SPANDIDOS PUBLICATIONS

WNT antagonist, SFRP1, is Hedgehog signaling target

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Received September 26, 2005; Accepted October 31, 2005

Abstract. Hedgehog and WNT signaling pathways network together during embryogenesis and carcinogenesis. Hedgehog signaling in intestinal epithelium represses canonical WNT signaling to restrict expression of WNT target genes to stem or progenitor cells; however, the mechanism remains unclear. The Hedgehog signal is transduced to GLI family transcription factors though Patched receptor, Smoothened signal transducer, and other signaling components, such as KIF27, KIF7, STK36, SUFU, and DZIP1. Here, we searched for the GLI-binding site within the promoter region of genes encoding secreted-type WNT signal inhibitors, including *SFRP1, SFRP2, SFRP3, SFRP4, SFRP5, DKK1, DKK2, DKK3, DKK4*, and *WIF1*. The GLI-binding site was identified within the human *SFRP1* promoter based on bioinformatics and human intelligence. The chimpanzee *SFRP1* gene was identified within the NW_110515.1 genome sequence. The GLI-binding site of the human *SFRP1* promoter was conserved in chimpanzee *SFRP1*, mouse *Sfrp1*, and rat *Sfrp1* promoters. *SFRP1* is the evolutionarily conserved target of the Hedgehog-GLI signaling pathway. Expression domain analyses based on text mining revealed that *Indian Hedgehog* (*IHH*), *SFRP1*, and *WNT6* are expressed in differentiated intestinal epithelial cells, mesenchymal cells, and stem/progenitor cells, respectively. Hedgehog is secreted from differentiated epithelial cells to induce SFRP1 expression in mesenchymal cells, which keeps differentiated epithelial cells away from the effects of canonical WNT signaling. These facts indicate that SFRP1 is the Hedgehog target to confine canonical WNT signaling within stem or progenitor cells. Therefore, epigenetic CpG hypermethylation of the *SFRP1* promoter during chronic persistent inflammation and aging leads to the occurrence of gastrointestinal cancers, such as colorectal cancer and gastric cancer, through the breakdown of Hedgehog-dependent WNT signal inhibition.

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Key words: bioinformatics, comparative genomics, comparative proteomics, Hedgehog, WNT, integrome network

Introduction

The Hedgehog signaling pathway is implicated in embryogenesis and carcinogenesis (1-3). Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH) are Hedgehog family ligands (4-6). PTCH1, PTCH2, DISP1, DISP2 and DISP3 are multi-transmembrane proteins sharing two PTCH/ DISP homologous domains (7,8). PTCH1 and PTCH2 are Hedgehog receptors while seven-transmembrane protein, Smoothened (SMO), is the Hedgehog signal transducer (9-13). KIF27, KIF7, STK36, SUFU, and DZIP1 are Hedgehog signaling components (10-17). GLI1, GLI2, and GLI3 are GLI family transcription factors to activate the transcription of Hedgehog target genes (1,18). *PTCH1, CCND2, IGFBP6*, and *FOXM1* genes are direct transcriptional targets of the Hedgehog-GLI signaling pathway (19,20).

Hedgehog and WNT signaling pathways network together during embryogenesis and carcinogenesis (1). Hedgehog signaling in intestinal epithelium represses canonical WNT signaling to restrict expression of WNT target genes to stem or progenitor cells (2); however, the mechanism remains unclear. Induction of the secreted-type WNT inhibitor by the Hedgehog signaling pathway is one reasonable explanation for the Hedgehog-dependent WNT signal inhibition.

SFRP1, SFRP2, SFRP3, SFRP4, SFRP5, DKK1, DKK2, DKK3, DKK4, and *WIF1* genes encode secreted-type WNT inhibitors (21-27). We have previously reported comparative genomics analyses on *SFRP1, SFRP2, DKK1, DKK2*, and *DKK4* orthologs (24-27). Here, we searched for GLI-binding site within the promoter region of WNT inhibitor genes. The *SFRP1* gene was identified as the evolutionarily conserved target of the Hedgehog-GLI signaling pathway.

