Comparative integromics on Angiopoietin family members

YURIKO KATOH¹ and MASARU KATOH²

¹M&M Medical BioInformatics, Hongo 113-0033; ²Genetics and Cell Biology Section, National Cancer Center Research Institute, Tokyo 104-0045, Japan

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Abstract. Angiopoietin-1 (ANGPT1), Angiopoietin-4 (ANGPT4), VEGF, FGF2, FGF4, HGF, Ephrin, IL8 and CXCL12 (SFD1) are pro-angiogenic factors (angiogenic activators), while Angiopoietin-2 (ANGPT2), Angiostatin, Endostatin, Tumstatin, Canstatin, THBS1, THBS2, TNFSF15 (VEGI) and Vasohibin (VASH1) are anti-angiogenic factors (angiogenic inhibitors). ANGPT1 and ANGPT2 are ligands for TIE family receptor tyrosine kinases, TIE1 and TIE2 (TEK). Angiopoietin family consists of ANGPT1, ANGPT2, ANGPT4, ANGPTL1 (ANGPT3), ANGPTL2, ANGPTL3 (ANGPT5), ANGPTL4, ANGPTL5, ANGPTL6 and ANGPTL7. TCF/LEF binding sites within the promoter region of human Angiopoietin family members were searched for by using bioinformatics and human intelligence (Humint). Because four TCF/LEF-binding sites were identified within the human ANGPTL7 promoter, comparative genomics analyses on ANGPTL7 orthologs were further performed. ANGPTL7 gene at human chromosome 1p36.22 was located within intron 28 of FRAP1 gene encoding mTOR protein. Chimpanzee ANGPTL7 gene, consisting of five exons, was located within NW_101546.1 genome sequence. Chimpanzee ANGPTL7 showed 99.4% and 86.1% total-amino-acid identity with human ANGPTL7 and mouse Angptl7, respectively. Human ANGPTL7 mRNA was expressed in neural tissues, keratoconus cornea, trabecular meshwork, melanotic melanoma and uterus endometrial cancer, while mouse Angptl7 mRNA was expressed in four-cell embryo, synovial fibroblasts, thymus, uterus and testis. Four TCF/LEF-binding sites within human ANGPTL7 promoter were conserved in chimpanzee ANGPTL7 promoter; however, only an unrelated TCF/LEF-binding site occurred in mouse and rat Angptl7 promoters. Human ANGPTL7, characterized as potent target gene of WNT/ ß-catenin signaling pathway, is a pharmacogenomics target in the fields of oncology and regenerative medicine.

Correspondence to: Dr Masaru Katoh, Genetics and Cell Biology Section, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan E-mail: mkatoh@ncc.go.jp

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Introduction

Angiogenesis is regulated by the balance between pro-angiogenic factors (angiogenic activators) and anti-angiogenic factors (angiogenic inhibitors) (1-6). Angiopoietin-1 (ANGPT1), Angiopoietin-4 (ANGPT4), VEGF, FGF2, FGF4, HGF, Ephrin, IL8 and CXCL12 (SFD1) are pro-angiogenic factors, while Angiopoietin-2 (ANGPT2), Angiostatin, Endostatin, Tumstatin, Canstatin, THBS1, THBS2, TNFSF15 (VEGI) and Vasohibin (VASH1) are anti-angiogenic factors (1-17).

ANGPT1, ANGPT2, ANGPT4 are ligands for TIE family receptor tyrosine kinases, TIE1 and TIE2 (TEK) (7-9). ANGPTL1 (ANGPT3), ANGPTL2, ANGPTL3 (ANGPT5), ANGPTL4, ANGPTL5, ANGPTL6 and ANGPTL7 (18-23) are related to ANGPT1, ANGPT2 and ANGPT4. Angiopoietin family consists of ANGPT1, ANGPT2, ANGPT4, ANGPTL1, ANGPTL2, ANGPTL3, ANGPTL4, ANGPTL5, ANGPTL6 and ANGPTL7.

WNT, FGF, Notch and Hedgehog signaling pathways network together during embryogenesis, tissue regeneration and carcinogenesis (24-33). Canonical WNT signals are transduced to the transcriptional complex consisting of TCF/LEF, β-catenin, BCL9/BCL9L and PYGO1/PYGO2 to activate transcription of target genes, such as *DKK1*, *DKK4*, *FGF18* and *FGF20* (34-43); however, WNT-dependent transcriptional regulation of Angiopoietin family members remains unclear.

Here, TCF/LEF binding sites within the promoter region of human Angiopoietin family members were searched for by using bioinformatics and human intelligence (Humint). Because four TCF/LEF-binding sites were identified in the 5'-promoter region of human *ANGPTL7* gene, comparative genomics analyses on *ANGPTL7* orthologs were further performed.

