

Transcriptional mechanisms of *WNT5A* based on NF- κ B, Hedgehog, TGF β , and Notch signaling cascades

MASUKO KATOH¹ and MASARU KATOH²

¹M&M Medical BioInformatics, Hongo 113-0033; ²Genetics and Cell Biology Section, National Cancer Center, Tokyo 104-0045, Japan

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Abstract. *WNT5A* is a cancer-associated gene involved in invasion and metastasis of melanoma, breast cancer, pancreatic cancer, and gastric cancer. *WNT5A* transduces signals through Frizzled, ROR1, ROR2 or RYK receptors to β -catenin-TCF/LEF, DVL-RhoA-ROCK, DVL-RhoB-Rab4, DVL-Rac-JNK, DVL-aPKC, Calcineurin-NFAT, MAP3K7-NLK, MAP3K7-NF- κ B, and DAG-PKC signaling cascades in a context-dependent manner. *SNAIL* (*Snail*), *CD44*, *G3BP2*, and *YAP1* are *WNT5A* target genes. We and other groups previously reported that IL6- or LIF-induced signaling through JAK-STAT3 signaling cascade is involved in *WNT5A* upregulation (STAT3-*WNT5A* signaling loop). Here, refined integrative genomic analyses of *WNT5A* were carried out to elucidate other mechanisms of *WNT5A* transcription. The *WNT5A* gene was found to encode two isoforms by using alternative first exons 1A and 1B. Quadruple Smad-binding elements (SBEs), single Sp1-binding site (GC-box), PPAR γ -binding site, C/EBP-binding site and bHLH-binding site within the promoter A region, 5'-adjacent to exon 1A, were conserved in human *WNT5A*, chimpanzee *WNT5A*, mouse *Wnt5a*, and rat *Wnt5a*. NF- κ B-binding site, CUX1-binding site, double SBEs and double GC-boxes within the promoter B region, 5'-adjacent to exon 1B, were conserved in mammalian *WNT5A* orthologs. Quadruple FOX-binding sites and double SBEs within ultra-conserved intron 1 were also conserved in mammalian *WNT5A* orthologs. Conserved NF- κ B-binding site within the *WNT5A* promoter B region elucidated the mechanisms that TNF α and toll-like receptor (TLR) signals upregulate *WNT5A* via MAP3K7. Quadruple FOX-binding sites rather than GLI-binding site revealed that Hedgehog signals induce *WNT5A* upregulation indirectly via FOX family members, such as FOXA2, FOXC2, FOXE1, FOXF1 and FOXL1. TGF β signals

were found to upregulate *WNT5A* expression directly through the Smad complex, and also indirectly through Smad-induced CUX1 and MAP3K7-mediated NF- κ B. Together these facts indicate that *WNT5A* is transcribed based on multiple mechanisms, such as NF- κ B, Hedgehog, TGF β , and Notch signaling cascades.

Introduction

WNT signaling cascades are involved in a variety of cellular processes during embryogenesis and carcinogenesis (1-4). Because biological functions of human genes and those of model-animal orthologs are not always conserved due to protein evolution and promoter evolution, we have carried out the human WNTome project for the comprehensive characterization of human genes encoding WNT signaling molecules during the genomic era before 2003 (5). Nineteen WNT family genes in the human genome are conserved in the mammalian genomes (6).

The *WNT5A* gene at human chromosome 3p14.3 (7,8) is the paralog of the *WNT5B* gene at human chromosome 12p13.33 (9). Human *WNT5A* shows 98.7% total amino-acid identity with rodent *Wnt5a* (10,11), indicating that mammalian *WNT5A* orthologs are ultra-conserved. Frizzled family members (12-14), ROR1 (15), ROR2 (16), and RYK (17) are *WNT5A* receptors. *WNT5A* signals are transduced to the β -catenin-TCF/LEF (12), DVL-RhoA-ROCK (18), DVL-RhoB-Rab4 (19), DVL-Rac-JNK (16), DVL-aPKC (20), Ca²⁺-Calcineurin-NFAT (21), Ca²⁺-MAP3K7-NLK (18,22), Ca²⁺-MAP3K7-NF- κ B (15), and DAG-PKC (23) signaling cascades in a context-dependent manner (13,24,25).

