Comparative integromics on BMP/GDF family

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Abstract. WNT, Notch, FGF, Hedgehog and BMP signaling pathways network together during embryogenesis, tissue regeneration, and carcinogenesis. *BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8A, BMP8B, BMP10, BMP15, AMH, GDF1, GDF2, GDF3, GDF5, GDF6, GDF7, GDF8, GDF9, GDF10, GDF11*, and *GDF15* are *BMP/GDF* family genes within the human genome; however, transcriptional regulation of *BMP/GDF* family members by the canonical WNT signaling pathway remains unclear. We searched for the TCF/LEF-binding site within the promoter region of *BMP/GDF* family genes by using bioinformatics and human intelligence. Because four TCF/LEF-binding sites were identified within human *GDF10* promoter, comparative genomics analyses on *GDF10* orthologs were further performed. Chimpanzee *GDF10* gene, encoding a 477-amino-acid protein, was identified within NW_112875.1 genome sequence. AY412135.1 was not the correct coding sequence for chimpanzee GDF10. Chimpanzee GDF10 showed 99.2%, 83.2% and 47.4% total amino-acid identity with human GDF10, mouse Gdf10 and human BMP3, respectively. *RASGEF1A-GDF10-PRKG1* locus at human chromosome 10q11 and *BMP3-PRKG2-RASGEF1B* locus at human chromosome 4q21 were paralogous regions with insertions/deletions and recombination. Human *GDF10* mRNA was expressed in fetal cochlea, fetal lung, testis, retina, pineal gland, other neural tissues, head and neck tumors, while mouse *Gdf10* mRNA was expressed in fetal liver, inner ear, cerebellum, other neural tissues, prostate and blood vessels. Four TCF/LEF-binding sites in human *GDF10* promoter were conserved in chimpanzee *GDF10* promoter, but not in the mouse *Gdf10* promoter; however, another TCF/LEF-binding site occurred in mouse *Gdf10* promoter. Four bHLH-binding sites in human *GDF10* promoter were conserved in chimpanzee *GDF10* promoter, but only one in mouse *Gdf10* promoter. Primate *GDF10* promoters were divergent from mouse *Gdf10* promoter. Because *GDF10* was characterized as a potential target of canonical WNT signaling pathway in neural tissues, GDF10 is one of the targets of systems medicine, especially in the field of regenerative medicine.

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Introduction

WNT, Notch, FGF, Hedgehog, and BMP signaling pathways network together during embryogenesis, tissue regeneration, and carcinogenesis (1-8). Canonical WNT signals are transduced through Frizzled receptors and LRP5/6 co-receptors to activate transcription of target genes, such as *FGF18, FGF20, DKK1* and *DKK4*, based on the transcriptional complex consisting of TCF/LEF, ß-catenin, BCL9/BCL9L, and PYGO1/PYGO2 (9-19).

TGFß superfamily members are classified into TFGß/Nodal/Activin group and BMP/GDF group. TFGß/Nodal/Activin signals are transduced through type I and type II receptors for each member to R-SMAD proteins, such as SMAD2 and SMAD3, while BMP/GDF signals are transduced through type I and type II receptors for each member to R-SMAD proteins, such as SMAD1, SMAD5 and SMAD8. Phosphorylated R-SMADs associated with SMAD4 are then translocated to the nucleus to activate transcription of target genes (20-24).

BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8A, BMP8B, BMP10, BMP15, AMH, GDF1, GDF2, GDF3, GDF5, GDF6, GDF7, GDF8, GDF9, GDF10, GDF11, and *GDF15* are *BMP/GDF* family genes within the human genome (http://www.gene.ucl.ac.uk); however, transcriptional regulation of *BMP/GDF* family members by the canonical WNT signaling pathway remains unclear. We searched for the TCF/LEF-binding site within the promoter region of *BMP/GDF* family genes by using bioinformatics and human intelligence. Because four TCF/LEF-binding sites were identified within human *GDF10* promoter, comparative genomics analyses on *GDF10* orthologs were further performed.

