FGF signaling inhibitor, SPRY4, is evolutionarily conserved target of WNT signaling pathway in progenitor cells

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Received December 7, 2005; Accepted January 3, 2006

Abstract. WNT, FGF and Hedgehog signaling pathways network together during embryogenesis, tissue regeneration, and carcinogenesis. FGFr16, FGFr18, and FGFr20 genes are targets of WNT-mediated TCF/LEF-ß-catenin-BCL9/BCL9L-PYGO transcriptional complex. SPROUTY (SPRY) and SPRED family genes encode inhibitors for receptor tyrosine kinase signaling cascades, such as those of FGF receptor family members and EGF receptor family members. Here, transcriptional regulation of SPRY1, SPRY2, SPRY3, SPRY4, SPRED1, SPRED2, and SPRED3 genes by WNT/ß-catenin signaling cascade was investigated by using bioinformatics and human intelligence (humint). Because double TCF/LEF-binding sites were identified within the 5'-promoter region of human SPRY4 gene, comparative genomics analyses on SPRY4 orthologs were further performed. SPRY4-FGF1 locus at human chromosome 5q31.3 and FGF2-NUDT6-SPATA5-SPRY1 locus at human chromosome 4q27-q28.1 were paralogous regions within the human genome. Chimpanzee SPRY4 gene was identified within NW_107083.1 genome sequence. Human, chimpanzee, rat and mouse SPRY4 orthologs, consisting of three exons, were well conserved. SPRY4 gene was identified as the evolutionarily conserved target of WNT/ß-catenin signaling pathway in progenitor cells.

Introduction

WNT, FGF, and Hedgehog signaling pathways network together in a variety of cellular processes, such as stem cell differentiation cascade, body axis formation, angiogenesis, organogenesis during embryogenesis, adult tissue regeneration during chronic persistent inflammation, cell fate determination and cancer cell proliferation during carcinogenesis (1-11).

Canonical WNT signals are transduced through Frizzled receptors and LRP5/6 co-receptors to activate transcription of target genes, such as FGFr18, FGFr20, CCND1 and MYC, based on the transcriptional complex consisting of TCF/LEF, ß-catenin, BCL9/BCL9L, and PYGO1/PYGO2 (12-21). FGF signals are transduced through FGF receptors and FRS2 docking protein to activate RAS-RAF-MAPKK-MAPK signaling cascade as well as PI3K-AKT signaling cascade (4,5,22-24). Hedgehog signals are transduced through Patched receptors and Smoothened signal transducers to activate transcription of target genes, such as PTCH1, SFRP1, CCND2 and FOXM1 genes, based on active form of GLI family transcription factors (6-9,25-28). WNT, FGF, and Hedgehog signaling pathways network together during embryogenesis, tissue regeneration, and carcinogenesis.

SPROUTY (SPRY) and SPRED family genes encode inhibitors for receptor tyrosine kinase signaling cascades, such as those of FGF receptor family members and EGF receptor family members (29-35). Here, transcriptional regulation of SPRY1, SPRY2, SPRY3, SPRY4, SPRED1, SPRED2, and SPRED3 genes by WNT/ß-catenin signaling pathway was investigated by using bioinformatics and human intelligence. SPRY4 gene was identified as the evolutionarily conserved target of the WNT/ß-catenin signaling pathway in progenitor cells.
the 5'-flanking promoter region of the above genes were searched for based on bioinformatics and human intelligence.

**Identification of chimpanzee ortholog.** Chimpanzee genome sequence homologous to human SPRY4 was searched for with BLAST programs as described previously (38,39). 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 (40,41). Coding sequence of chimpanzee SPRY4 was determined by assembling exonic regions.

**Comparative genomics analyses.** Promoter region of human, chimpanzee, rat and mouse SPRY4 orthologs were aligned by using the Genetyx program and manual curation. TCF/LEF-binding sites within the promoter regions were determined as mentioned above. Other transcription factor-binding sites were searched for by using the Match program as described previously (42,43).

**Results**

**Screening of the TCF/LEF-binding site within promoter region of SPRY/SPRED family genes.** By using human RefSeqs as query sequences for the BLAST programs, the 5'-flanking promoter region of human SPRY1, SPRY2, SPRY3, SPRY4, SPRED1, SPRED2 and SPRED3 genes were identified within AC026402.3, AL354668.13, AC026724.12, AC026724.13, AC012370.8, AC005789.1, and AC005789.1 genome sequences, respectively. TCF/LEF-binding sites within the promoter regions were determined as mentioned above. Other transcription factor-binding sites were searched for by using the Match program as described previously (42,43).

