genomic clones revealed that the *WNT-2B1* mRNA consists of exons 1, 2, and 4–7, and also that the *WNT-2B2* mRNA consists of exons 3–7 (Fig. 2A). These results clearly indicate that the *WNT-2B1* and *WNT-2B2* mRNAs are transcribed due to alternative splicing.

Among human cancer cell lines derived from brain tumor, *WNT-2B* mRNA of 2.5-kb in size was detected in A-172 and SW1783 cell lines derived from glioblastoma by Northern blot analysis (Fig. 4). We have previously demonstrated *WNT-2B* mRNA expression in normal tissues as well as in cancer cell lines (1). The differential expression pattern of the *WNT-2B1* and *WNT-2B2* mRNAs in five normal tissues and five cancer cell lines was investigated by multiplex RT-PCR. *WNT-2B2* was predominantly expressed in five normal tissues and five cancer cell lines examined in this study (Fig. 5). *WNT-2B1* expression level was relatively higher in fetal brain and fetal lung than in other tissues or cell lines expressing *WNT-2B2* (Fig. 5). The distinct expression pattern of *WNT-2B* isoforms suggests that the *WNT-2B1* and *WNT-2B2* mRNAs might be transcribed by using alternative promoter.

The amino-acid sequence of WNT-2B1, WNT-2B2 (this study), WNT-1, WNT-2, WNT-5A, WNT-7A, WNT-8B, WNT-10B, WNT-11, and WNT-16 (12, 15, 16, 19, 20, 24–26) was aligned in the WNT-core domain (Fig. 3). WNT-2 is more homologous to WNT-2B isoforms than other WNTs, and WNT-2B and WNT-2 constitute a subfamily among the WNT family (1). We have previously mapped the *WNT-2B* gene to human chromosome 1p13, and another group mapped the *WNT-2* gene to human chromosome 7 (16). Although highly related *WNT* genes (e.g., *WNT-2B* and *WNT-2*) exist in the human genome, each *WNT* gene has been reported to encode a single isoform. In this report, we have demonstrated that the *WNT-2B* gene encodes two isoforms, completely divergent in the N-terminal part of the coding region, due to alternative splicing. This is the first report on the WNT isoforms derived from the same gene due to alternative splicing.

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