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SHORT COMMUNICATION

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Identification of a novel human angiopoietin-like gene expressed mainly in heart

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Abstract The angiopoietins are an important family of growth factors specific for vascular endothelium. Most of them bind to the *TIE2* receptor and are related to regulation of angiogenesis. During large-scale DNA sequencing of the human fetal brain cDNA library, we cloned a novel human angiopoietin-like cDNA and termed it human angiopoietin-like 5 (*ANGPTL5*). Like other members of the angiopoietin family, *ANGPTL5*-deduced protein also has an N-terminal cleavable signal peptide, a predicted coiled-coil domain, and a fibrinogen-like domain. The search against the human genome database indicated that *ANGPTL5* maps to 11q22. Expression analysis of *ANGPTL5* shows that it is mainly expressed in adult human heart.

Key words Angiopoietin · Coiled-coil domain · Fibrinogen-like domain · Heart · ANGPTL5

Angiogenesis is important to normal embryogenesis and is also related to several pathophysiological conditions such as the growth of solid tumor, neovascularization in the retina, and some inflammatory diseases (Nishimura et al. 1999). Angiogenesis requires proliferation and migration of endothelial cells, leading to sprouting, growth, and remodeling of the initial network. Although the basic mechanism underlying angiogenesis is not yet fully understood, regulation of angiogenesis seems to depend on a balance between positive and negative regulation factors (Hanahan and Folkman 1996).

The angiopoietins are an important family of growth factors that are closely related to angiogenesis. Angiopoietin 1 (ANGPT1) was found originally as a ligand for TIE2 (tyrosine kinase with immunoglobulin and epidermal growth factor homology domains 2, also known as TEK) to regulate later stages of vascular development, stabilization, and maturation (Davis et al. 1996; Suri et al. 1996). Angiopoietin 2 (ANGPT2) also binds to TIE2, but ANGPT2 acts as an antagonist of ANGPT1 and destabilizes vascular networks (Maisonpierre et al. 1997). Both the targeted disruption of the *ANGPT1* gene and the overexpression of *ANGPT2* lead to angiogenic deficits, which are similar to those seen in mice lacking *TIE2*. Angiopoietin 3 (ANGPT3, mouse) and angiopoietin 4 (ANGPT4) are probably interspecies orthologues, and both bind to TIE2. However, like ANGPT2, mouse ANGPT3 acts as an antagonist for TIE2, whereas, like ANGPT1, ANGPT4 acts as an agonist for TIE2 (Valenzuela et al. 1999). All members of angiopoietin are secreted proteins with a characteristic structure: an N-terminal cleavable signal peptide, an extended helical domain predicted to form dimeric or trimeric coiled-coils, a short linker peptide, and a fibrinogen-like domain in the C-terminal portion.

A novel cDNA clone was obtained from large-scale DNA sequencing of the human fetal brain cDNA library, which was constructed by our laboratory (Xu et al. 2001). The nucleotide sequence is available from GenBank under accession number AY169281. This 1.8-kb cDNA spans an open reading frame from nucleotide 55 to 1221, encoding a putative 388-amino acid protein with a predicted molecular mass of 44.1 kDa and a predicted isoelectric point of 6.02 (Fig. 1a). An in-frame stop codon is found at position 22–24, and two putative polyadenylation signal AATAAAs are found near the 3′ end of the sequence. Therefore, we conclude that the coding sequence is complete.

By using BlastP, the deduced protein was found to be highly homologous with the angiopoietins mentioned earlier. The protein is also homologous to some angiopoietin-like genes, such as *ANGPTL1* (Kim et al. 1999a), *ANGPTL2* (Kim et al. 1999b), *ANGPTL3* (Conklin et al. 1999), *ANGPTL4* (Kim et al. 2000) (Fig. 2), and some other members of the fibrinogen protein superfamily, sharing about 30%–40% amino acid identity. All angiopoietin-like