Materials and methods

WNT target gene screening. Genome sequences corresponding to human *BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8A, BMP8B, BMP10, BMP15, AMH, GDF1, GDF2, GDF3, GDF5, GDF6, GDF7, GDF8, GDF9, GDF10, GDF11*

A

Human gene	RefSeq ID	Genome clone	TCF/LEF-binding sites	Human gene	RefSeq ID	Genome clone	TCF/LEF-binding sites
BMP2	NM_001200.2	AL035668.15	0	*GDF1*	NM_001492.3	AC005197.1	0
BMP3	NM_001201.1	AC093883.2	1	*GDF2*	NM_016204.1	AL731561.12	2
BMP4	NM_001202.2	AL138479.4	1	*GDF3*	NM_020634.1	AC006927.27	3
BMP5	NM_021073.2	AL137178.7	2	*GDF5*	NM_000557.2	AL121586.31	0
BMP6	NM_001718.2	AL135778.9	0	*GDF6*	NM_001001557.1	AC015998.8	0
BMP7	NM_001719.1	AL122058.19	0	*GDF7*	NM_182828.2	AC012065.8	1
BMP8A	NM_181809.1	AL365277.23	0	*GDF8*	NM_005259.1	AC073120.5	0
BMP8B	NM_001720.3	AL033527.26	0	*GDF9*	NM_005260.2	AC004500.1	0
BMP10	NM_014482.1	AC097495.3	0	*GDF10*	NM_004962.2	AL731561.12	4
BMP15	NM_005448.1	AL359914.14	0	*GDF11*	NM_005811.2	AC073487.34	0
AMH	NM_000479.2	AC138127.1	0	*GDF15*	NM_004864.1	AC008397.7	1

