Transcriptional regulation of WNT2B based on the balance of Hedgehog, Notch, BMP and WNT signals

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Abstract. We cloned and characterized human WNT2B in 1996, and then others cloned and characterized mouse, chicken, and zebrafish WNT2B orthologs. WNT2B is expressed in several types of human cancer, such as basal cell carcinoma, gastric cancer, breast cancer, head/neck squamous cell carcinoma, cervical cancer and leukemia. WNT2B is one of canonical WNTs transducing signals through Frizzled (FZD) and LRP5/LRP6 receptors to β-catenin-TCF/LEF signaling cascade. Here, refined integrative genomic analyses on WNT2B orthologs were carried out to elucidate its transcriptional mechanisms. GLI-, double FOX-, HES/HEY-, bHLH-, and Sp1-binding sites within mammalian WNT2B promoter were well conserved. Because GLI1, FOXA2, FOXC2, FOXE1, FOXL1 and FOXL1 are direct target genes of Hedgehog-GLI2 signaling cascade, Hedgehog signals should induce WNT2B upregulation through GLI family members as well as FOX family members. Notch, BMP and Hedgehog signals inhibit WNT2B expression via HES/HEY-binding to N-box, whereas BMP and WNT signals inhibit bHLH transcription factor-induced WNT2B expression via ID1, ID2, ID3, MSX1 or MSX2. Together these facts indicate that Hedgehog signals and bHLH transcription factors are involved in WNT2B upregulation, which is counteracted by BMP, WNT and Notch signals. Mesenchymal BMP induces IHH expression in gastrointestinal epithelial cells, and then epithelial Hedgehog induces WNT2B and BMP4 expression in mesenchymal cells. NF-κB signals induce SHH upregulation, and WNT2B is upregulated in inflammatory bowel disease (IBD). BMP-IHx and inflammation-SHH signaling loops are involved in WNT2B upregulation during embryogenesis, adult tissue homeostasis, and carcinogenesis.

Introduction

WNT family members are lipid-modified glycoproteins involved in embryogenesis and carcinogenesis (1-5). We cloned and characterized human WNT2B/WNT13 as the 13th member of the mammalian WNT family in 1996 (6), and others then characterized mouse, chicken, and zebrafish WNT2B orthologs (7-9). Human WNT2B shows 66.9% total amino-acid identity with human WNT2, while mouse Wnt2b shows 71.1% total amino-acid identity with mouse Wnt2. The WNT2B-ST7L-CAPZA1 locus at human chromosome 1p13.2 and the WNT2-S7-CAPZA2 locus at human chromosome 7q31.2 are paralogous regions within the human genome (10). Human WNT2B gene consisting of seven exons encodes multiple isoforms due to alternative splicing using alternative promoters (11). WNT2B2 isoform rather than WNT2B1 is evolutionarily conserved.

WNT2B functions as a canonical WNT to transduce signal through Frizzled (FZD) receptor and LRP5/6 co-receptor (12). In the absence of canonical WNT signaling, β-catenin is phosphorylated by Casein kinase 1α (CK1α) and glycogen synthase kinase 3β (GSK3β), and then is ubiquitylated by βTRCP1 (FBXW1)- or βTRCP2 (FBXW11)-containing ubiquitin-ligase complex for its degradation in the proteasome system (13-17). Canonical WNT signals induce assembly of FZD-Dishevelled complex and LRP5/6-AXIN complex to release β-catenin from AXIN-APC degradation complex (18-20), which results in stabilization and nuclear translocation of β-catenin for the transcriptional activation of target genes, such as MYC and CCND1 (21-23).

WNT signaling cascades cross-talk with Hedgehog, BMP/ TGF-β, FGF, and Notch signaling cascades to constitute the stem-cell signaling network (24-26). Dysregulation of the stem-cell signaling network due to epigenetic and genetic alterations leads to congenital abnormality and carcinogenesis (27-29).

Human WNT2B is expressed in fetal brain, lung, kidney, adult heart, brain, placenta, lung, prostate, testis, ovary, small intestine, colon, and also in several types of human cancer, such as basal cell carcinoma, gastric cancer, breast cancer, head/neck squamous cell carcinoma, cervical cancer, and leukemia (6,11,30-34). WNT2B is upregulated in liver and biliary epithelial cells of patients with primary biliary cirrhosis (35), and in colonic mucosa of patients with inflammatory bowel disease (36). WNT2B is upregulated in some types of human cancer and in some tissues with chronic inflammation;
however, the mechanisms of WNT2B transcription remain unclear. Here, refined integrative genomic analyses are carried out to elucidate the transcriptional mechanisms of WNT2B with an emphasis on the stem-cell signaling network.

**Materials and methods**

*Comparative genomic analyses.* Human genome sequences corresponding to human WNT2B2 isoform (NM_024494.2) were searched for by using BLAST programs, as previously described (37-40). Conserved transcription factor-binding sites within the WNT2B2 promoter were then searched for based on manual inspection, as previously described (41-44).

