Integrative genomic analyses of *WNT11*: Transcriptional mechanisms based on canonical WNT signals and GATA transcription factors

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Abstract. We and others previously cloned and characterized vertebrate *WNT11* orthologs, which are involved in gastrulation, neurulation, cardiogenesis, nephrogenesis, and chondrogenesis during fetal development. WNT11 orthologs activate both canonical and non-canonical WNT signaling cascades depending on the expression profile of WNT receptors, such as Frizzled family members, LRP6, ROR2, and RYK. Human *WNT11* is expressed in breast cancer, gastric cancer, esophageal cancer, colorectal cancer, neuroblastoma, Ewing sarcoma, and prostate cancer. Canonical WNT signals and GATA family members are involved in *WNT11* transcription during embryogenesis of model animals; however, precise mechanisms of *WNT11* expression remain unclear. Here, refined integrative genomic analyses of *WNT11* are carried out to elucidate the mechanisms of *WNT11* transcription. The *WNT11* gene was found to encode two isoforms by using alternative first exons. WNT11 isoform A (NM_004626.2 RefSeq) consists of exons 2, 3, 4, 5 and 6, whereas WNT11 isoform B consists of exons 1, 2, 3, 4, 5 and 6. We identified double TCF/LEF-binding sites within the proximal promoter regions (-48-bp position from the TSS of human *WNT11* isoform B and -43-bp position from the TSS of human *WNT11* isoform A), and also double GATA-binding sites within intron 2 of human *WNT11* gene (+933-bp and +5001-bp positions from TSS of human *WNT11* isoform A). Double TCF/LEF- and double GATA-binding sites within the regulatory regions of human *WNT11* gene were conserved in other mammalian *WNT11* orthologs. These facts indicate that canonical WNT signals and GATA family members directly upregulate *WNT11* transcription. Canonical WNT-induced WNT11 activates non-canonical WNT signaling cascades to induce cellular movement, and also activates the Ca^{2+}-MAP3K7-NLK signaling cascade to break the canonical WNT signaling. Canonical WNT-to-WNT11 signaling loop is involved in cellular migration during embryogenesis as well as tumor invasion during carcinogenesis.

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Introduction

WNT family members are secreted proteins with glycolipid modifications, which are involved in embryogenesis, adult-tissue homeostasis, and carcinogenesis (1-5). *WNT1, WNT2, WNT2B, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11,* and *WNT16* genes are conserved in the mammalian genomes (6), whereas additional *wnt* family genes other than the conserved 19 genes exist in the non-mammalian vertebrate genomes.

There is growing evidence that WNT signals are transduced to the canonical and non-canonical WNT signaling cascades in a context-dependent manner (7-10). LRP5, LRP6, and Frizzled family members are involved in the canonical WNT signaling cascade to activate the transcription of target genes based on the ß-catenin-TCF/LEF complex, whereas ROR1, ROR2, RYK, and Frizzled family members are involved in the non-canonical WNT signaling cascades, including DVL-RhoA-ROCK, DVL-RhoB-Rab4, DVL-Rac-JNK, DVL-aPKC, Ca^{2+}-Calcineurin-NFAT, Ca^{2+}-MAP3K7-NLK, Ca^{2+}-MAP3K7-NF-κB, and DAG-PKC signaling cascades (1-5,11-17).

We and other groups have cloned and characterized vertebrate *WNT11* orthologs (18-23). WNT11-related gene, *wnt11r*, exists in the zebrafish, *Xenopus*, and chicken genomes, but not in the mammalian genomes (24-26). WNT11 orthologs activate both canonical and non-canonical WNT signaling cascades depending on the expression profile of WNT receptors, such as Frizzled family members, LRP6, and RYK (27-34). Vertebrate *WNT11* homologs are involved in fetal development, especially in gastrulation (23,35-37), neural crest migration (26,38), cardiogenesis (39-43), nephrogenesis (44,45), and chondrogenesis (46,47).

WNT11 is expressed in several types of human cancer. We reported expression of human *WNT11* in breast cancer, gastric cancer, esophageal cancer, and embryonal tumor (1,19). We also reported upregulation of *WNT11* in some cases