WNT5A is expressed in a variety of human tumors (Table I), including breast cancer (8,26-28), lung cancer (26,29), melanoma (26,30), osteosarcoma (26), prostate cancer (26, 31,32), endometrial uterine cancer (33,34), colorectal cancer (34-36), pancreatic cancer (37,38), gastric cancer (34), esophageal cancer (39), embryonal tumor (39), Ewing sarcoma (40), neuroblastoma (41), skin basal cell carcinoma (BCC) (42), skin squamous cell carcinoma (SCC) (43), and leukemia (44,45). *SNAIL* (*Snail*), *CD44*, *G3BP2*, and *YAP1* are *WNT5A* target genes (23). *WNT5A* is involved in invasion, peritoneal dissemination, and distant metastasis of tumor cells via RhoB and Snail (18,19,23,46).

We and other groups previously reported that IL6- or LIF-induced signaling through JAK-STAT3 signaling cascade

Correspondence to: Dr Masaru Katoh, Genetics and Cell Biology Section, National Cancer Center, 5-1-1 Tsukiji, Chuo Ward, Tokyo 104-0045, Japan
E-mail: mkatoh-kkr@umin.ac.jp

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Table I. WNT5A expression in human cancer.

Type of cancer	Notes	Author/(Refs.)
Breast cancer	Upregulation in primary tumors	Lejeune <i>et al</i> (8)
	Upregulation in primary tumors	Iozzo <i>et al</i> (26)
	Downregulation in primary tumors	Leris <i>et al</i> (27)
	Stromal expression	Pukrop <i>et al</i> (28)
Lung cancer	Upregulation in primary tumors	Iozzo <i>et al</i> (26)
	Upregulation in primary NSCLC	Huang <i>et al</i> (29)
Melanoma	Expression in a tumor cell line	Iozzo <i>et al</i> (26)
	Upregulation in primary tumors	Weeraratna <i>et al</i> (30)
Osteosarcoma	Expression in a tumor cell line	Iozzo <i>et al</i> (26)
Prostate cancer	Expression in primary tumors	Iozzo <i>et al</i> (26)
	Downregulation by BMP	Kumagai <i>et al</i> (31)
	Promoter hypomethylation	Wang <i>et al</i> (32)
Uterine cancer	Downregulation in primary tumors	Bui <i>et al</i> (33)
	Some up, others down	Saitoh <i>et al</i> (34)
Colorectal cancer	Stromal expression	Smith <i>et al</i> (35)
	Some up, others down	Saitoh <i>et al</i> (34)
	Epigenetic silencing in primary tumors	Ying <i>et al</i> (36)
Pancreatic cancer	Downregulation in primary tumors	Crnogorac-Jurcevic <i>et al</i> (37)
	Stromal expression	Pilarsky <i>et al</i> (38)
Gastric cancer	Upregulation in primary tumors	Saitoh <i>et al</i> (34)
Esophageal cancer	Upregulation in tumor cell lines	Saitoh and Katoh (39)
Embryonal tumor	Upregulation in tumor cell lines	Saitoh and Katoh (39)
Ewing sarcoma	Expression in tumor cell lines	Uren <i>et al</i> (40)
Neuroblastoma	Downregulation in primary tumors	Blanc <i>et al</i> (41)
Skin BCC	Upregulation in primary tumors	Bonifas <i>et al</i> (42)
Skin SCC	Upregulation in primary tumors	Haider <i>et al</i> (43)
Leukemia	Expression in B-ALL	Khan <i>et al</i> (44)
	Epigenetic silencing in ALL	Roman-Gomez <i>et al</i> (45)

is involved in *WNT5A* upregulation (STAT3-*WNT5A* signaling loop) (46-48). Here, refined integrative genomic analyses of *WNT5A* were carried out to elucidate the mechanisms of *WNT5A* transcription other than the STAT3-*WNT5A* signaling loop.

Materials and methods

Comparative genomic analyses. Human genome sequences corresponding to human *WNT5A* RefSeq (NM_003392.3)

were searched for by using BLAST programs, as previously described (49,50). *WNT5A* expressed sequence tags (ESTs) were also searched for to identify *WNT5A* splicing variants (51,52). Conserved transcription factor-binding sites within *WNT5A* promoters were then searched for based on manual inspection, as previously described (53,54).