Materials and methods

Hedgehog target gene screening. Genome sequences corresponding to human *SFRP1, SFRP2, SFRP3, SFRP4, SFRP5, DKK1, DKK2, DKK3, DKK4* and *WIF1* genes were searched for using BLAST programs (http://www.ncbi.nlm.nih.gov) as described previously (28,29). GLI-binding sites within the 5'-flanking promoter region of above genes were searched for based on bioinformatics and human intelligence.

Identification of chimpanzeee SFRP1 ortholog. Chipmanzee genome sequences homologous to human *SFRP1* were searched for using BLAST programs as described previously

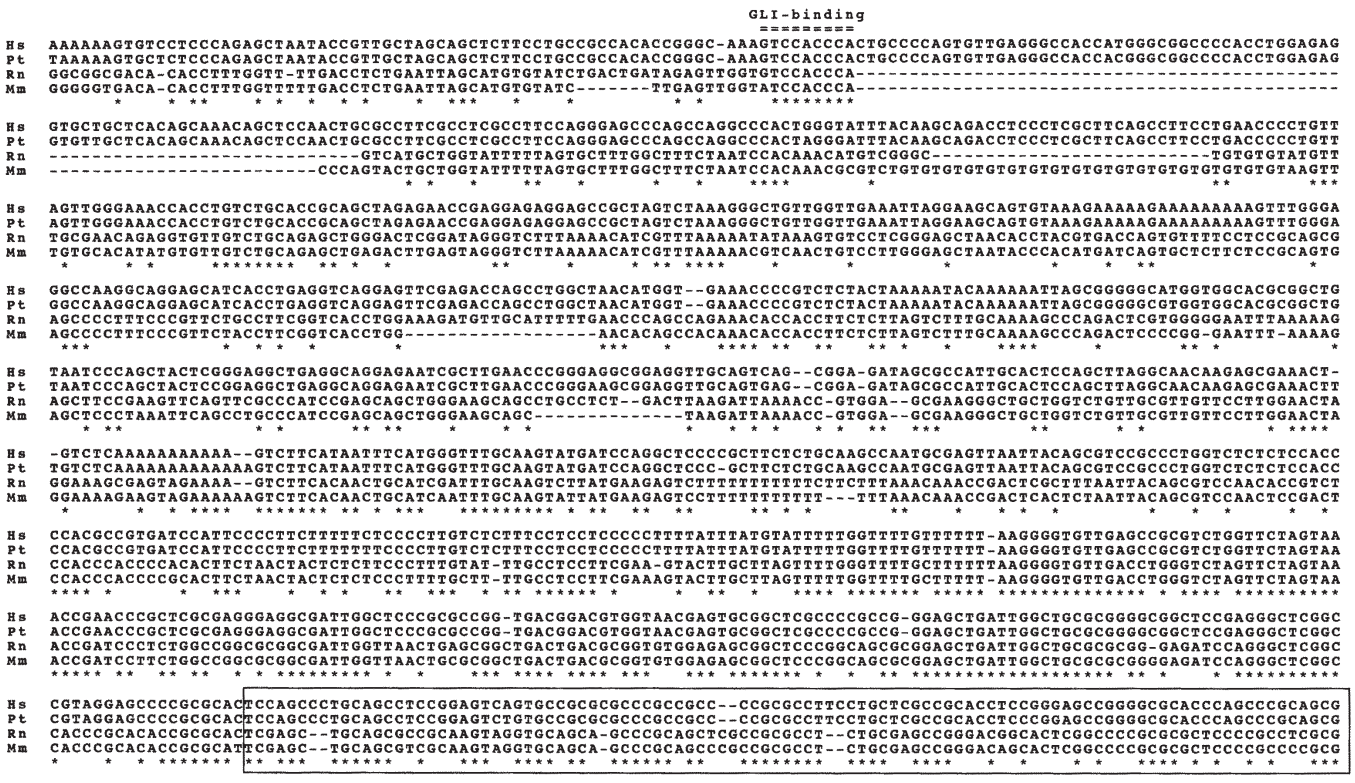

Figure 1. Comparative genomics on mammalian *SFRP1* promoters. Hs, human; Pt, chimpanzee; Rn, rat; Mm, mouse. The region corresponding to exon 1 of the rat *Sfrp1* gene is boxed. The GLI-binding site is indicated by a double over-line.