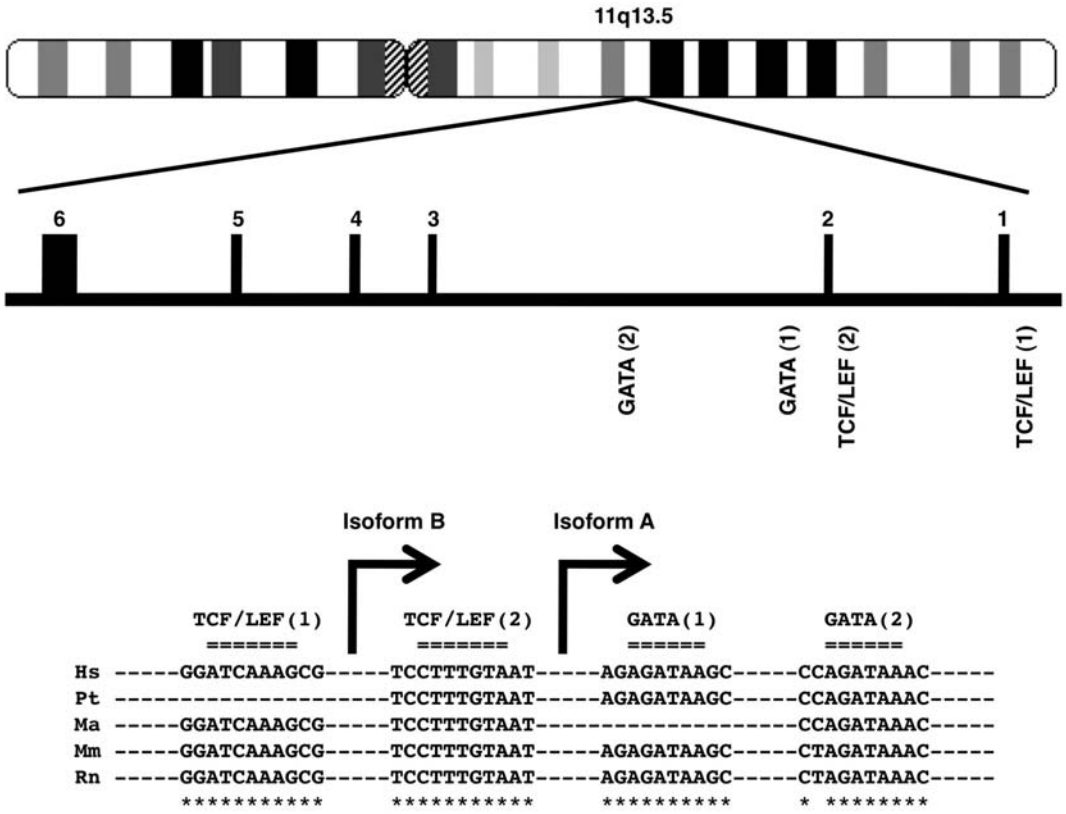

Figure 1. Integrative genomic analyses of *WNT11*. Schematic representation of *WNT11* gene at human chromosome 11q13.5 is shown in the upper part. *WNT11* gene encodes two splicing variants by using alternative first exons. Conserved transcription factor-binding sites within *WNT11* regulatory regions are shown in the lower part. Hs, human; Pt, chimpanzee; Ma, macaque; Mm, mouse; Rn, rat.

of primary colorectal cancer (19). Other groups then reported expression of human *WNT11* in neuroblastoma (48), Ewing sarcoma (49), and prostate cancer (50). Because precise mechanisms of *WNT11* expression remain unclear, refined integrative genomic analyses of *WNT11* were carried out to elucidate the mechanisms of *WNT11* transcription.

Materials and methods

Comparative genomic analyses. Human genome sequences corresponding to human WNT11 RefSeq (NM_004626.2) were searched for by using BLAST programs, as previously described (51,52). *WNT11* expressed sequence tags (ESTs) were also searched for to identify *WNT11* splicing variants, and also to determine the putative transcription start site (TSS) (53,54). Conserved transcription factor-binding sites within *WNT11* promoters were then searched for based on manual inspection, as previously described (55,56).

Regulatory network analyses. The literature on WNT, Hedgehog, and Notch signaling molecules and GATA family transcription factors in PubMed and Medline databases was critically evaluated to extract knowledge on the regulation of TCF/ LEF, GLI, FOX, CSL, and GATA transcription factors. The mechanisms of *WNT11* transcription were then investigated based on our data of conserved transcription factor-binding sites within *WNT11* promoters and in-house knowledgebase of transcription factors regulated by the stem-cell signaling network.

Results

WNT11 splicing variants transcribed by using alternative promoters. BLAST programs using NM_004626.2 RefSeq as a query sequence revealed that human *WNT11* gene is located within human genome sequence AP000785.4, as previously described (57). BLAST programs using the *WNT11* genome sequence as a query sequence next revealed that 11 ESTs are transcribed from the same first exon of human *WNT11* gene as NM_004626.2 RefSeq, and that three ESTs are transcribed from alternative first exon located about 4-kb upstream position compared with NM_004626.2 RefSeq. DA812463.1, AW009544.1, and AW294719.1 ESTs transcribed from the alternative first exon are spliced to the first exon of NM_004626.2 RefSeq. To distinguish two alternative first exons of human *WNT11* gene, the first exon of NM_004626.2 RefSeq was renamed exon 2, and the alternative first exon was designated exon 1. WNT11 isoform A (NM_004626.2 RefSeq) consists of exons 2, 3, 4, 5 and 6, whereas WNT11 isoform B consists of exons 1, 2, 3, 4, 5 and 6 (Fig. 1).