Regulatory network analyses. Literature on *WNT5A*, Hedgehog, TGF β , Notch and inflammatory signaling molecules in PubMed and Medline databases was critically evaluated to

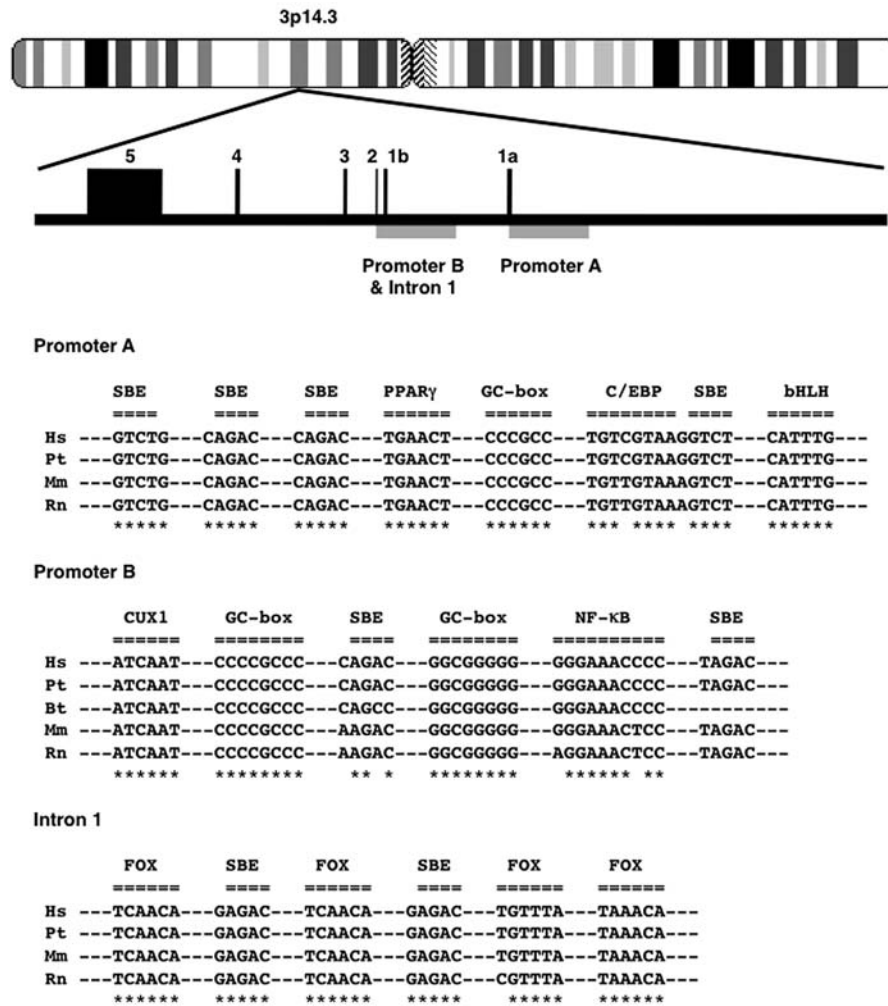


Figure 1. Integrative genomic analyses of *WNT5A*. Schematic representation of *WNT5A* gene at human chromosome 3p14.3 is shown in the upper part. *WNT5A* gene encodes two transcripts by using alternative first exons 1A and 1B. Conserved promoter and enhancer regions (gray bar) are shown. Conserved transcription factor-binding sites within *WNT5A* regulatory regions are shown in the lower part.

extract knowledge on the regulation of GLI, FOX, SMAD, and NF- κ B transcription factors. The mechanisms of *WNT5A* transcription were then investigated based on our data of conserved transcription factor-binding sites within *WNT5A* promoters and in-house knowledgebase of transcription factors regulated by the stem-cell signaling network.

Results

WNT5A splicing variants transcribed by using alternative promoters. Human *WNT5A* gene is located within human genome sequence AC121764.2, as previously described (11). BLAST programs using the *WNT5A* genome sequence revealed that human ESTs CB988958.1, CF994133.1, DA459745.1, DA874427.1 and DB276659.1 were transcribed from exon 1, and that human ESTs DA030580.1, DA650006.1 and DB224740.1 were transcribed from alternative first exon located between exon 1 and exon 2. To distinguish two alternative first exons of human *WNT5A* gene, exon 1 was renamed exon 1a, and alternative first exon was designated exon 1b (Fig. 1). *WNT5A* isoform A (NM_003392.3 RefSeq) consists of exons 1a, 2, 3, 4 and 5, whereas *WNT5A* isoform B (AK290869.1 cDNA) consists of exons 1b, 2, 3, 4 and 5.