**Comparative genomics analyses.** Phylogenetic analyses on SPRY/SPRED family members revealed that SPRY4 was more homologous to SPRY1 and SYRY2 (Fig. 1B). Intraspecies comparative genomics analyses revealed that SPRY4 gene at human chromosome 5q31.3 was linked to FGF1 gene, and that SPRY1 gene at human chromosome 4q28.1 was linked to FGF2 gene (Fig. 1C). These facts indicate that SPRY4-FGF1 locus at human chromosome 5q31.3 and FGF2-NUDT6-SPATA5-SPRY1 locus at human chromosome 4q27-q28.1 were paralogous regions within the human genome.

**Identification and characterization of chimpanzee SPRY4 gene.** BLAST programs using human SPRY4 RefSeq revealed that chimpanzee SPRY4 gene was located within NW_107083.1 genome sequence. Exon-intron boundaries of chimpanzee SPRY4 gene were determined based on the consensus sequence of exon-intron junctions. exon 1 corresponded to nucleotide position 1968269-1968113 of chimpanzee genome sequence NW_107083.1, exon 2 to 1962961-1962911, and exon 3 to 1958281-1953557. Complete coding sequence of chimpanzee SPRY4 was determined by assembling nucleotide sequences of three exons (Fig. 2A). Chimpanzee SPRY4 and human SPRY4 with 6-amino-acid substitutions showed 98.1% total-amino-acid identity.

**Comparative genomics analyses on SPRY4 promoters.** Human and chimpanzee SPRY4 promoters were located within AC091825.4 and NW_107083.1 genome sequences, respectively, as mentioned above. BLAST programs next revealed that rat and mouse Spry4 promoters were located within AC099211.8 and AC151580.2 genome sequences, respectively. GC content of human SPRY4 promoter, chimpanzee SPRY4 promoter, rat Spry4 promoter, and mouse Spry4 promoter was 63.3%, 63.3%, 60.7%, and 58.9%, respectively.

Human SPRY4 promoter, chimpanzee SPRY4 promoter, rat Spry4 promoter and mouse Spry4 promoter were aligned by using the Genetyx program and manual curation. Human SPRY4 promoter showed 99.0% nucleotide identity with chimpanzee SPRY4 promoter, and 80.6% nucleotide identity with rat Spry4 promoter. Double TCF/LEF-binding sites were conserved among 5'-promoter regions of mammalian SPRY4 orthologs (Fig. 2B). These facts indicate that SPRY4 is the evolutionarily conserved target of the WNT/β-catenin signaling pathway.
Expression of SPRY4 mRNA. Human expressed sequence tags (ESTs) derived from SPRY4 gene were searched for with the BLAST programs. Sources of SPRY4 ESTs were then listed up. In silico expression analyses revealed that human SPRY4 mRNA was expressed in embryonic stem cells, brain, pancreatic islet, colon cancer, head and neck tumor, melanoma, and pancreatic cancer.

Discussion

Transcriptional regulation of SPRY1, SPRY2, SPRY3, SPRY4, SPRED1, SPRED2, and SPRED3 genes by the WNT/β-catenin signaling cascade was investigated in this study. Because double TCF/LEF-binding sites were identified within the 5'-promoter region of human SPRY4 gene (Fig. 1A), comparative genomics analyses on SPRY4 orthologs were performed. SPRY4-FGF1 locus at human chromosome 5q31.3 and FGF2-NUDT6-SPATA5-SPRY1 locus at human chromosome 4q27-q28.1 were paralogous regions within the human genome (Fig. 1C). Human SPRY4 mRNA was expressed in embryonic stem cells, brain, pancreatic islet, colon cancer, melanoma, and pancreatic cancer.

Chimpanzee SPRY4 gene, encoding a 322-amino-acid protein, was identified within NW_107083.1 genome sequence.
(Fig. 2A). Chimpanzee SPRY4 showed 98.1% total-amino-acid identity with human SPRY4. Human, chimpanzee, rat and mouse SPRY4 orthologs, consisting of three exons, were well conserved.

Based on the comparative genomics analyses, we successfully identified evolutionarily conserved TCF/LEF-binding sites within the S'-promoter region of mammalian SPRY4 orthologs (Fig. 2B). Although Ding et al. reported conservation of human SPRY4 and mouse Spry4 promoters, they failed to identify TCF/LEF-binding sites (44). Therefore, this is the first report on the characterization of SPRY4 as the target gene of WNT/b-catenin signaling pathway.

WNT/b-catenin signaling pathway is activated in progenitor cells (45), and SPRY4 (Fig. 2B) as well as FGFs (18-20) are its target genes. WNT signaling activation in progenitor cells leads to the growth regulation of progenitor cells themselves through SPRY4 induction, and also to the growth stimulation of proliferating cells through FGF induction (Fig. 3). Epigenetic silencing and loss-of-function mutations of SPRY4 gene in progenitor cells could lead to carcinogenesis. Therefore, SPRY4 is the pharmacogenomics target in the fields of oncology and regenerative medicine.

References