Comparative genomics on mammalian WNT11 orthologs. Chimpanzee *WNT11* gene, macaque *WNT11* gene, mouse *Wnt11* gene, and rat *Wnt11* gene are located within NW_001222304.1, NW_001100387.1, AC093351.8 and NW_047561.1 genome sequences, respectively. Comparative genomic analyses of mammalian *WNT11* orthologs revealed that the *WNT11* promoter B region located at the 5'-adjacent position of exon 1, the *WNT11* promoter A region located at

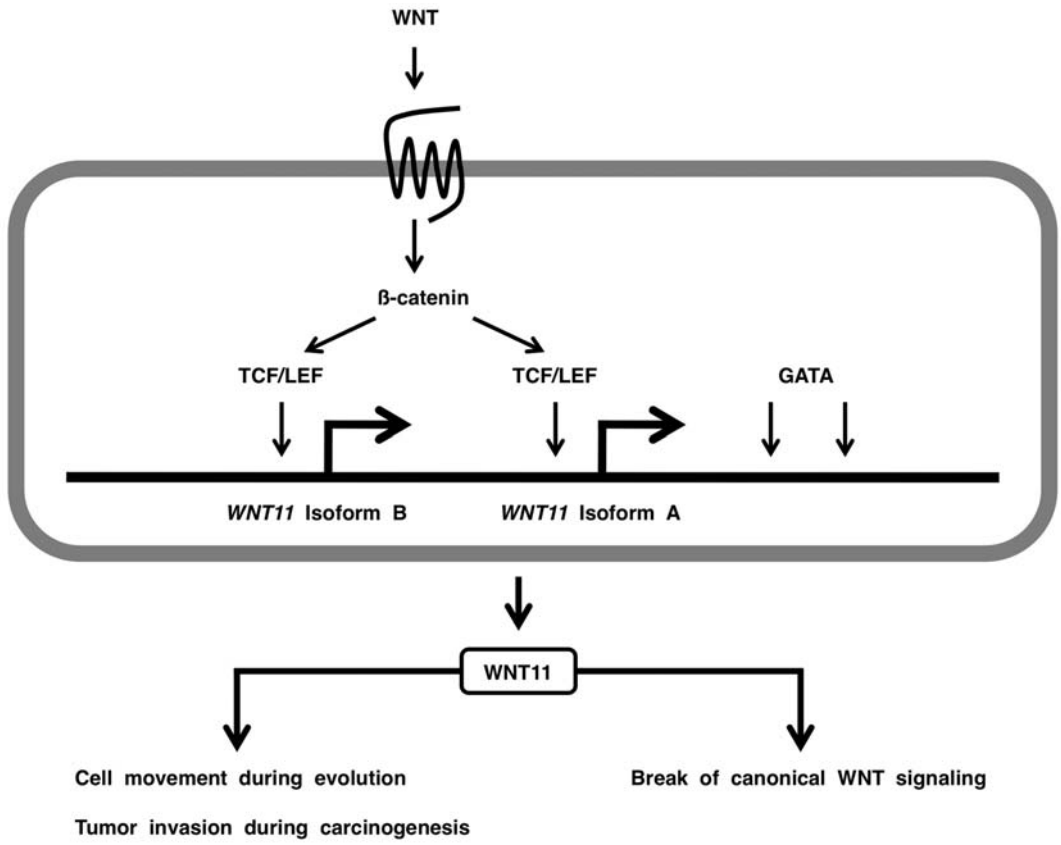

Figure 2. Canonical WNT-to-WNT11 signaling loop. Canonical WNT signals directly induce *WNT11* upregulation. WNT11 activates non-canonical WNT signaling cascades to induce cellular movement. WNT11 also activates the Ca^{2+}-MAP3K7-NLK signaling cascade to break the canonical WNT signaling. Canonical WNT-to-WNT11 signaling loop is involved in cellular migration during embryogenesis, and tumor invasion during carcinogenesis.

the 5'-adjacent position of exon 2, and *WNT11* intron 2 were well conserved in mammalian *WNT11* orthologs (data not shown). However, the genome sequence around the *WNT11* promoter B region was absent in the NW_001222304.1 chimpanzee genome draft sequence due to sequencing gap, and the genome sequence corresponding to a part of *WNT11* intron 2 was absent in the NW_001100387.1 macaque genome draft sequence due to sequencing gap.