Comparative genomics on mammalian WNT5A orthologs. Rat *Wnt5a* gene is located within AC095764.5 incomplete genome sequence with gaps, as previously described (11). To our surprise, AC095764.5 draft sequence had not been replaced by complete genome sequence since September 22, 2002. BLAST programs next revealed that chimpanzee *WNT5A* gene and mouse *Wnt5a* gene were located within NW_001232823.1 draft sequence and CT025649.7 complete sequence, respectively (data not shown).

Comparative genomic analyses of mammalian *WNT5A* orthologs revealed that the *WNT5A* promoter A region located at the 5'-adjacent position of exon 1a, the *WNT5A* promoter B region located at the 5'-adjacent position of exon 1b and *WNT5A* intron 1 located between exon 1b and exon 2 were well conserved in mammalian *WNT5A* orthologs (Fig. 1).

Conserved transcription factor-binding sites within WNT5A regulatory regions. We previously identified the evolutionary conservation between the promoter A region of human *WNT5A* gene and that of rat *Wnt5a* gene (11), whereas Danielson *et al* identified putative transcription factor-binding sites within the promoter B of human *WNT5A* gene without any information on evolutionary conservation (7). Because database of

mammalian genome sequences and knowledgebase of transcription factor-binding sites have been quantitatively and qualitatively improved during the past three years, refined integrative genomic analyses of *WNT5A* regulatory regions were next carried out.

WNT5A promoter A without TATA-box is predicted to show 'broad peak' pattern of transcription start sites (TSSs). We utilized the most 5'-TSS of *WNT5A* in our previous study (11); however, TSS of NM_003392.3 RefSeq was 338-bp 3'-position compared with the most 5'-TSS. Based on manual inspection of the promoter A region, we found that PPAR γ -binding site, Sp1-binding site (GC-box), C/EBP-binding site, bHLH-binding site and quadruple Smad-binding elements (SBEs) within the promoter A region were completely conserved in human *WNT5A*, chimpanzee *WNT5A*, mouse *Wnt5a*, and rat *Wnt5a* genes (Fig. 1).

WNT5A promoter B was less conserved compared with *WNT5A* promoter A (data not shown). CUX1 (CUTL1)-binding site, double SBEs, and double GC-boxes within the promoter B region were completely conserved in human *WNT5A*, chimpanzee *WNT5A*, mouse *Wnt5a*, and rat *Wnt5a* genes (Fig. 1). NF- κ B-binding site within the promoter B region was completely conserved in human *WNT5A*, chimpanzee *WNT5A*, and mouse *Wnt5a* genes, and was almost conserved in rat *Wnt5a* gene except one-base substitution (Fig. 1). Because rat genome sequence AC095764.5 was incomplete as mentioned above, we next searched for the existence of the NF- κ B-binding site within other mammalian *WNT5A* orthologs, and found that the NF- κ B-binding site was also conserved in the cow *WNT5A* ortholog (Fig. 1).

WNT5A intron 1 was ultra-conserved among mammals. For example, intron 1 of human *WNT5A* gene showed 87.9% total nucleotide identity with that of mouse *Wnt5a* gene (data not shown). Manual inspection of transcription factor-binding sites then revealed that quadruple FOX-binding sites and double SBEs were completely conserved in human *WNT5A*, chimpanzee *WNT5A*, and mouse *Wnt5a* genes (Fig. 1). Most of these transcription factor-binding sites, except the third FOX-binding site, were also completely conserved in the rat *Wnt5a* gene (Fig. 1).

Conserved NF- κ B-binding site, FOX-binding sites, SBEs and CUX1-binding site within the regulatory regions of *WNT5A* orthologs were novel findings in this study (Fig. 1).

NF- κ B signaling cascades and *WNT5A*. Chronic inflammation is involved in tumor progression via activation of STAT3 and NF- κ B transcription factors (46,48,55-59). We have previously reported the mechanisms of IL6- or LIF-induced *WNT5A* upregulation via STAT3 (46), and McCall *et al* reported the involvement of the IL6-STAT3 signaling loop in *WNT5A* upregulation in papillary thyroid carcinoma (48). Mechanisms of NF- κ B-mediated *WNT5A* upregulation will be described in this section.

TNF receptors (TNFRs), Toll-like receptors (TLRs), IL1 receptors (IL1Rs), and TGF β receptors (TGF β Rs) are involved in MAP3K7-mediated IKK activation (58). IKK then phosphorylates I κ B to induce ubiquitin-mediated degradation, which results in stabilization and activation of NF- κ B (56). We reported TNF α -induced *WNT5A* upregulation in gastric cancer cells in 2002 (39). Blumenthal *et al* reported TLR-mediated

NF- κ B activation and *WNT5A* upregulation in macrophages in 2006 (60). Because NF- κ B-binding site within *WNT5A* promoter B was conserved in mammals (Fig. 1), it was concluded that *WNT5A* is upregulated by TNF α , TLR, IL1, and TGF β signaling activation via NF- κ B (Fig. 2).