(30,31). 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 (32,33). The coding sequence of chimpanzee SFRP1 was determined by assembling exonic regions.

Comparative genomics analyses. Promoter regions of human, chimpanzee, rat and mouse *SFRP1* orthologs were aligned by using the Genetyx program and manual curation. The GLI-binding site within the promoter regions was determined as mentioned above. Other transcription factor-binding sites were searched for by using the Match program as mentioned previously (34,35).

Results

Screening of GLI-binding site within promoter region of WNT inhibitor genes. By using human RefSeqs as query sequences for the BLAST programs, the 5'-flanking promoter regions of human *SFRP1, SFRP2, SFRP3, SFRP4, SFRP5, DKK1, DKK2, DKK3, DKK4* and *WIF1* genes were identified within AC016868.8, AC020703.7, AC108514.3, AC018634.3, AL358938.8, AC009986.10, AP001819.2, AC124276.5, AF170802.6 and AC026124.35 genome sequences, respectively. The GLI-binding sequences within the promoter regions of human *SFRP1, SFRP2, SFRP3, SFRP4, SFRP5, DKK1, DKK2, DKK3, DKK4* and *WIF1* genes were then searched for using manual inspection. The GLI-binding site was identified within the 5'-flanking promoter region of the human *SFRP1* gene (Fig. 1).

Identification of the chimpanzee SFRP1 gene. The Blastn program using human SFRP1 RefSeq revealed that the chimpanzee *SFRP1* gene was located within chimpanzee genome sequence NW_110515.1. Exon-intron boundaries of the chimpanzee *SFRP1* gene were determined based on the consensus sequence of exon-intron junctions. Exon 1 corresponded to 562669-561824 of chimpanzee genome sequence NW_110515.1, exon 2 to 556735-556658, and exon 3 to 518156-514629. Although nucleotide position 377-768 of chimpanzee cDNA was a sequence gap, nucleotide identity between human and chimpanzee SFRP1 cDNAs in other parts was 98.3%. Based on these facts, it was concluded that the chimpanzee *SFRP1* gene was located around nucleotide position 562669-514629 of the chimpanzee genome sequence NW_110515.1. A partial coding sequence of chimpanzee SFRP1 was determined by assembling the nucleotide sequences of three exons (Fig. 2).

Comparative genomics analyses on SFRP1 promoters. The human *SFRP1*, rat *Sfrp1* and mouse *Sfrp1* promoters were located within AC016868.8, AC112899.4 and AC139848.4 genome sequences, respectively, as described previously (24). The chimpanzee *SFRP1* promoter was located within the NW_110515.1 genome sequence as mentioned above. The GC content of the human *SFRP1*, chimpanzee *SFRP1*, rat *Sfrp1*, and mouse *Sfrp1* promoters was 56.3%, 56.1%, 50.3%, and 49.5%, respectively.

The human *SFRP1*, chimpanzee *SFRP1*, rat *Sfrp1* and mouse *Sfrp1* promoters were aligned by using the Genetics program and manual curation. The human *SFRP1* promoter showed 98.7% nucleotide identity with the chimpanzee *SFRP1*