Conserved transcription factor-binding sites within WNT11 regulatory regions. Based on manual inspection, we identified TCF/LEF-binding sites within the promoter B region (-48-bp position from the TSS of human *WNT11* isoform B), and also within the promoter A region (-43-bp position from the TSS of human *WNT11* isoform A). We also found double GATA-binding sites within intron 2 of human *WNT11* gene (+933-bp and +5001-bp positions from TSS of human *WNT11* isoform A). Double TCF/LEF- and double GATA-binding sites within the regulatory regions of human *WNT11* gene were conserved in other mammalian *WNT11* orthologs (Fig. 1).

Canonical WNT signals involved in WNT11 upregulation. Lin *et al* reported that ß-catenin is required for *Wnt11* up-regulation in cardiac progenitors based on the observation in conditional *Ctnnb1* knockout mice (58). Ueno *et al* reported that recombinant Wnt3a protein induces *Wnt11* upregulation in embryoid bodies derived from mouse embryonic stem (ES) cells (42). Gros *et al* reported that electroporation of plasmid containing activated form of ß-catenin cDNA in the lateral domain of newly formed somites induces *Wnt11* upregulation in the lateral somites (59). Together these facts indicate that the canonical WNT signals are involved in *WNT11* upregulation during embryogenesis; however, precise mechanism of *WNT11* upregulation by the canonical WNT signals remained unclear.

In this study, we identified conserved TCF/LEF-binding sites within proximal promoter region of *WNT11* orthologs (Fig. 1). Based on these facts, it was concluded that the canonical WNT signals directly upregulate *WNT11* transcription (Fig. 2).

GATA family members involved in WNT11 upregulation. Afouda *et al* reported that gata4 and gata6 are involved in *wnt11* upregulation during *Xenopus* cardiogenesis (60). Afouda *et al* suggested that *wnt11* is a direct target of gata family members, because gata-induced *wnt11* upregulation is resistant to cycloheximide treatment. However, precise mechanism of *WNT11* upregulation by GATA family members remained unclear.

In this study, we identified conserved GATA-binding sites within intron2 of *WNT11* orthologs (Fig. 1). Based on these facts, it was concluded that GATA family members directly upregulate *WNT11* transcription.

Other regulatory signaling cascades. WNT signaling cascades cross-talk with Hedgehog, Notch, FGF/RTK, and TGFß/BMP signaling cascades (61-65). There are several reports that Hedgehog and Notch signaling cascades are involved in *WNT11* upregulation (66-68). GLI and CSL are representative

transcription factors involved in the regulation of Hedgehog (69-71) and Notch (72-74) target genes, respectively. However, conserved GLI- or CSL-binding site was not identified within the regulatory regions of mammalian *WNT11* orthologs (data not shown). Hedgehog and Notch signaling cascades might be involved in *WNT11* transcription through indirect mechanisms.

Discussion

Refined integrative genomic analyses of *WNT11* were carried out to elucidate the mechanisms of *WNT11* transcription in this study. The *WNT11* gene at human chromosome 11q13.5 was found to encode two isoforms by using alternative first exons. WNT11 isoform A consists of exons 2, 3, 4, 5 and 6, whereas WNT11 isoform B consists of exons 1, 2, 3, 4, 5 and 6 (Fig. 1). Because the open reading frame spans exons 2-6, two WNT11 isoforms encode an identical WNT11 protein.

Canonical WNT signals and GATA family members are involved in WNT11 transcription during embryogenesis of model animals (42,58-60); however, precise mechanisms of *WNT11* transcription remained unclear. TCF/LEF-binding site within the proximal promoter regions, and double GATA-binding sites within intron 2 of human *WNT11* gene were identified in this study (Fig. 1). In addition, these TCF/LEF- and GATA-binding sites within the regulatory regions of human *WNT11* gene were conserved in other mammalian *WNT11* orthologs (Fig. 1). Together these facts indicate that *WNT11* transcription is directly upregulated by canonical WNT signals and GATA family members.

Canonical WNT signals induce *WNT11* upregulation, and then WNT11 activates non-canonical WNT signaling cascades to induce cellular movement. WNT11 also activates the Ca^{2+}-MAP3K7-NLK signaling cascade to attenuate the canonical WNT signaling. Canonical WNT-to-WNT11 signaling loop is involved in cellular migration during embryogenesis as well as tumor invasion during carcinogenesis (Fig. 2). The canonical WNT-to-WNT11 signaling loop is a potent target of cancer therapeutics, especially for the inhibition of invasion and metastasis.

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