Hedgehog signaling cascades and *WNT5A*. Hedgehog signals are transduced through Patched family receptors and Smoothed signal transducer to GLI transcriptional activators (61-63). Hedgehog signals are activated in gastric cancer (64,65), basal cell carcinoma (66), and other tumors. *WNT5A* is expressed in gastric cancer (34), basal cell carcinoma (42), and other tumors, as mentioned above. Due to the co-existence of Hedgehog signaling activation and *WNT5A* expression in human tumors, causal relationship between Hedgehog signaling activation and *WNT5A* expression was investigated.

Consensus GLI-binding site was not located within *WNT5A* promoters and *WNT5A* gene (data not shown). *FOXA2*, *FOXC2*, *FOXE1*, *FOXF1* and *FOXLI* are direct target genes of Hedgehog-GLI signaling cascade (63), and quadruple FOX-binding sites within human *WNT5A* intron 1 were conserved in chimpanzee *WNT5A*, cow *WNT5A*, and mouse *Wnt2b* (Fig. 1). Together these facts indicate that Hedgehog signals induce *WNT5A* upregulation indirectly through FOX family members (Fig. 2).

TGF β signaling cascades and *WNT5A*. TGF β signals are transduced through receptor-type serine/threonine kinases to Smad2/3-Smad4 transcriptional complex and MAP3K7-NF- κ B signaling cascade (67,68). Smad2/3-Smad4 complex, binding to the SBE of target genes, is associated with other transcription factors, such as Sp1 and ETS for transcriptional regulation of TGF β -target genes. TGF β -induced upregulation of *CDNK1A* (*P16*) and *CDKN2B* (*P15*) is involved in growth inhibition of tumor cells, whereas TGF β -induced upregulation of EMT regulators is involved in invasion, peritoneal dissemination, and distant metastasis of tumor cells. Roarty and Serra reported that TGF β -induced *Wnt5a* upregulation is required for proper mammary gland development (69). Ripka *et al* reported that TGF β induces *WNT5A* upregulation via CUX1 (70); however, CUT1-binding site within the *WNT5A* regulatory regions remained unclear. Causal link between TGF β signaling activation and *WNT5A* expression was next investigated.

Quadruple SBEs within the promoter A region, double SBEs within the promoter B region, and double SBEs within intron 1 were conserved in mammalian *WNT5A* orthologs; single GC-box within the promoter A region and double GC-boxes within the promoter B region were conserved in mammalian *WNT5A* orthologs; single CUX1-binding site within the promoter B region was conserved in mammalian *WNT5A* orthologs (Fig. 1). In addition, NF- κ B-binding site within the promoter B region was conserved in mammalian *WNT5A* orthologs, as discussed above. Together these facts indicate that TGF β signals upregulate *WNT5A* expression directly through the Smad complex, and also indirectly through Smad-induced CUX1 and MAP3K7-mediated NF- κ B (Fig. 2).

Notch signaling cascades and *WNT5A*. Notch ligands-induced Notch signaling activation leads to transcriptional activation of Notch target genes via NICD-CSL complex or NICD-