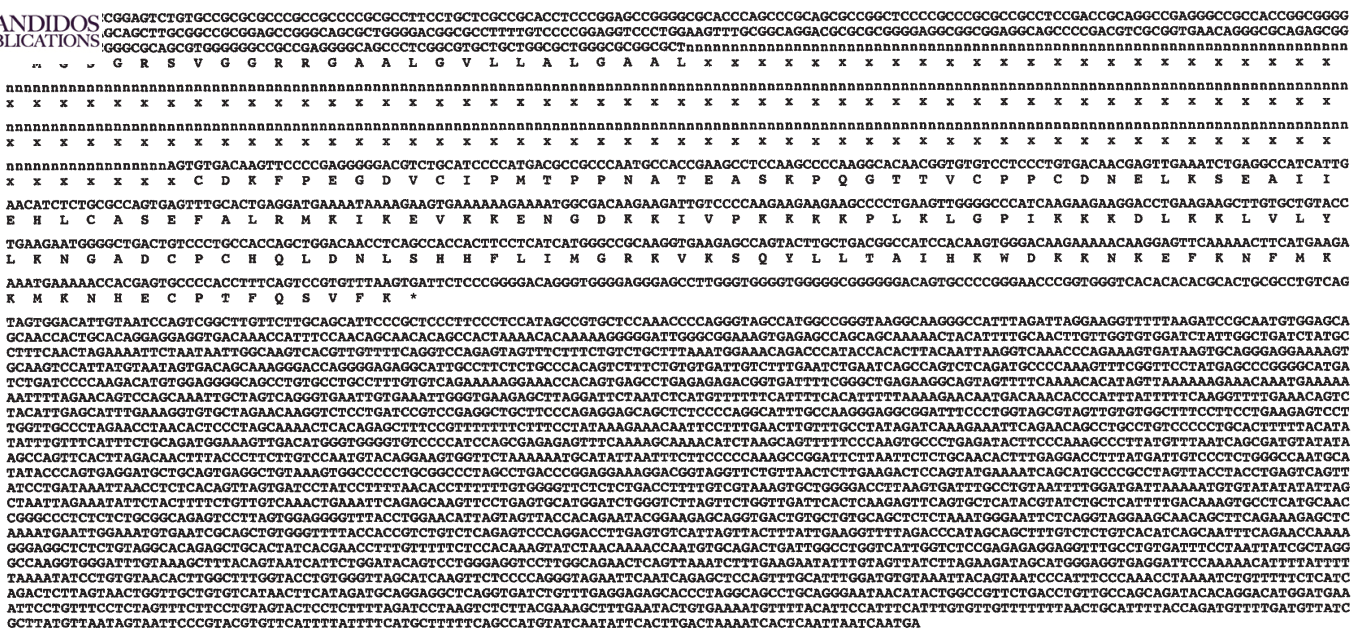

Figure 2. Partial nucleotide and amino-acid sequences of chimpanzee SFRP1.

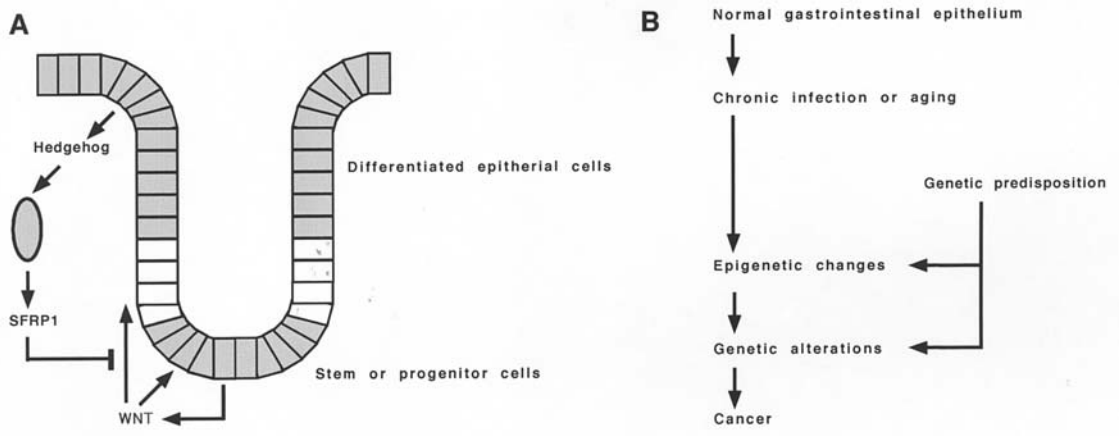

Figure 3. (A) Hedgehog, SFRP1 and WNT signaling network in intestinal epithelium. (B) Chronic persistent inflammation and cancer. Chronic persistent inflammation induces epigenetic changes and genetic alterations during multi-stage gastrointestinal carcinogenesis.

promoter, and 45.0% nucleotide identity with the mouse *Sfrp1* promoter. The GLI-binding site was conserved among promoter regions of mammalian *SFRP1* orthologs (Fig. 1). These facts indicate that *SFRP1* is the evolutionarily conserved target of the Hedgehog-GLI signaling pathway.