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CACACACGGGCGCACGCACACGGCAGACGGGCCAGGGACGACCCTGTCAGCTGCAGCCCCAGAGGTCCGGGGGCGCGCAGCCGGGTCCCCTCGAGGTCGCAGCTGGCCGCCCCGCCCCGCCCCTCGAAGCAGCCGGGCCGGGCGCGCAGTG  150
GGCTACAAACTTTCGCCGCGCGAGTCCGCCAAGGCAGCGCCGACTCGGGCTCGGTTCGGCTCTGCGCTGCTCCGGACGGCTGTGACCGCTGGCCGGGGGCTCGGGGCCGCCGGTACCCACGGACCGCGCGCCCCGGGTGCCCTGCTCCGCT  300
AAGCCCCTCGCCCCGCGCGGACCCCGATATCCAGCGCCCTGCTGCCCCGGGCTCTCCCCGCGCGCCCTACTGCCGCGAGGTCAGTCCGCAGCCTCCGGTGCGCCAGCGCTCGCCTTCCTCCTCCTGGACTTCGGCCCTTTGCCGCCCTCAC  450
CACGCCATGGCTCATGTCCCCGCTCGGACCAGCCCGGGACCCGGGCCCCAGCTGCTGCTGCTGCCGTTGTTTCTGCTGTTGCTCCGGGACGTGGCCGGCAGCCACAGGGCCCCCGCCTGGTCCGCACTGCCCGCGGCCGCCGACGGT  600
        M  A  H  V  P  A  R  T  S  P  G  P  G  P  Q  L  L  L  L  L  P  L  F  L  L  L  L  R  D  V  A  G  S  H  R  A  P  A  W  S  A  L  P  A  A  A  D  G   48
CTGCAGGGGGACAGGGATCCCCAGCGGCACCCTGGGGACGCGGCCGCCACGTTGGGCCCCAGCGCCCAGGACATGGTCGCTGTCCACATGCATAGGCTCTATGAGAAGTACAGCCGGCAGGGCGCGCGGCCGGGAGGGGGCAACACGGTC  750
  L  Q  G  D  R  D  P  Q  R  H  P  G  D  A  A  A  T  L  G  P  S  A  Q  D  M  V  A  V  H  M  H  R  L  Y  E  K  Y  S  R  Q  G  A  R  P  G  G  G  N  T  V   98
CGCAGCTTCAGGGCCAGGCTGGAAGTGGTCGACCAGAAGGCCGTGTATTTCTTCAACCTGACTTCCATGCAAGACTCGGAAATGATCCTTACGGCCACTTTCCACTTCTACTCAGAGCCGCCTCGGTGGCCTCGAGCGCTCGAGGTGCTA  900
  R  S  F  R  A  R  L  E  V  V  D  Q  K  A  V  Y  F  F  N  L  T  S  M  Q  D  S  E  M  I  L  T  A  T  F  H  F  Y  S  E  P  P  R  W  P  R  A  L  E  V  L  148
TGCAAGCCGCGGGCCAAGAACGCTTCAGGCCGCCCGCTGCCCCTGGGCCCGCCCACACGCCAGCACCTGCTCTTCCGCAGCCTCTCACAGAACACGGCCACACAGGGGCTACTCCGCGGTGCCATGGCCCTGGCGCCCCCACCGCGCGGC  1050
  C  K  P  R  A  K  N  A  S  G  R  P  L  P  L  G  P  P  T  R  Q  H  L  L  F  R  S  L  S  Q  N  T  A  T  Q  G  L  L  R  G  A  M  A  L  A  P  P  P  R  G  198
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  L  W  Q  A  K  D  I  S  P  I  V  K  A  A  R  R  D  G  E  L  L  L  S  A  Q  L  D  S  E  E  R  D  P  G  V  P  Q  P  S  P  Y  A  P  Y  I  L  V  Y  A  N  248
GATCTGGCCATCTCGGAGCCCAACAGCGTGGCGGTGACGCTGCAGAGATACGACCCCTTCCCTGCCGGAGACCCCGAGCCCCGCGGAGCCCCCAACAACTCAGCGGACCCCCGCGTGCGCCGAGCCGCGCAGGCCACTGGGCCCCTCCAG  1350
  D  L  A  I  S  E  P  N  S  V  A  V  T  L  Q  R  Y  D  P  F  P  A  G  D  P  E  P  R  G  A  P  N  N  S  A  D  P  R  V  R  R  A  A  Q  A  T  G  P  L  Q  298