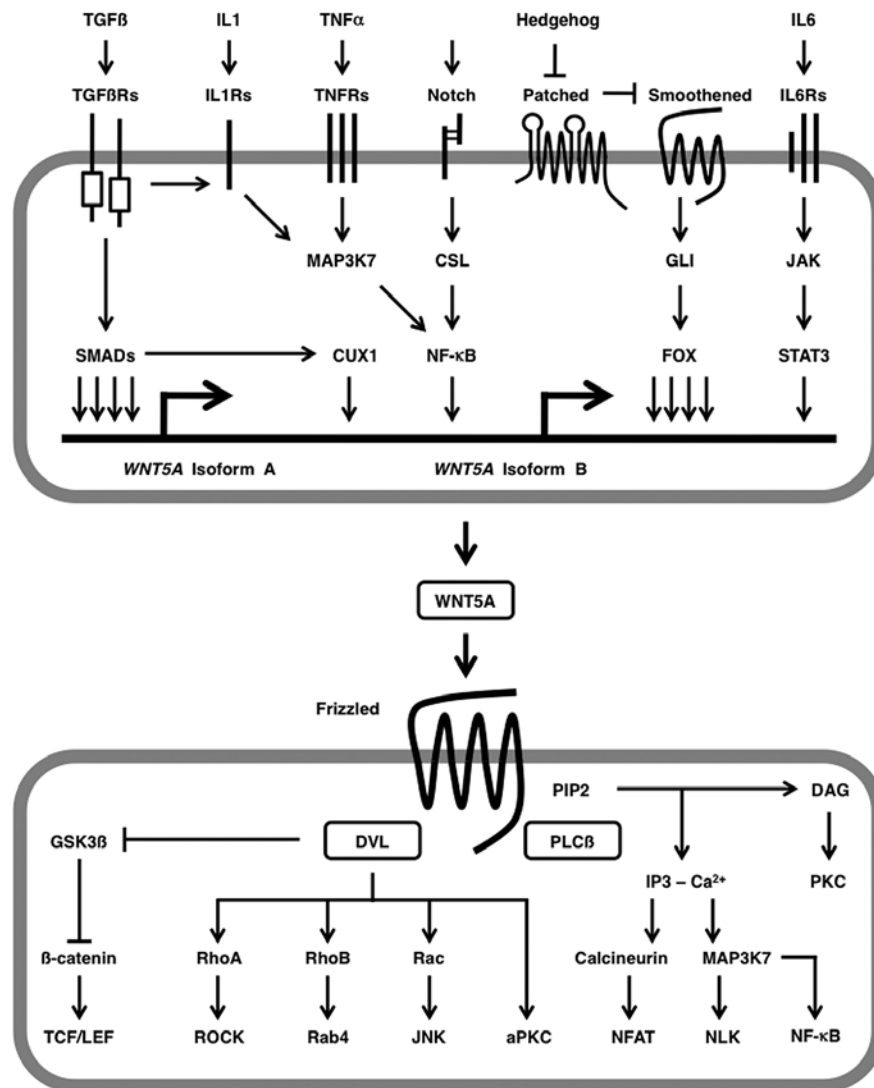


Figure 2. WNT5A at the cross roads of regulatory signaling cascades. Transcriptional mechanisms of *WNT5A* are shown in the upper part, and WNT5A signaling cascades are shown in the lower part.

NF-κB complex (71-73). Koyanagi *et al* reported Notch ligand-induced WNT5A upregulation in endothelial progenitor cells (74); however, the mechanism of Notch-mediated WNT5A upregulation remained to be elucidated. Manual inspection in this study revealed that consensus CSL-binding site within WNT5A regulatory regions was not conserved in mammals (data not shown). On the other hand, NF-κB-binding site within the promoter B region was conserved in mammalian WNT5A orthologs, as discussed above. Together these facts indicate that Notch signals upregulate WNT5A expression via the NICD-NF-κB complex (Fig. 2).

Discussion

Refined integrative genomic analyses of WNT5A orthologs were carried out in this study. The WNT5A gene was found to encode two transcripts by using alternative first exons 1A and 1B (Fig. 1). Comparative genomic analyses revealed that the promoter A region, promoter B region, and intron 1 were well conserved in mammalian WNT5A orthologs (Fig. 1).

Transcription factor-binding sites within the conserved WNT5A regulatory regions were searched for in this study. Quadruple SBEs, single GC-box, PPARγ-binding site, C/EBP-binding site, and bHLH-binding site within the promoter A region were conserved in human WNT5A, chimpanzee WNT5A, mouse *Wnt5a*, and rat *Wnt5a*; NF-κB-binding site, CUX1-binding site, double SBEs and double GC-boxes within the promoter B region were conserved in mammalian WNT5A orthologs; quadruple FOX-binding sites and double SBEs within ultra-conserved intron 1 were also conserved in the mammalian WNT5A orthologs (Fig. 1).

Conserved NF-κB-binding site within WNT5A promoter B region elucidated the mechanisms that TNFα and TLR signals upregulate WNT5A via MAP3K7. Quadruple FOX-binding sites within the WNT5A enhancer region and absence of GLI-binding site around and within the WNT5A gene revealed that Hedgehog signals induce WNT5A upregulation indirectly via FOX family members rather than directly via GLI activators. TGFβ signals were found to upregulate WNT5A expression directly through the Smad complex, and

also indirectly through Smad-induced CUX1 and MAP3K7-mediated NF- κ B. Together these facts indicate that *WNT5A* is transcribed due to multiple mechanisms, such as NF- κ B, Hedgehog, TGF β , and Notch signaling activation.