*Regulatory network analyses.* Literature on WNT2B, Hedgehog, BMP, and Notch signaling molecules in PubMed and Medline databases was critically evaluated to extract knowledge on the regulation of GLI, FOX, bHLH, ID and MSX family transcription factors. The mechanisms of WNT2B transcription were then investigated based on our data of conserved transcription factor-binding sites within the WNT2B promoter and in-house knowledgebase of transcription factors regulated by the stem-cell signaling network.

**Results**

*Comparative genomics on WNT2B promoter.* Because the region around the WNT2B2 promoter is GC-rich (45), transcription start sites (TSSs) of human WNT2B2 mRNA have been predicted to show 'broad peak' pattern, as is usual for other GC-rich promoters. Indeed, NM_024494.2 WNT2B2 RefSeq was extended to the 496-bp 5'-position compared with NM_024494.1 WNT2B2 RefSeq. Comparative integromics analyses of the WNT2B promoter were carried out again in this study by using up-to-date in-house knowledgebase on the transcription-factor network.

BLAST programs using the NM_024494.2 WNT2B2 RefSeq as a query sequence revealed that the TSS of human WNT2B2 mRNA was located within AL354760.11 and AC140714.1 genome sequences. BLAST programs next revealed that the distal promoter region (-4636 ~ -4628 position from TSS) and the proximal promoter region (~701 ~ -213 position from TSS) were well conserved between human WNT2B2 genome sequence and mouse Wnt2b genome sequence AC166156.4 (Fig. 1).

Consensus transcription factor-binding sites within the conserved promoter regions were searched for with manual inspection based on in-house knowledgebase. Double bHLH-binding sites [bHLH(1) and bHLH(2)] and double FOX-binding sites [FOX(1) and FOX(2)] within the distal promoter region of human WNT2B gene were conserved in mouse Wnt2b promoter (Fig. 1). HES-binding site, bHLH-binding site [bHLH(3)], and Sp1-binding site within the proximal promoter region of human WNT2B gene were also conserved in mouse Wnt2b promoter (Fig. 1).

Conservation of transcription factor-binding sites in other mammalian species was then searched for. Rat Wnt2b gene was located within AC106372.5 genome sequence, as previously described (45). Chimpanzee WNT2B gene was located within NW_001229591.1 genome sequence. The bHLH(1)-binding site was not conserved in chimpanzee WNT2B promoter, whereas the bHLH(2)-binding site was not conserved in rat Wnt2b promoter. Together these facts indicate that FOX(1)-, FOX(2)-, HES-, bHLH(3)-, and Sp1-binding sites were conserved in human WNT2B, chimpanzee WNT2B, mouse Wnt2b, and rat WNT2B promoters (Fig. 1).

*Hedgehog signaling cascades and WNT2B.* Hedgehog signals are transduced through Patched family receptors to activate Smoothened signal transducer, which results in stabilization and nuclear accumulation of GLI family members for transcriptional activation of Hedgehog target genes (29,46-48). Hedgehog signaling cascade is aberrantly activated in basal cell carcinoma (49-51), gastric cancer (52,53), breast cancer (54), and other tumors. WNT2B is expressed in basal cell carcinoma (32), gastric cancer (31), breast cancer (31), and other tumors, as mentioned above. Because Hedgehog signaling activation and WNT2B expression co-existed in several types of human cancer, we investigated the causal link between Hedgehog signaling activation and WNT2B expression.

Consensus GLI-binding site was located at the position -4636 ~ -4628 from the human WNT2B TSS (Fig. 1).
GLI-binding site at about 5 kb upstream of human WNT2B TSS was completely conserved in chimpanzee WNT2B and rat WNT2b promoters, and was almost conserved in mouse Wnt2b promoter except one base substitution (Fig. 1). GLI1, FOXA2, FOXC2, FOXE1, FOXF1 and FOXL1 are direct target genes of Hedgehog-GLI signaling cascade (29), and double FOX-binding sites within human WNT2B promoter were completely conserved in chimpanzee WNT2B, rat WNT2b, and mouse Wnt2b promoters, as mentioned above. Hedgehog signals should induce WNT2B upregulation through GLI and FOX family members (Fig. 2).

BMP signaling cascades and WNT2B. BMP signals are transduced through BMP receptors to SMAD1, SMAD5 or SMAD8 effectors to regulate transcription of target genes in cooperation with SMAD4 (55). ID1, ID2, ID3, MSX1, MSX2, HES1, HEY1, and IHH are representative target genes of BMP signaling cascades (56-58). ID1, ID2 and ID3 are HLH transcription factors functioning as inhibitors of tissue-specific bHLH transcription factors. MSX1 and MSX2 are homeobox transcription factors functioning as inhibitors of tissue-specific bHLH transcription factors. HES1 and HEY1 are N-box-binding transcriptional repressors to downregulate tissue-specific bHLH transcription factors. BMP signals downregulate WNT2B expression due to the repression of bHLH-induced transcription via ID1, ID2, ID3, MSX1, MSX2, HES1 or HEY1.