Materials and methods

WNT target gene screening. Genome sequences corresponding to human ANGPT1, ANGPT2, ANGPT4, ANGPTL1, ANGPTL2, ANGPTL3, ANGPTL4, ANGPTL5, ANGPTL6 and ANGPTL7 genes were searched for with BLAST programs (http://www.ncbi.nlm.nih.gov) as described previously (44-47). 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 (38-42,48).

Identification of chimpanzee and cow ANGPTL7 orthologs. Chimpanzee and cow genome sequences homologous to



Figure 1. (A), Human Angiopoietin gene family. Gene symbol, complete coding sequence, genome sequence and the number of TCF/LEF-binding sites within promoter region of Angiopoietin family genes are listed. Four TCF/LEF-binding sites exist within the human *ANGPTL7* promoter. (B), Phylogenetic analysis on human and mouse Angiopoietin family members. Hs, human; Mm, mouse. (C), *ANGPTL7* locus at human chromosome 1p36.22. *ANGPTL7* gene, consisting of five exons, is located within intron 28 of the *FRAP1* gene.

human *ANGPTL7* were searched for with BLAST programs as described previously (49-52). TCF/LEF-binding sites within the 5'-flanking promoter region of *ANGPTL7* orthologs were also searched for.

Comparative proteomics analysis. Phylogenetic analysis on ANGPT family proteins was performed by using the CLUSTALW program.

Comparative genomics analyses. Phylogenetic analysis on promoter of *ANGPTL7* orthologs was performed by using the CLUSTALW program. Promoter region of human and chimpanzee *ANGPTL7* orthologs were aligned by using the Genetyx program and manual curation as described previously (53-56).

In silico expression analyses. Expressed sequence tags (ESTs) derived from human *ANGPTL7* gene and mouse *Angptl7* gene were searched for by using the BLAST programs. The sources of human ANGPTL7 ESTs and those of mouse Angptl7 ESTs were listed up for *in silico* expression analyses.

Results

Screening of the TCF/LEF-binding site within promoter region of Angiopoietin family genes. Human ANGPT1 RefSeq (NM_001146.3), ANGPT2 RefSeq (NM_001147.1), ANGPT4 RefSeq (NM_015985.2), ANGPTL1 RefSeq (NM_004673.3), ANGPTL2 RefSeq (NM_012098.2), ANGPTL3 RefSeq (NM_014495.2), ANGPTL4 RefSeq (NM_139314.1), ANGPTL5 RefSeq (NM_178127.2), ANGPTL6 RefSeq (NM_031917.2) and ANGPTL7 RefSeq (NM_021146.2) were used as query sequences for the BLAST programs to identify genome clones corresponding to Angiopoietin family genes. The 5'-flanking promoter region of human ANGPT1, ANGPT2, ANGPT4, ANGPTL1, ANGPTL2, ANGPTL3, ANGPTL4, ANGPTL5, ANGPTL6 and ANGPTL7 genes were identified within AC053479.8, AC018398.10, AL050325.20, AL355520.8, AL356862.10, AL138847.14, AC010323.8, AP002372.4, AC020931.6 and AL391561.20 genome sequences, respectively (Fig. 1A). TCF/LEF-binding sites within the 5'-promoter region of human Angiopoietin family genes were then searched for based on manual inspection. Four TCF/LEF-binding sites were identified within human ANGPTL7 promoter (Fig. 1A).

Identification of chimpanzee ANGPTL7 ortholog. BLAST programs using human ANGPTL7 RefSeq revealed that chimpanzee ANGPTL7 gene was located within NW_101546.1 genome sequence (Fig. 2A). Exon-intron boundaries of the chimpanzee ANGPTL7 gene were determined based on the consensus sequence of exon-intron junctions. Chimpanzee ANGPTL7 gene was found consisting of five exons.

Compared with human ANGPTL7 RefSeq, one-base insertion occurred at exon 3 of chimpanzee *ANGPTL7* gene within NW_101546.1 genome sequence. Re-sequencing of the genome sequence around exon 3 of chimpanzee *ANGPTL7* gene should be done in the future to correct the sequencing error.

Complete coding sequence (CDS) of chimpanzee ANGPTL7 was determined in this study by assembling nucleotide sequences of five exons (Fig. 2A). Genetyx program revealed that nucleotide position 241-1281 was the coding region of chimpanzee ANGPTL7 complete CDS. Chimpanzee *ANGPTL7* gene was found to encode a 346-amino-acid ANGPTL7 protein (Fig. 2A).

Comparative proteomics analysis on ANGPTL7 orthologs. Phylogenetic analysis on human and mouse Angiopoietin family members revealed that ANGPTL7 orthologs were

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Figure 2. Chimpanzee ANGPTL7. (A), Nucleotide and amino-acid sequences of chimpanzee ANGPTL7 complete CDS. Nucleotides and amino-acid residues are numbered on the right. (B), Alignment of ANGPTL7 orthologs. Hs, human; Pt, chimpanzee; Mm, mouse. Conserved amino-acid residues are shown by asterisks.