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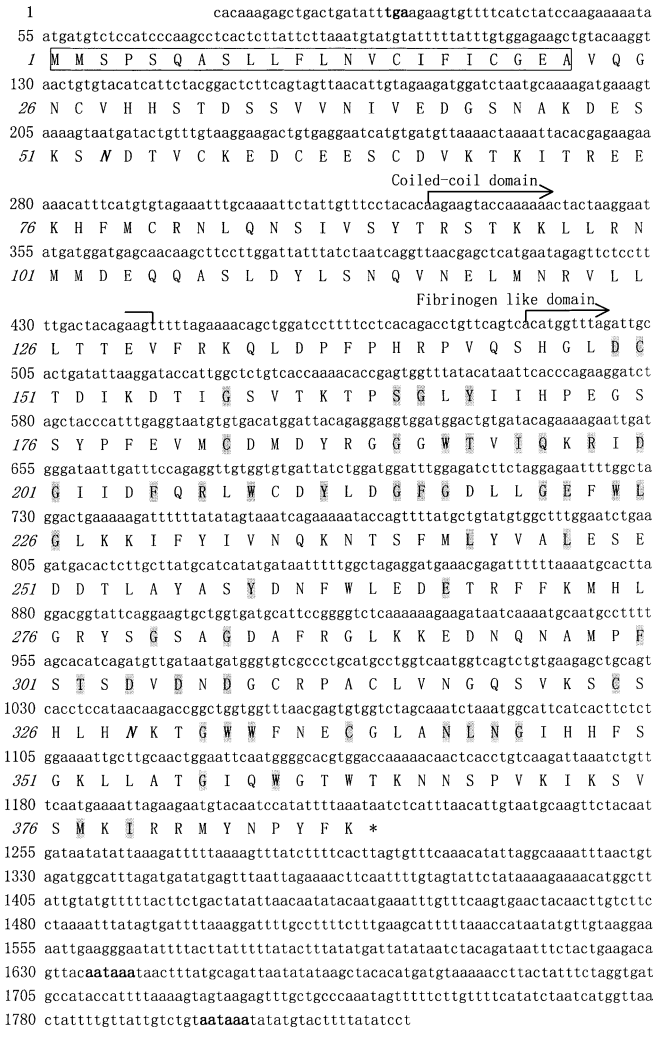

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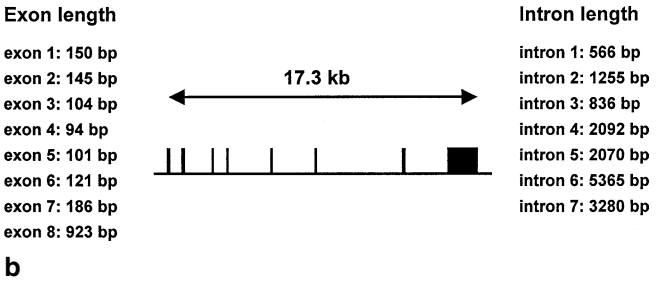

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Fig. 1a,b. The sequence and structure of *ANGPTL5*. **a** The nucleotide sequence and deduced amino acid sequence of *ANGPTL5*. The in-frame codon and polyadenylation signals are shown in *bold*. Amino acids are represented *below the DNA sequences*. The asterisk represents the stop codon. The signal peptide sequence predicted by computer analysis using the SignalP program is shown in the **box**. Amino acid residues identical to at least seven of the following eight most related proteins are *shaded*: ANGPT1, ANGPT2, mANGPT3, ANGPT4, ANGPTL1, ANGPTL2, ANGPTL3, and ANGPTL4. The two *arrows* limit the coiled-coil domain and fibrinogen-like domain, respectively. Two potential glycosylation sites predicted by NetNGlyc 1.0 are shown in *bold italic*. **b** Genomic organization of the *ANGPTL5* gene. The length of each exon and each intron is shown. The start codon is present in the first exon

proteins show the common characteristic structure of angiopoietins and show homology with them. However, they do not bind to *TIE2* and might have a different function from angiopoietins. For example, *ANGPTL4* (also known as *pGAR* or *FIAF*), which is regulated by the peroxisome proliferator-activated receptor α (*PPARα*), was identified as a fasting-induced adipose factor (Kersten et al. 2000). Recently, both ANGPTL3 and ANGPTL4 were found to function as a new class of lipid metabolism modulators (Koishi et al. 2002; Shimizugawa et al. 2002; Yoshida et al. 2002). Thus, we term this gene human angiopoietin-like 5 (*ANGPTL5*) in agreement with the HUGO Nomenclature Committee.

Like the other members of the angiopoietin family, *ANGPTL5*-deduced protein has also been predicted to have an N-terminal cleavable signal peptide (PSORT II server, http://psort.nibb.ac.jp: 8800), a predicted coiled-coil domain (COILS program web server, http://www1.york.ac.uk/depts/biol/units/coils/coilcoil.html), and a fibrinogen-like domain (Bioinformatics Web server, http://www.isrec.isb-sib.ch/software/PFSCAN_form.html) (Fig. 1a). The fibrinogen-like domain is conserved and is supposed to be the receptor-binding site (Valenzuela et al. 1999). The coiled-coil domain is related to oligomerization, which is very important to angiopoietins because oligomerization appears to be required for the functioning of angiopoietins (Maisonpierre et al. 1997).