In adult mouse intestine, Wnt2b is expressed in mesenchymal cells (59), whereas Indian Hedgehog (Ihh) is expressed in differentiated epithelial enterocytes (60). We previously underscored the role of BMP-IHH signaling loop in the gastrointestinal homeostasis and carcinogenesis (61). Because epithelial IHH is able to induce mesenchymal WNT2B expression as mentioned above, we propose the BMP-IHH-WNT2B signaling loop in this study.

Based on these facts, it was concluded that BMP signals directly downregulate WNT2B expression in intestinal epithelial cells through the impediment of the bHLH-driven transcription.
and also that BMP signals indirectly upregulate WNT2B expression in intestinal mesenchymal cells through Hedgehog-mediated transcription (Fig. 2).

Canonical WNT signaling cascades and WNT2B. Canonical WNT signals are transduced through FZD and LRPs receptors to activate transcription of target genes via the β-catenin-TCF/LEF complex, as mentioned above. MYC is a key target of canonical WNT signaling activation in colorectal cancer (22). MYC is one of the bHLH-LZ transcription factors binding to the CACGTG motif to regulate cellular proliferation and differentiation (62). Manual inspection in this study revealed that consensus TCF/LEF- or MYC-binding site within WNT2B promoter was not conserved in mammals (data not shown). WNT and BMP signals synergistically induce up-regulation of ID2, MSX1, and MSX2 mRNAs (63). Because ID2, MSX1 and MSX2 are involved in the inhibition of bHLH-mediated transcription as mentioned above, it was predicted that canonical WNT signals downregulate WNT2B expression via ID and MSX family members (Fig. 2).

Notch signaling cascades and WNT2B. Notch ligands induce Notch signaling activation through the NICD- CSL complex, and HES1, HES5, HEY1, HEY2 and HEYL are representative target genes of Notch signaling cascades (64-67). Manual inspection in this study revealed that the consensus CSL-binding site within WNT2B promoter was not conserved among mammals (data not shown). On the other hand, HES/HEY-binging N-box within proximal promoter region of WNT2B was conserved in mammals (Fig. 1). Together these facts indicate that Notch signals downregulate WNT2B expression via HES/HEY family members (Fig. 2).

Chronic inflammation and WNT2B. Chronic inflammation in tumor microenvironment is advantageous for tumor progression, because mediators or effectors of chronic inflammation support the proliferation and survival of tumor cells (68, 69). NF-κB and STAT3 are representative transcription factors involved in inflammation-associated carcinogenesis. Because WNT2B is upregulated in chronic inflammation-associated diseases such as primary biliary cirrhosis (35) and inflammatory bowel disease (35, 36), we next investigated the causal link between chronic inflammation and WNT2B expression. Consensus STAT3-binding site was not identified conserved in the WNT2B promoter region in this study (data not shown), while in silico prediction of NF-κB-binding site is not practical at present. Recently, Sonic Hedgehog (Shh) was identified as an NK-κB target gene (70). Because epithelial Hedgehog induces WNT2B upregulation in mesenchymal cells as mentioned above, we propose the inflammation-SHH-WNT2B signaling loop (Fig. 2).

Discussion

Refined integrative genomic analyses on WNT2B orthologs were carried out to elucidate the transcriptional mechanisms of WNT2B in this study. GLI- and double FOX-binding sites within the distal promoter region as well as HES/HEY-, bHLH- and Sp1-binding sites within the proximal promoter region were well conserved in mammalian WNT2B orthologs (Fig. 1).

GLI-binding site in human WNT2B promoter was completely conserved in chimpanzee WNT2B and rat Wnt2b promoters, and was almost conserved in mouse Wnt2b promoter except one base substitution (Fig. 1). Double FOX-binding sites within human WNT2B promoter were completely conserved in chimpanzee WNT2B, rat Wnt2b, and mouse Wnt2b promoters (Fig. 1). Hedgehog signals initially stabilize the GLI2 activator to upregulate primary Hedgehog target genes, such as GLI1, FOXA2, FOXC2, FOXE1, FOXF1 and FOXL1. Hedgehog-GLI2-induced GLI1 upregulation augments the Hedgehog signaling quantitatively as well as qualitatively (29). Based on the conserved GLI- and double FOX-binding sites within distal WNT2B promoter region, it was concluded that Hedgehog signals should induce WNT2B upregulation through GLI and FOX family members (Fig. 2).

HES/HEY- and bHLH-binding sites in human WNT2B promoter were completely conserved in chimpanzee WNT2B, rat Wnt2b, and mouse Wnt2b promoters (Fig. 1). Notch and BMP signals upregulate HES/HEY family members to repress WNT2B transcription, whereas BMP and WNT signals upregulate ID and MSX family members to impede bHLH-induced WNT2B upregulation. Notch, BMP, and WNT signals synergistically repress WNT2B transcription due to the impediment of the bHLH-induced WNT2B transcription (Fig. 2).

We emphasized the BMP-IHH signaling loop to maintain the homeostasis of stem-cell signaling network (61), and Kasperczyk et al reported the NF-κB-SHH signaling loop during chronic inflammation (70). These facts were combined with the results of this study to propose that the BMP-IHH and inflammation-SHH signaling loops are involved in WNT2B upregulation during embryogenesis, adult tissue homeostasis, and carcinogenesis.

References


