

Notch ligand, JAG1, is evolutionarily conserved target of canonical WNT signaling pathway in progenitor cells

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Abstract. WNT, Notch, FGF, and Hedgehog signaling pathways network together during embryogenesis, tissue regeneration, and carcinogenesis. Association of Notch ligands with Notch receptors on neighboring cells leads to cleavage of Notch receptors by metalloprotease and γ -secretase to induce nuclear translocation of Notch intracellular domain (NICD). Nuclear complex, consisting of CSL (RBPSUH), NICD, Mastermind (MAML), p300 and histone acetyltransferase (HAT), then induces transcriptional activation of Notch target genes, such as *HES1*, *HES5*, *HES7*, *HEY1*, *HEY2* and *HEYL*. Here, we searched for TCF/LEF-binding site within the promoter region of Notch ligand genes, including *DLL1*, *DLL3*, *DLL4*, *JAG1* and *JAG2*. Because TCF/LEF-binding sites were identified within human *JAG1* promoter based on bioinformatics and human intelligence, comparative genomics analyses on *JAG1* orthologs were further performed. Chimpanzee *JAG1* gene, consisting of 26 exons, was identified within NW_120319.1 genome sequence. XM_525264.1 and XM_514517.1 were not the correct coding sequences for chimpanzee *JAG1*. Chimpanzee *JAG1* gene was found to encode a 1218-amino-acid protein showing 99.5% and 96.2% total-amino-acid identity with human *JAG1* and mouse *Jag1*, respectively. Phylogenetic analysis revealed that *JAG1* orthologs were more conserved than those of other Notch ligands. *JAG1* gene was identified as evolutionarily conserved target of WNT/ β -catenin signaling pathway based on the conservation of double TCF/LEF-binding sites within 5'-promoter region of mammalian *JAG1* orthologs. Human *JAG1* mRNA was expressed in embryonic stem (ES) cells, neural tissues, lung carcinoid, gastric cancer, pancreatic cancer, colon cancer, and also in squamous cell carcinoma (SCC) of skin, oral cavity, esophagus, head and neck. *JAG1* expression on progenitor cells due to canonical WNT signaling activation

induces self-renewal of stem cells due to Notch signaling activation. *JAG1*, functioning as WNT-dependent Notch signaling activator, is the key molecule maintaining the homeostasis of stem and progenitor cells.

Introduction

WNT, Notch, FGF, Hedgehog, and BMP signaling pathways network together during embryogenesis, tissue regeneration, and carcinogenesis (1-24). Because *Wnt1*, *Wnt3*, *Wnt10b*, *Notch4*, *Fgf3*, *Fgf4* and *Fgf8* are up-regulated during mouse mammary carcinogenesis due to MMTV integration, WNT, Notch ligand, and FGF are predicted as key regulators of stem and progenitor cells.

Notch signaling pathway is implicated in the maintenance of self-renewal potential in stem cells, binary cell-fate determination in progenitor cells, and induction of terminal differentiation in proliferating cells (9,10). Delta homologs (*DLL1*, *DLL3*, *DLL4*) and Serrate homologs (*JAG1*, *JAG2*) are transmembrane-type ligands for Notch family receptors, including *NOTCH1*, *NOTCH2*, *NOTCH3* and *NOTCH4* (25-33). Association of Notch ligands with Notch receptors on the neighboring cells leads to the cleavage of Notch receptors by metalloprotease and γ -secretase to induce nuclear translocation of Notch intracellular domain (NICD). Nuclear complex, consisting of CSL (RBPSUH), NICD, Mastermind (MAML), p300 and histone acetyltransferase (HAT), then induces transcriptional activation of Notch target genes, such as *HES1*, *HES5*, *HES7*, *HEY1*, *HEY2* and *HEYL*. Because HES/HEY family members are bHLH-type transcriptional repressors, Notch signaling activation in stem cells leads to the maintenance of self-renewal potential through the down-regulation of tissue-specific transcription factors (9,10,34-36).

Here, we searched for TCF/LEF-binding site within the promoter region of Notch ligand genes, including *DLL1*, *DLL3*, *DLL4*, *JAG1* and *JAG2*. Because double TCF/LEF-binding sites were identified within human *JAG1* promoter based on bioinformatics and human intelligence, comparative genomics analyses on *JAG1* orthologs were further performed.