WNT signaling cascades cross-talk with FGF, Notch, Hedgehog, and TGF β /BMP signaling cascades constituting the stem cell signaling network (75-77). Chronic inflammation is involved in carcinogenesis through tumor-stromal interaction activating STAT3 and NF- κ B signaling cascades, and also through epigenetic alterations of the stem-cell signaling molecules (55-57). Results obtained in this study clearly indicate that *WNT5A* is the key molecule at the crossroads of inflammation and carcinogenesis. Because next-generation sequence technology and peta-scale supercomputer are emerging to open up the frontier of personalized medicine (78), large scale analyses of single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) of the *WNT5A* gene in melanoma, breast cancer, lung cancer, pancreatic cancer and gastric cancer should be carried out with the stratification based on the co-existence of environmental insults, such as ultraviolet exposure, tobacco smoking, and chronic infection.

References

- Katoh M: Regulation of WNT signaling molecules by retinoic acid during neuronal differentiation in NT2 cells: Threshold model of WNT action. *Int J Mol Med* 10: 683-687, 2002.
- Gregorieff A and Clevers H: Wnt signaling in the intestinal epithelium: from endoderm to cancer. *Genes Dev* 19: 877-890, 2005.
- Mikels AJ and Nusse R: Wnts as ligands: processing, secretion and reception. *Oncogene* 25: 7461-7468, 2006.
- Chien AJ and Moon RT: WNTs and WNT receptors as therapeutic tools and targets in human disease processes. *Front Biosci* 12: 448-457, 2007.
- Katoh M: Networking of WNT, FGF, Notch, BMP, and Hedgehog signaling pathways during carcinogenesis. *Stem Cell Rev* 3: 30-38, 2007.
- Katoh M: *WNT* and *FGF* gene clusters. *Int J Oncol* 21: 1269-1273, 2002.
- Danielson KG, Pillarisetti J, Cohen IR, *et al*: Characterization of complete genomic structure of human *WNT5A* gene, functional analysis of its promoter, chromosomal mapping, and expression in early human embryogenesis. *J Biol Chem* 270: 31225-31234, 1995.
- Lejeune S, Huguet EL, Hamby A, Poulsom R and Harris AL: *Wnt5a* cloning, expression, and up-regulation in human primary breast cancers. *Clin Cancer Res* 1: 215-222, 1995.
- Saitoh T and Katoh M: Molecular cloning and characterization of human *WNT5B* on chromosome 12p13.3 region. *Int J Oncol* 19: 347-351, 2001.
- Parr BA, Shea MJ, Vassileva G and McMahon AP: Mouse *Wnt* genes exhibit discrete domains of expression in the early embryonic CNS and limb buds. *Development* 119: 247-261, 1993.
- Katoh M and Katoh M: Comparative genomics on *Wnt5a* and *Wnt5b* genes. *Int J Mol Med* 15: 749-753, 2005.
- He X, Saint-Jeannet JP, Wang Y, Nathans J, Dawid I and Varmus H: A member of the Frizzled protein family mediating axis induction by *Wnt5A*. *Science* 275: 1652-1654, 1997.
- Mikels AJ and Nusse R: Purified *Wnt5a* protein activates or inhibits β -catenin-TCF signaling depending on receptor context. *PLoS Biol* 4: e115, 2006.
- Lagerström MC and Schiöth HB: Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov* 7: 339-357, 2008.
- Fukuda T, Chen L, Endo T, *et al*: Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal antigen and receptor for *Wnt5a*. *Proc Natl Acad Sci USA* 105: 3047-3052, 2008.
- Oishi I, Suzuki H, Onishi N, *et al*: Ror2 is involved in non-canonical *Wnt5a*/JNK signalling pathway. *Genes Cells* 8: 645-654, 2003.
- Keeble TR, Halford MM, Seaman C, *et al*: Wnt receptor Ryk is required for *Wnt5a*-mediated axon guidance on contralateral side of corpus callosum. *J Neurosci* 26: 5840-5848, 2006.
- Katoh M: WNT/PCP signaling pathway and human cancer. *Oncol Rep* 14: 1583-1588, 2005.