GACAACGAGCTGCCGGGGCTGGATGAGAGGCCGCCGCGCGCCCACGCACAGCACTTCCACAAGCACCAGCTGTGGCCCAGCCCCTTCCGGGCGCTGAAACCCCGGCCAGGGCGCAAAGACCGCAGGAAGAAGGGCCAGGAGGTGTTCATG  1500
  D  N  E  L  P  G  L  D  E  R  P  P  R  A  H  A  Q  H  F  H  K  H  Q  L  W  P  S  P  F  R  A  L  K  P  R  P  G  R  K  D  R  R  K  K  G  Q  E  V  F  M  348
GCCGCCTCGCAGGTGCTGGACTTTGACGAGAAGACGATGCAGAAAGCCCGGAGGAAGCAGTGGGATGAGCCGAGGGTGTGCTCCCGGAGGTACCTGAAGGTGGACTTCGCAGACATCGGCTGGAATGAATGGATAATCTCACCGAAATCT  1650
  A  A  S  Q  V  L  D  F  D  E  K  T  M  Q  K  A  R  R  K  Q  W  D  E  P  R  V  C  S  R  R  Y  L  K  V  D  F  A  D  I  G  W  N  E  W  I  I  S  P  K  S  398
TTTGATGCCTACTACTGCGCGGGAGCATGTGAGTTCCCCATGCCTAAGATCGTTCGTCCATCCAACCATGCCACCATCCAGAGCATTGTCAGGGCTGTGGGCATCATCCCTGGCATCCCAGAGCCCTGCTGTGTTCCCGATAAGATGAAC  1800
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TCCCTTGGGGTCCTCTTCCTGGATGAGAGTCGGAATGTGGTTCTGAAGGTGTACCCCAACATGTCCGTGGACACCTGTGCCTGCCGGTGAGACCACTCCAGGGTGGAAAGAAGCCACGCCCAGCAGAGCCGCCTTCTCGGAGCCTTCTGC  1950
  S  L  G  V  L  F  L  D  E  S  R  N  V  V  L  K  V  Y  P  N  M  S  V  D  T  C  A  C  R  *                                                                   477
AACCAGGACTTGTGGTGCAGCTGCAGACACAGAGCACAGCTCATGGGCAACATCACTGGGGCCCAGAGAGAGCTGTCCGCCAGTGCATCATTAGGGGATCTTTCATTGCTAGTGACTAGCCCCTTAAATGCCAGCCTGAGTACCTGAAGG  2100
AATCTGGGAATTAGCCCTGGCCTGAAAGTGGCCCATCATTCATACCCACTGTTCTGAAGGCTTGAAAACAAAACATATCCACAACATTGGCTTGATGTGATCATCATCTCATAACTGAGCAAGAAGACTATGCAAATCTTAGGGCGCTCG  2250
CTCCCTGCACACGGAAAGAACTCTGTTTAAATGCTCAGTTCAGAACACTTTGGGCCACATAGTGATTTTGGAAAACAGGATAATCGTGGTGTAAATGGGTGTTTCCTTTCAAAGTCCACTGCAGAGCTTTTATCCATATGGTATGCACAT  2400
GTAACCAATATTGGTTTCTTTTTCTTTAATATATATTTTATTTAAAACAACAAAAAAGGAGGGCATTGACACCATTCCCCACAGAGATAGTCATGCTGAGTGTGGGTTGTTTAAACATGCATATTGAAATAACACATATAGTAACGTG  2550
GGAATACTAAAAAATAACCAAGATTTTATATTTTTGTAAATTATACTTTCTATACTGTAGATTGTGTATGTTATGTGTTTTTATGGAAAGCTAATAAATTAAAGGTACAGTGGTATCTTG  2670
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Figure 1. (A), Human *BMP/GDF* gene family. Genome sequences corresponding to 5'-promoter region of 22 *BMP/GDF* family members are listed up. Four TCF/LEF-binding sites occur within the *GDF10* promoter. (B), Nucleotide and amino-acid sequences of chimpanzee GDF10. Nucleotides and amino-acid residues are numbered on the right.