Materials and methods

WNT target gene screening. Genome sequences corresponding to human *DLL1*, *DLL3*, *DLL4*, *JAG1* and *JAG2* genes were searched for with BLAST programs (<http://www.ncbi.nlm.nih.gov/>).

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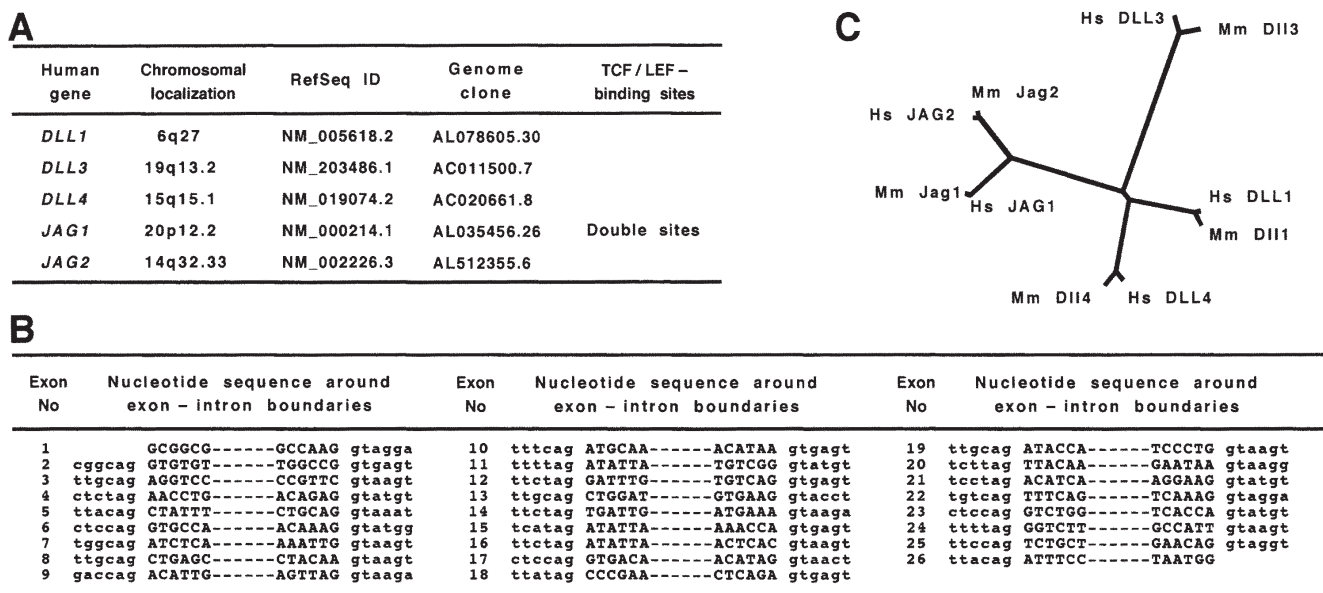


Figure 1. (A) Human Notch ligand family. *DLL1*, *DLL3*, *DLL4*, *JAG1* and *JAG2* genes constitute Notch ligand family. Double TCF/LEF-binding sites are located within the 5'-promoter region of *JAG1* gene. (B) Exon-intron structure of chimpanzee *JAG1* gene. Nucleotide sequences around exon-intron boundaries are shown by upper-case letters (exon) and by lower-case letters (intron). (C) Phylogenetic tree of human and mouse Notch ligands. *JAG1* orthologs are more conserved than those of other Notch ligands.