Hedgehog, SFRP1 and WNT signaling network. Expression domains of Hedgehog, SFRP1, and canonical WNTs were searched for using text mining. Van den Brink *et al* reported IHH expression in differentiated epithelial cells within human and rat colons (2). We reported *WNT6* mRNA expression in human colon and small intestine (36), and conservation between the human *WNT6* promoter and rodent *Wnt6* promoter (37). Gregorieff *et al* reported *Sfrp1* mRNA expression in mesenchymal cells within mouse small intestine and colon, and *Wnt6* mRNA expression in stem or progenitor cells (38). These facts indicate that IHH is secreted from differentiated epithelial cells to induce SFRP1 expression in mesenchymal cells and that SFRP1 keeps differentiated epithelial cells away from the effects of canonical WNT signaling (Fig. 3A).

Discussion

The GLI-binding site within the promoter region of genes encoding secreted-type WNT signal inhibitors, including *SFRP1, SFRP2, SFRP3, SFRP4, SFRP5, DKK1, DKK2, DKK3, DKK4* and *WIF1*, was searched for by using bio-informatics and human intelligence in this study. The GLI-binding site was identified within the human *SFRP1* promoter (Fig. 1).

We next investigated evolutionary conservation of the GLI-binding site within *the SFRP1* promoter by using comparative genomics. We had previously identified and characterized the rat *Sfrp1* gene (24). To improve the accuracy of the comparative genomics analyses on the *SFRP1* promoters, we identified the chimpanzee *SFRP1* gene within the

NW_110515.1 genome sequence (Fig. 2). The GC contents of human and chimpanzee *SFRP1* promoters were higher than those of rat and mouse *Sfrp1* promoters. Nucleotide identity between the human *SFRP1* promoter and chimpanzee *SFRP1* promoter was 98.7%, while that between the human *SFRP1* promoter and mouse *Sfrp1* promoter was 45.0%. Although GC content and nucleotide identity were relatively divergent between primate *SFRP1* promoters and rodent *Sfrp1* promoters, the GLI-binding site was conserved among mammalian *SFRP1* promoters (Fig. 1). These facts indicate that the *SFRP1* gene is the evolutionarily conserved target of the Hedgehog-GLI signaling pathway.

Expression domain analyses based on text mining revealed that *IHH, SFRP1*, and *WNT6* are expressed in differentiated intestinal epithelial cells, mesenchymal cells, and stem/progenitor cells, respectively (Fig. 3A). Hedgehog is secreted from differentiated epithelial cells to induce SFRP1 expression in mesenchymal cells, which keeps differentiated epithelial cells away from the effects of canonical WNT signaling. These facts indicate that SFRP1 is the Hedgehog target to confine canonical WNT signaling within stem or progenitor cells (Fig. 3A).

The *SFRP1* tumor suppressor gene at human chromosome 8p11.21 is inactivated in colorectal cancer, gastric cancer, and other tumors by epigenetic CpG hypermethylation and deletion (24,39-41). Inflammatory bowel disease and *Helicobacter pylori* infection are risk factors for colorectal and gastric cancers, respectively (42-45). Although the precise mechanism to induce epigenetic changes remains to be elucidated, epigenetic changes are associated with chronic persistent inflammation and aging (44-46). Accumulation of epigenetic changes and genetic alterations leads to gastrointestinal carcinogenesis (Fig. 3B). Epigenetic CpG hypermethylation of the *SFRP1* promoter during chronic persistent inflammation and aging leads to the occurrence of gastrointestinal cancers, such as colorectal cancer and gastric cancer, through the breakdown of Hedgehog-dependent WNT signal inhibition.

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