and *GDF15* genes were searched for with BLAST programs (http://www.ncbi.nlm.nih.gov) as described previously (25-28). TCF/LEF-binding sites within the 5'-flanking promoter region of the above genes were searched for based on bioinformatics and manual inspection as described previously (29,30).

Identification of the chimpanzee GDF10 ortholog. Chimpanzee genome sequences homologous to human *GDF10* were searched for with BLAST programs as described previously (31-34). Exon-intron boundaries were determined based on the consensus sequence of exon-intron junctions ('gt ... ag' rule of intronic sequence) and codon usage within the coding region as described previously (35-38). Coding sequence of chimpanzee GDF10 was determined by assembling exonic regions.

Comparative proteomics analysis. Phylogenetic analyses on mammalian BMP/GDF family members were performed by using the CLUSTALW program.

Comparative genomics analyses. Promoter region of mammalian *GDF10* orthologs were aligned by using the Genetyx program and manual curation. TCF/LEF-binding sites within the promoter region were determined as mentioned above.

In silico expression analysis. Expressed sequence tags (ESTs) derived from *GDF10* orthologs were searched for by using the BLAST programs. The sources of ESTs were listed up for *in silico* expression analysis on *GDF10* orthologs.

Results

Screening of TCF/LEF-binding site within promoter region of BMP/GDF family genes. Human BMP2 Refseq (NM_001200.2), BMP3 Refseq (NM_001201.1), BMP4 Refseq (NM_001202.2), BMP5 Refseq (NM_021073.2), BMP6 Refseq (NM_001718.2), BMP7 Refseq (NM_001719.1), BMP8A Refseq (NM_181809.1), BMP8B Refseq (NM_001720.3), BMP10 Refseq (NM_014482.1), BMP15 Refseq (NM_005448.1), AMH Refseq (NM_000479.2), GDF1 Refseq (NM_001492.3), GDF2 Refseq (NM_016204.1), GDF3 Refseq (NM_020634.1), GDF5 Refseq (NM_000557.2), GDF6 Refseq (NM_001001557.1), GDF7 Refseq (NM_182828.2), GDF8 Refseq (NM_005259.1), GDF9 Refseq (NM_005260.2), GDF10 Refseq (NM_004962.2), GDF11 Refseq (NM_005811.2) and GDF15 Refseq (NM_004864.1) were used as query sequences for the BLAST programs to identify genome clones corresponding to *BMP/GDF* family genes. The 5'-flanking promoter region of human *BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8A, BMP8B, BMP10, BMP15, AMH, GDF1, GDF2, GDF3, GDF5, GDF6, GDF7, GDF8, GDF9, GDF10, GDF11* and *GDF15* genes were identified within AL035668.15, AC093883.2, AL138479.4, AL137178.7, AL135778.9, AL122058.19, AL365277.23, AL033527.26, AC097495.3, AL359914.14, AC138127.1, AC005197.1, AL731561.12, AC006927.27, AL121586.31, AC015998.8, AC012065.8, AC073120.5, AC004500.1, AL731561.12, AC073487.34 and AC008397.7 genome sequences, respectively (Fig. 1A). TCF/LEF-binding sites within the 5'-promoter region