By searching against the human EST database and the human genome database, we found that the *ANGPTL5* gene is represented by six ESTs and three genomic sequences (accession no. NT_009151.12, AP002372.3, and AP003383.2) from chromosome 11q22. Comparison of the

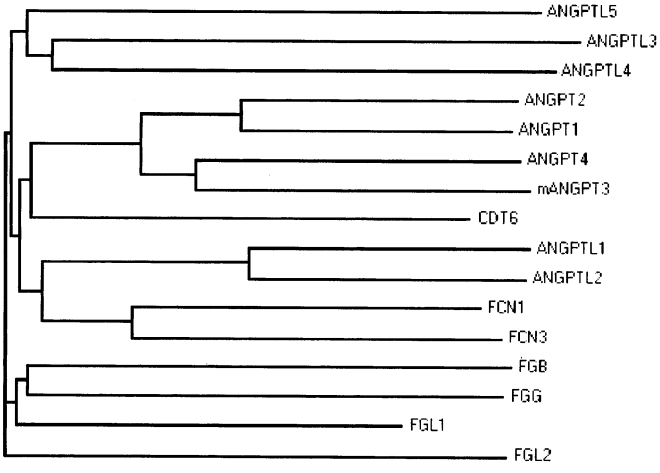

Fig. 2. A gene tree representing ANGPTL5 and its related sequences, including angiopoietins, angiopoietin-like genes, fibrinogen (beta and gamma polypeptide), and fibrinogen-like genes. The gene tree was generated using the AlignX program of Vector NTI suite 5.5. Sequence data were derived from GenBank as the following accession numbers: ANGPTL5 (our sequence), NP_055310, NP_057193, O15123, Q15389, NP_057069, AAD21586, CAA76078, NP_004664, NP_036230, NP_001994, NP_003656, NP_005132, NP_068656, NP_004458, and NP_006673 (from top to bottom)

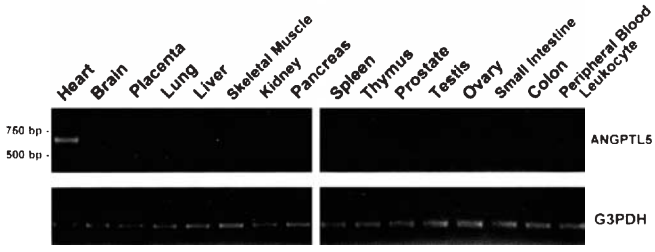

Fig. 3. Reverse transcription-polymerase chain reaction analysis of human adult tissue cDNA for ANGPTL5 and G3PDH (positive control). Results of 35 cycles (for ANGPTL5) and 24 cycles (for G3PDH) of amplification are shown

cDNA sequence of *ANGPTL5* to the genomic sequence revealed that this gene spans about 17.3 kb of genomic DNA and consists of eight exons (Fig. 1b). All sequences of the exon–intron junctions are consistent with the AG–GT rule. Moreover, a CpG island (−835 to −629) and a potential transcription promotor region (−312 to −262) were found just before the first exon (predicted at http://125.itba.mi.cnr.it/genebin/wwwcpg.pl and http://www.fruitfly.org/seq_tools/promotor.html, respectively).

To investigate the expression pattern of *ANGPTL5* in different tissues, we used two human multiple tissue cDNA (MTC, Clontech, Palo Alto, CA, USA) panels as Polymerase Chain Reaction (PCR) templates according to the manufacturer's protocol. The *ANGPTL5*-specific primer pairs (ANGPTL5F: 5′-aggttgtggtgtgattatctggatgg-3′ and ANGPTL5R: 5′-actcatatcatctaaatgccatctacag-3′) were designed to amplify a 0.7-kb fragment. A glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) control primer pair included in the panels was used to verify the normalization of the MTC panels. A total of 35 cycles of amplification was performed using rTaq DNA polymerase (TaKaRa, Shiga, Japan) in a total volume of 50 μl. All the reactions were paused after a total of 24 cycles, 27 cycles, 30 cycles, 33 cycles, and 35 cycles. At each pause, a 5-μl sample of every reaction mixure was removed to run on a gel, and the rest were put back in the thermal cycler. The cycling conditions were as follows: 5 min at 94°C, followed by cycles of 30 s at 94°C and 90 s at 68°C, with a 5-min 68°C step to finish. When 24 cycles of amplification had been performed, *G3PDH* (positive control) reverse transcriptase (RT)-PCR products were detected in all tissues tested. After 35 cycles, *ANGPTL5* RT-PCR products were only detected in adult human heart (Fig. 3). Further study should be made to clarify the precise role of the *ANGPTL5* gene.

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