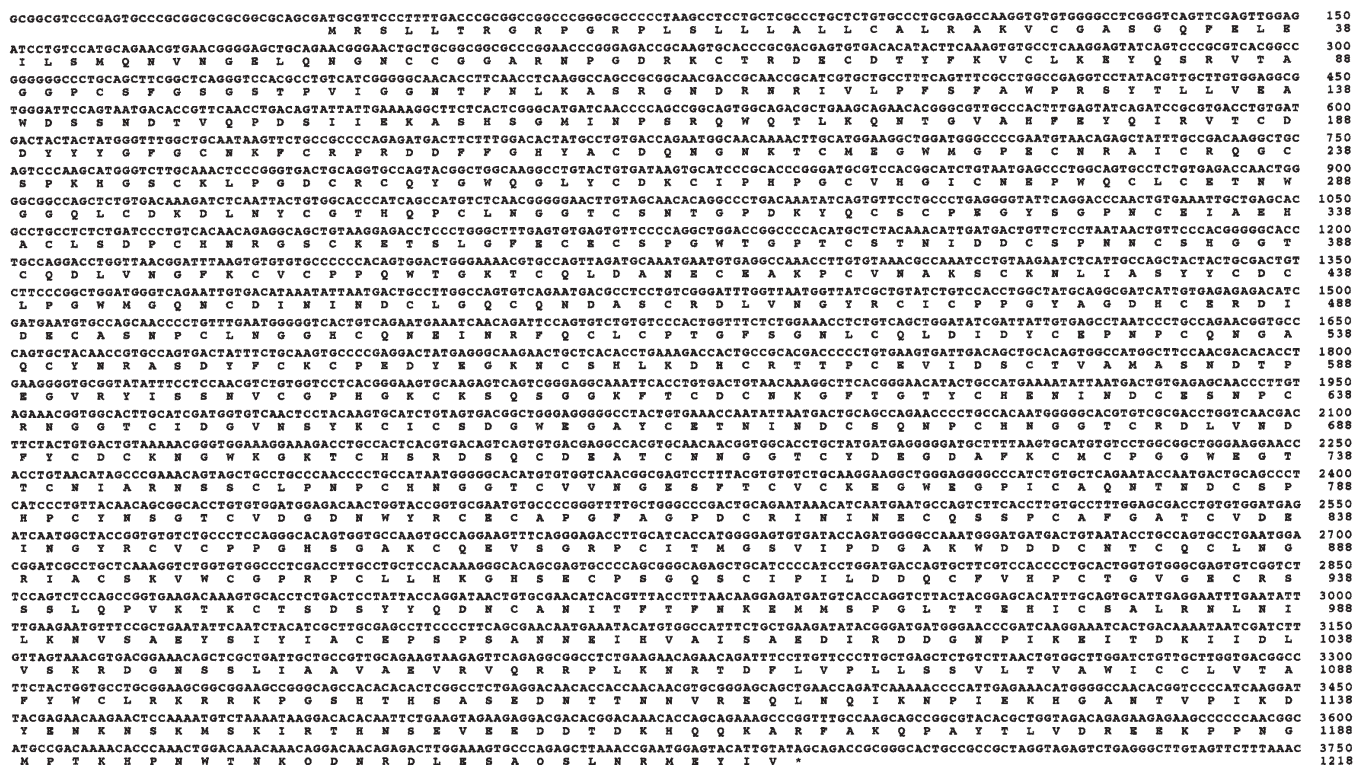


Figure 2. Nucleotide and amino-acid sequences of chimpanzee JAG1 complete CDS. Nucleotides and amino-acid residues are numbered on the right.

nih.gov) as described previously (37-39). TCF/LEF-binding sites within the 5'-flanking promoter region of above genes were searched for based on bioinformatics and human intelligence as described previously (40-42).

Identification of the chimpanzee *JAG1* ortholog. Chimpanzee genome sequence homologous to human *JAG1* was searched

for with BLAST programs as described previously (43-45). Exon-intron boundaries were determined by examining the consensus sequence of exon-intron junctions ('gt ag' rule of intronic sequence) and the codon usage within the coding region as described previously (46-48). Coding sequence of chimpanzee JAG1 was determined by assembling exonic regions.