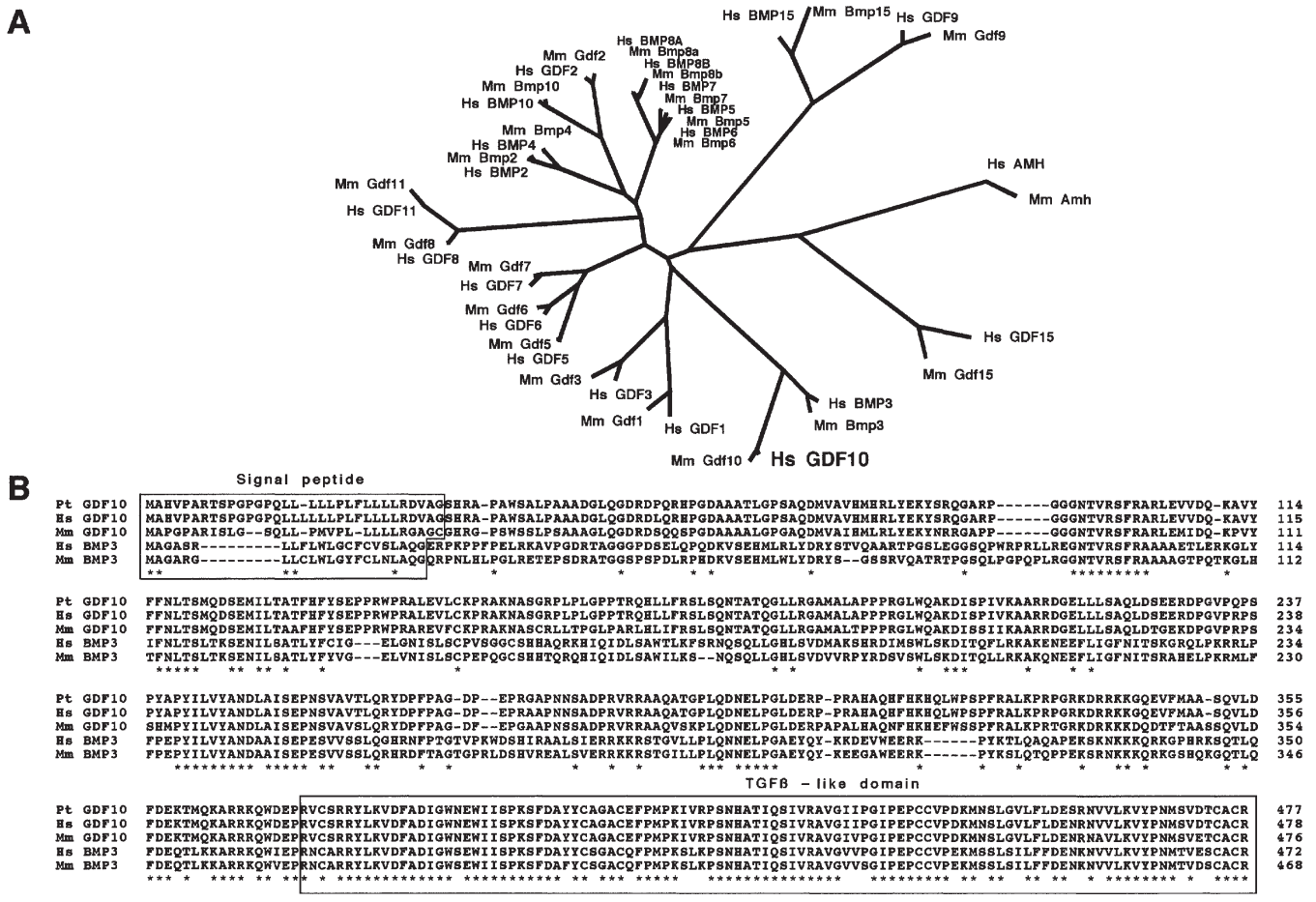

Figure 2. (A), Phylogenetic analyses on BMP/GDF family. GDF10 and BMP3 are paralogs. (B), Alignment of GDF10 orthologs and BMP3 orthologs. Amino-acid residues are numbered on the right. Conserved amino-acid residues are shown by asterisks. GDF10 and BMP3 orthologs are well conserved within the C-terminal TGFß-like domain (boxed).

of human *BMP/GDF* family genes were then searched for based on manual inspection. Four TCF/LEF-binding sites were identified within the human *GDF10* promoter (Fig. 1A).

Identification of the chimpanzee GDF10 gene. BLAST programs using human GDF10 RefSeq revealed that chimpanzee *GDF10* gene was located within NW_112875.1 genome sequence. Exon-intron boundaries of chimpanzee *GDF10* gene were determined based on the consensus sequence of exon-intron junctions. Exon 1 corresponded to nucleotide position 152574-151803 of NW_112875.1 genome sequence, exon 2 to nucleotide position 143019-142962 and 142824-141957, and exon 3 to nucleotide position 140077-139106. Chimpanzee *GDF10* gene was found consisting of three exons.

Because AY412135.1 was a partial sequence derived from chimpanzee *GDF10* gene, complete coding sequence (CDS) of chimpanzee GDF10 was determined by assembling nucleotide sequences of three exons in this study (Fig. 1B). Genetyx program revealed that nucleotide position 457-1890 was the coding region of chimpanzee GDF10 complete CDS (Fig. 1B). Chimpanzee *GDF10* gene was found to encode a 477-amino-acid protein.

Comparative integromics analysis on mammalian BMP/GDF family members. Phylogenetic analysis on human and mouse BMP/GDF family members revealed that GDF10 and BMP3 were paralogs (Fig. 2A). Chimpanzee GDF10 showed 99.2%, 83.2% and 47.4% total amino-acid identity with human GDF10, mouse Gdf10 and human BMP3, respectively. Alignment of human GDF10, BMP3, chimpanzee GDF10, mouse Gdf10 and Bmp3 revealed that GDF10 and BMP3 homologs were well conserved, especially within the C-terminal TGFß-like domain (Fig. 2B).

Intra-species comparative genomics next revealed that *RASGEF1A-GDF10-PRKG1* locus at human chromosome 10q11 and *BMP3-PRKG2-RASGEF1B* locus at human chromosome 4q21 were paralogous regions with insertions/deletions and recombination.

Expression of human GDF10 mRNA. In silico expression analyses were performed to investigate expression of human *GDF10* and mouse *Gdf10* mRNAs. Human *GDF10* mRNA was expressed in fetal cochlea, fetal lung, testis, retina, pineal gland, other neural tissues, head and neck tumors, while mouse *Gdf10* mRNA was expressed in fetal liver, inner ear, cerebellum, other neural tissues, prostate and blood vessels.