Figure 3. Alignment of the human and chimpanzee *ANGPTL7* promoters. Hs, human; Pt, chimpanzee. Region corresponding to exon 1 of the human *ANGPTL7* gene is boxed. TCF/LEF-binding sites are shown by double over-lines.

relatively well conserved (Fig. 1B). Chimpanzee ANGPTL7 showed 99.4% and 86.1% total-amino-acid identity with human ANGPTL7 and mouse Angptl7, respectively (Fig. 2B).

Intra-species comparative genomics analysis on ANGPTL7 orthologs. ANGPTL7 gene was located within FRAP1 gene at human chromosome 1p36.22 (Fig. 1C). Paralog of FRAP1 gene, encoding mTOR protein, was not identified within the human genome. ANGPTL7 gene was found consisting of 5 exons, while FRAP1 gene was found consisting of 58 exons. ANGPTL7 gene was located within intron 28 of FRAP1 gene in the anti-sense direction (Fig. 1C). These facts indicate that ANGPT7L gene was inserted into the FRAP1 gene during evolution.

Expression profile of human ANGPTL7 and mouse Angpt17 mRNAs. In silico expression analyses were performed to compare the expression profiles of human *ANGPTL7* and mouse *Angpt17* mRNAs. Human *ANGPTL7* mRNA was expressed in neural tissues, keratoconus cornea, trabecular

meshwork, melanotic melanoma and uterus endometrial cancer, while mouse *Angptl7* mRNA was expressed in four-cell embryo, synovial fibroblasts, thymus, uterus and testis.

Comparative genomics analyses on ANGPTL7 promoters. Human and chimpanzee *ANGPTL7* promoters were located within AL391561.20 and NW_101546.1 genome sequences, respectively, as mentioned above. BLAST programs revealed that cow, mouse and rat *Angptl7* promoters were located within AC174033.2, AC108508.2 and AC125863.3 genome sequences, respectively. Cow *Angptl7* promoter was not used for the following comparative genomics analyses, because one of sequencing gaps within the AC174033.2 genome sequence corresponded to the cow *Angptl7* promoter region.

GC contents of human, chimpanzee, mouse and rat *ANGPTL7* promoters were 41.5%, 41.4%, 50.2% and 50.6%, respectively. GC contents of primate *ANGPTL7* promoters were lower than those of rodent *Angptl7* promoters.

Four TCF/LEF-binding sites within human VEGFD promoter were located about 1050, 900, 600, and 550 bp upstream of the transcription start site (Fig. 3). Four TCF/ LEF-binding sites within the human ANGPTL7 promoter were conserved in chimpanzee ANGPTL7 promoter; however, only an unrelated TCF/LEF-binding site occurred in the mouse and rat Angptl7 promoters.

Discussion

TCF/LEF binding sites within the promoter region of human ANGPT1, ANGPT2, ANGPT4, ANGPTL1, ANGPTL2, ANGPTL3, ANGPTL4, ANGPTL5, ANGPTL6 and ANGPTL7 genes were searched for in this study. Because four TCF/ LEF-binding sites were identified within the human ANGPTL7 promoter (Fig. 1A), comparative genomics analyses on ANGPTL7 orthologs were further performed.

ANGPTL7 gene at human chromosome 1p36.22 was located within intron 28 of the *FRAP1* gene encoding mTOR protein (Fig. 1C). Chimpanzee ANGPTL7 gene, consisting of five exons, was located within the NW_101546.1 genome sequence (Fig. 2A). Chimpanzee ANGPTL7 showed 99.4% and 86.1% total-amino-acid identity with human ANGPTL7 and mouse Angptl7, respectively (Fig. 2B).

Four TCF/LEF-binding sites within the human ANGPTL7 promoter were conserved in chimpanzee ANGPTL7 promoter (Fig. 3); however, only an unrelated TCF/LEF-binding site occurred in the mouse and rat Angptl7 promoters. GC contents of primate ANGPTL7 promoters were lower than those of rodent Angptl7 promoters. Human ANGPTL7 mRNA was expressed in neural tissues, keratoconus cornea, trabecular meshwork, melanotic melanoma and uterus endometrial cancer, while mouse Angptl7 mRNA was expressed in four-cell embryo, synovial fibroblasts, thymus, uterus and testis. Together these facts indicate that expression profiles of primate ANGPTL7 orthologs and rodent Angptl7 orthologs differ due to promoter evolution.

Human ANGPTL7, characterized as a potent target gene of WNT/ β -catenin signaling pathway, is a pharmacogenomics target in the fields of oncology and regenerative medicine.

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