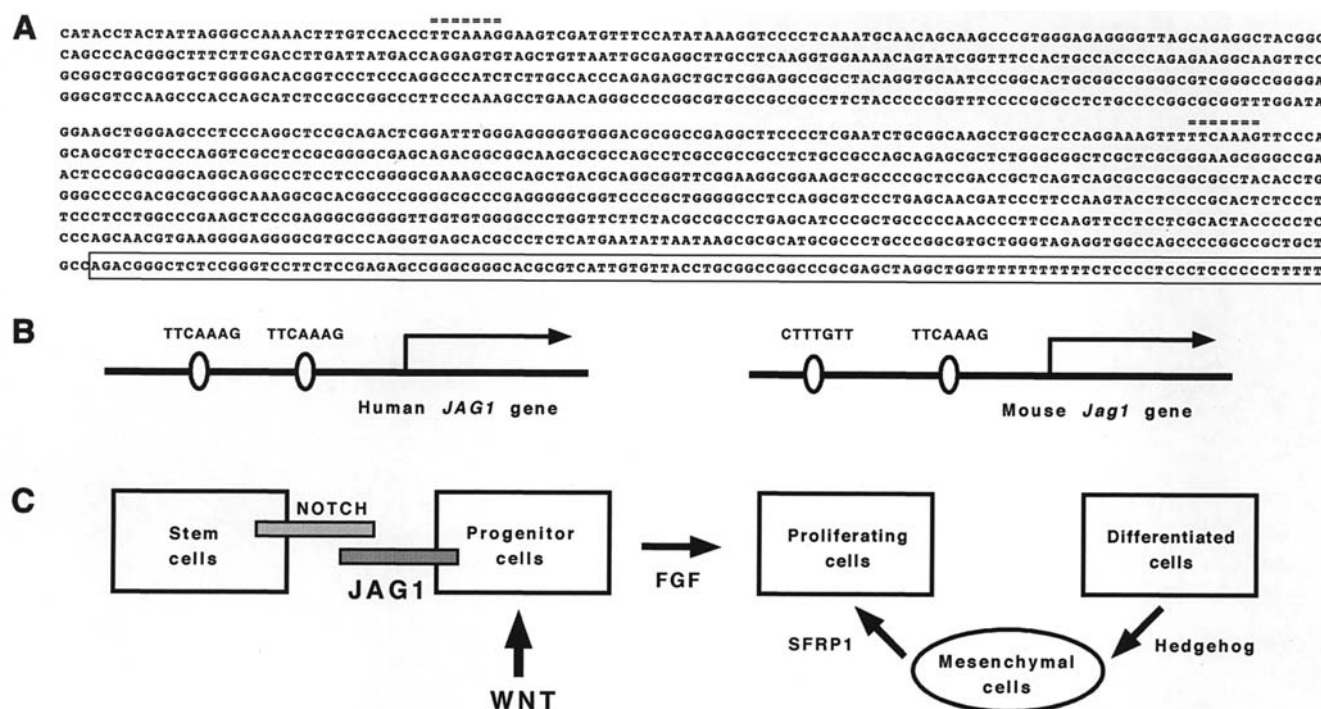


Figure 3. (A) Nucleotide sequence of human *JAG1* promoter region. TCF/LEF-binding sites are shown by double over-lines. Region corresponding to exon 1 of human *JAG1* gene is boxed. (B) Schematic representation of promoter regions of human *JAG1* and mouse *Jag1*. Double TCF/LEF-binding sites are conserved between human *JAG1* promoter and mouse *Jag1* promoter. (C) WNT and Notch signaling networks for the homeostasis of stem and progenitor cells. Canonical WNT activates the WNT/ β -catenin signaling pathway to induce JAG1 expression in progenitor cells. JAG1 on progenitor cells then activates the Notch signaling pathway in stem cells to maintain self-renewal potential.

Comparative proteomics analysis. Phylogenetic analyses on mammalian Notch ligand family members were performed by using the CLUSTALW program.

Comparative genomics analyses. Promoter region of human and mouse *JAG1* 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 human *JAG1* gene were searched for by using the BLAST programs. The sources of JAG1 ESTs were listed up for *in silico* expression analysis on *JAG1* mRNA.

Results

Screening of TCF/LEF-binding site within promoter region of Notch ligand genes. Human *DLL1* RefSeq (NM_005618.2), *DLL3* RefSeq (NM_203486.1), *DLL4* RefSeq (NM_019074.2), *JAG1* RefSeq (NM_000214.1) and *JAG2* RefSeq (NM_002226.3) were used as query sequences for the BLAST programs to identify genome clones corresponding to Notch ligand family genes. The 5'-flanking promoter region of human *DLL1*, *DLL3*, *DLL4*, *JAG1* and *JAG2* genes were identified within AL078605.30, AC011500.7, AC020661.8, AL035456.26 and AL512355.6 genome sequences, respectively (Fig. 1A). TCF/LEF-binding sites within the 5'-promoter region of human *DLL1*, *DLL3*, *DLL4*, *JAG1* and *JAG2* genes were then searched for. Double TCF/LEF-binding sites were identified within human *JAG1* promoter based on bioinformatics and human intelligence (Fig. 1A).

Identification of the chimpanzee *JAG1* gene. BLAST programs using human *JAG1* RefSeq revealed that chimpanzee *JAG1* gene was located within NW_120319.1 genome sequence. Exon-intron boundaries of chimpanzee *JAG1* gene were determined based on the consensus sequence of exon-intron junctions. Chimpanzee *JAG1* gene was found consisting of 26 exons (Fig. 1B).