Comparative genomics analyses on GDF10 promoters. Human *GDF10* promoter and chimpanzee *GDF10* promoter were located within AL731561.12 and NW_112875.1 genome sequences, respectively, as mentioned above. BLAST programs

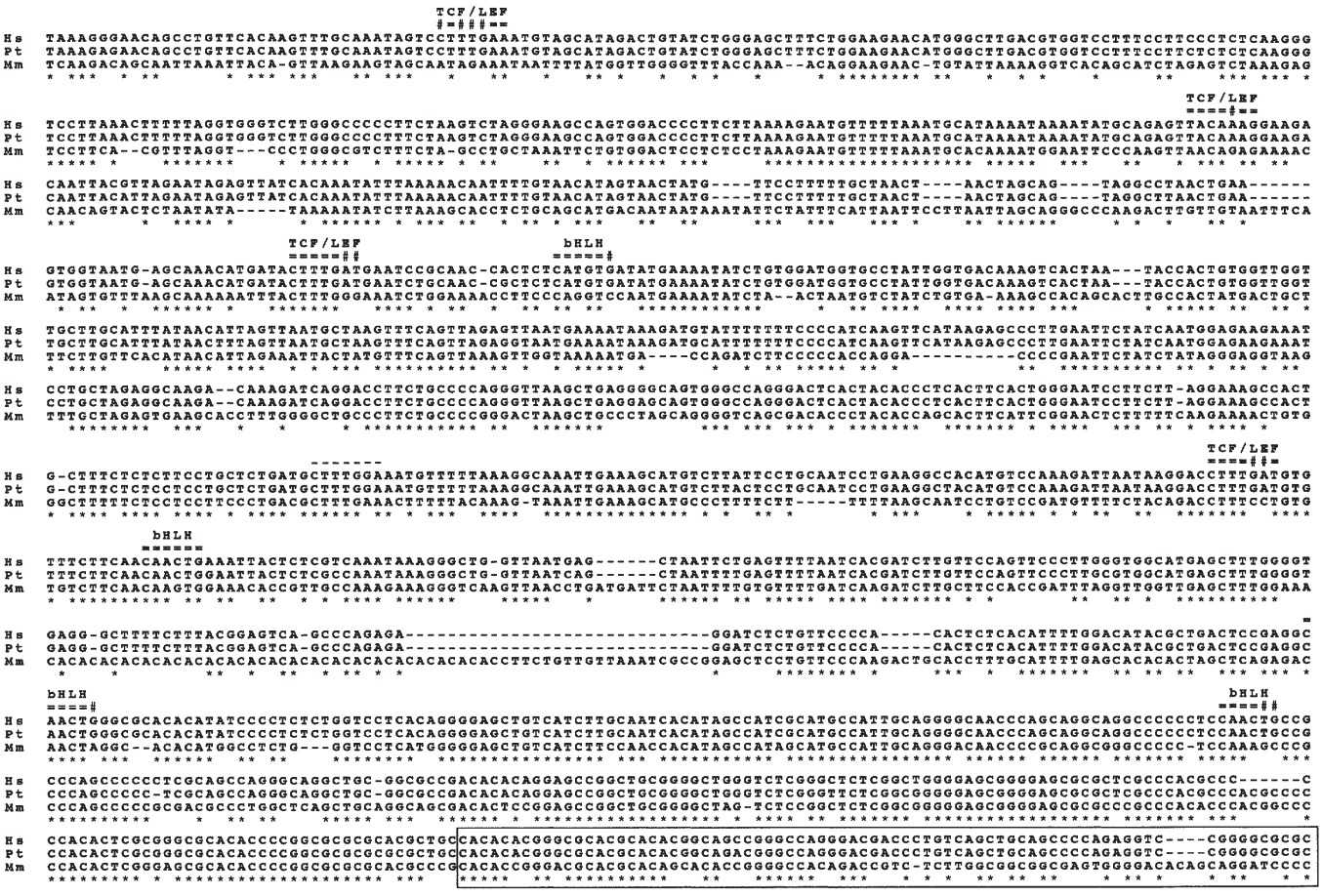

Figure 3. Mammalian *GDF10* promoter. Hs, human; Pt, chimpanzee; Mm, mouse. The region corresponding to exon 1 of human *GDF10* gene is boxed. Four TCF/LEF-binding sites and four bHLH-binding sites, conserved between human and chimpanzee *GDF10* promoters, are shown by over-lines. Nucleotide changes to disrupt the conserved TCF/LEF- and bHLH-binding sites in mouse *Gdf10* promoter are shown by a sharp. TCF/LEF-binding site in mouse *Gdf10* promoter is shown by an over-line.

revealed that mouse *Gdf10* promoter and rat *Gdf10* promoter were located within AC166828.3 and AC106060.5 genome sequences, respectively. Because sequence gap occurred within the *Gdf10* promoter region in rat AC106060.5 genome sequence, rat *Gdf10* promoter was not used for the following comparative genomics analyses on *GDF10* promoters.

GC content of human *GDF10* promoter was 53.9%, that of chimpanzee *GDF10* promoter was 53.8%, and that of mouse *Gdf10* promoter was 51.4. No significant change in the GC content was observed among mammalian *GDF10* promoters.

Four TCF/LEF-binding sites within human and chimpanzee *GDF10* promoters were not conserved in mouse *Gdf10* promoter; however, another TCF/LEF-binding site occurred in mouse *Gdf10* promoter (Fig. 3). In addition, four bHLH-binding sites within human *GDF10* promoter were conserved in chimpanzee *GDF10* promoter, but only one in mouse *Gdf10* promoter (Fig. 3). These facts indicate that primate *GDF10* promoters and mouse *Gdf10* promoter were significantly divergent.

Discussion

TCF/LEF-binding sites within the promoter region of 22 members of *BMP/GDF* gene family were searched for in this study. Because four TCF/LEF-binding sites were identified within human *GDF10* promoter (Fig. 1A), comparative genomics analyses on *GDF10* orthologs were further performed. Chimpanzee *GDF10* gene, consisting of three exons, was identified within NW_112875.1 genome sequence (Fig. 1B). AY412135.1 was not the correct coding sequence for chimpanzee GDF10. Chimpanzee *GDF10* gene, encoding a 477-amino-acid protein, showed 99.2% and 83.2% total amino-acid identity with human GDF10 and mouse Gdf10, respectively.

Phylogenetic analysis revealed that GDF10 and BMP3 were paralogs (Fig. 2A). GDF10 and BMP3 homologs were well conserved, especially within the C-terminal TGFß-like domain (Fig. 2B). Intra-species comparative genomics next revealed that *RASGEF1A-GDF1-PRKG1* locus at human chromosome 10q11 and *BMP3-PRKG2-RASGEF1B* locus at human chromosome 4q21 were paralogous regions with insertions/deletions and recombination.

Human *GDF10* mRNA was expressed in fetal cochlea, fetal lung, testis, retina, pineal gland, other neural tissues, head and neck tumors, while mouse *Gdf10* mRNA was expressed in fetal liver, inner ear, cerebellum, other neural tissues, prostate and blood vessels. Four TCF/LEF-binding sites in human *GDF10* promoter were conserved in chimpanzee *GDF10* promoter, but not in mouse *Gdf10* promoter, however, another

TCF/LEF-binding site occurred in mouse *Gdf10* promoter (Fig. 3). Four bHLH-binding sites within human *GDF10* promoter were conserved in chimpanzee *GDF10* promoter, but only one in mouse *Gdf10* promoter (Fig. 3). Primate *GDF10* promoters were divergent from mouse *Gdf10* promoter. Because *GDF10* was characterized as a potential target of the canonical WNT signaling pathway in neural tissues, GDF10 is one of targets of systems medicine, especially in the field of re-generative medicine.

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