Chimpanzee *JAG1* predicted sequence XM_525264.1 corresponded to 5'-flanking fragments, exons 2-4, 6-16 and introns of chimpanzee *JAG1* gene. Another chimpanzee *JAG1* predicted sequence XM_514517.1 corresponded to exons 17, 18, 20-26 of chimpanzee *JAG1* gene. Because XM_525264.1 and XM_514517.1 were not the correct chimpanzee *JAG1* sequences, complete coding sequence (CDS) of chimpanzee *JAG1* was determined in this study by assembling nucleotide sequences of 26 exons (Fig. 2).

Genetyx program revealed that nucleotide position 37-3693 was the coding region of chimpanzee *JAG1* complete CDS (Fig. 2). Chimpanzee *JAG1* gene was found to encode a 1218-amino-acid protein showing 99.5% and 96.2% total-amino-acid identity with human *JAG1* and mouse *Jag1*, respectively.

Comparative proteomics analysis on mammalian Notch ligand family members. Phylogenetic analysis revealed that *JAG1* orthologs were more conserved than other orthologs of Notch ligand family members (Fig. 1C).

Expression of human *JAG1* mRNA. *In silico* expression analysis revealed that human *JAG1* mRNA was expressed in embryonic stem (ES) cells, neural tissues, lung carcinoid,

gastric cancer, pancreatic cancer, colon cancer, and also in squamous cell carcinoma (SCC) of skin, oral cavity, esophagus, as well as head and neck.

Comparative genomics analyses on JAG1 promoters. Human *JAG1* promoter and mouse *Jag1* promoter were located within AL035456.26 and AL713981.17 genome sequences, respectively. Because chimpanzee *JAG1* promoter was located within the sequencing gap of NW_120319.1 genome sequence, 5'-promoters of human and mouse *JAG1* orthologs were compared.

Double TCF/LEF-binding sites within human *JAG1* promoter were located about 1200 bp and 600 bp upstream of the transcription start site (Fig. 3A). The region corresponding to about 1100 upstream of the transcription start site of human *JAG1* gene, including the proximal TCF/LEF-binding site, was well conserved in mouse (Fig. 3B). The distal TCF/LEF-binding site of human *JAG1* promoter corresponded to inverted TCF/LEF-binding site about 1400 bp upstream of the transcription start site of the mouse *Jag1* gene (Fig. 3B). Therefore, double TCF/LEF-binding sites were conserved among the human *JAG1* and mouse *Jag1* promoters.

Discussion

TCF/LEF-binding site within the promoter region of genes encoding Notch ligands, including *DLL1*, *DLL3*, *DLL4*, *JAG1* and *JAG2*, were searched for. Because double TCF/LEF-binding sites were identified within human *JAG1* promoter based on bioinformatics and human intelligence (Fig. 1A), comparative genomics analyses on *JAG1* orthologs were further performed in this study.

Chimpanzee *JAG1* gene, consisting of 26 exons, was identified within NW_120319.1 genome sequence (Fig. 1B). Because XM_525264.1 and XM_514517.1 were not the correct coding sequences for chimpanzee *JAG1*, complete CDS for chimpanzee *JAG1* was determined by assembling nucleotide sequences of exonic regions (Fig. 2). Chimpanzee *JAG1* gene was found to encode a 1218-amino-acid protein showing 99.5% total-amino-acid identity with human *JAG1*. Phylogenetic analyses revealed that *JAG1* orthologs were more conserved than those of other Notch ligands (Fig. 1C).

JAG1 gene was identified as evolutionarily conserved target of WNT/ β -catenin signaling pathway based on the conservation of double TCF/LEF-binding sites within the 5'-promoter region (Fig. 3B). WNT/ β -catenin signaling pathway is activated in progenitor cells (23). *JAG1* is expressed on progenitor cells within the epithelium of adult gastrointestinal tract (49). *JAG1* is the ligand for Notch receptors in stem cells (10). These facts indicate that *JAG1* expression on progenitor cells induced by canonical WNT signaling activation leads to the maintenance of self-renewal potential of stem cells through Notch signaling activation (Fig. 3C).

Expression of human *JAG1* mRNA was detected in ES cells, neural tissues, lung carcinoid, gastric cancer, pancreatic cancer, colon cancer, and also in SCC of skin, oral cavity, esophagus, as well as head and neck in this study. Because *JAG1* functioning as WNT-dependent Notch signaling

activator is the key molecule maintaining the homeostasis of stem and progenitor cells, *JAG1* is a pharmacogenomics target in the fields of oncology and regenerative medicine.

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