- Witze ES, Litman ES, Argast GM, Moon RT and Ahn NG: *Wnt5a* control of cell polarity and directional movement by polarized redistribution of adhesion receptors. *Science* 320: 365-369, 2008.
- Zhang X, Zhu J, Yang GY, *et al*: Dishevelled promotes axon differentiation by regulating aPKC. *Nat Cell Biol* 9: 743-754, 2007.
- Dejmek J, Säfholm A, Kamp Nielsen C, Andersson T and Leandersson K: *Wnt5a*/Ca²⁺-induced NFAT activity is counteracted by *Wnt5a*/Yes-Cdc42-CK1 α signaling in human mammary epithelial cells. *Mol Cell Biol* 26: 6024-6036, 2006.
- Ishitani T, Kishida S, Hyodo-Miura J, *et al*: TAK1-NLK cascade functions in *Wnt5a*/Ca²⁺ pathway. *Mol Cell Biol* 23: 131-139, 2003.
- Dissanayake SK, Wade M, Johnson CE, *et al*: The *Wnt5A*/protein kinase C pathway mediates motility in melanoma cells via the inhibition of metastasis suppressors and initiation of an epithelial to mesenchymal transition. *J Biol Chem* 282: 17259-17271, 2007.
- Swain RK, Katoh M, Medina A and Steinbeisser H: *Xenopus* frizzled-4S, a splicing variant of Xfz4, is a context-dependent activator and inhibitor of Wnt/ β -catenin signaling. *Cell Commun Signal* 3: 12, 2005.
- Katoh M and Katoh M: WNT signaling pathway and stem cell signaling network. *Clin Cancer Res* 13: 4042-4045, 2007.
- Iozzo RV, Eichstetter I and Danielson KG: Aberrant expression of the growth factor *Wnt5A* in human malignancy. *Cancer Res* 55: 3495-3499, 1995.
- Leris AC, Roberts TR, Jiang WG, Newbold RF and Mokbel K: *WNT5A* expression in human breast cancer. *Anticancer Res* 25: 731-734, 2005.
- Pukrop T, Klemm F, Hagemann T, *et al*: *Wnt5a* signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proc Natl Acad Sci USA* 103: 5454-5459, 2006.
- Huang CL, Liu D, Nakano J, *et al*: *Wnt5a* expression is associated with the tumor proliferation and the stromal vascular endothelial growth factor: an expression in non-small-cell lung cancer. *J Clin Oncol* 23: 8765-8773, 2005.
- Weeraratna AT, Jiang Y, Hostetter G, *et al*: *Wnt5a* signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell* 1: 279-288, 2002.
- Kumagai T, Tomari K, Shimizu T and Takeda K: Alteration of gene expression in response to BMP2 in androgen-dependent human prostate cancer LNCaP cells. *Int J Mol Med* 17: 285-291, 2006.
- Wang Q, Williamson M, Bott S, *et al*: Hypomethylation of *WNT5A*, *CRIP1* and *S100P* in prostate cancer. *Oncogene* 26: 6560-6565, 2007.
- Bui TD, Zhang L, Rees MC, Bicknell R and Harris AL: Expression and hormone regulation of *Wnt2*, 3, 4, 5a, 7a, 7b and 10b in normal human endometrium and endometrial carcinoma. *Br J Cancer* 75: 1131-1136, 1997.
- Saitoh T, Mine T and Katoh M: Frequent up-regulation of *WNT5A* mRNA in primary gastric cancer. *Int J Mol Med* 9: 515-519, 2002.
- Smith K, Bui TD, Poulsom R, Kaklamanis L, Williams G and Harris AL: Up-regulation of macrophage *Wnt* gene expression in adenoma-carcinoma progression of human colorectal cancer. *Br J Cancer* 81: 496-502, 1999.
- Ying J, Li H, Yu J, *et al*: *WNT5A* exhibits tumor-suppressive activity through antagonizing the Wnt/ β -catenin signaling, and is frequently methylated in colorectal cancer. *Clin Cancer Res* 14: 55-61, 2008.
- Crnogorac-Jurcevic T, Efthimiou E, Capelli P, *et al*: Gene expression profiles of pancreatic cancer and stromal desmoplasia. *Oncogene* 20: 7437-7446, 2001.
- Pilarsky C, Ammerpohl O, Sipos B, *et al*: Activation of Wnt signalling in stroma from pancreatic cancer identified by gene expression profiling. *J Cell Mol Med* 12: 2823-2835, 2008.
- Saitoh T and Katoh M: Expression and regulation of *WNT5A* and *WNT5B* in human cancer. *Int J Mol Med* 10: 345-349, 2002.
- Uren A, Wolf V, Sun YF, Azari A, Rubin JS and Toretsky JA: *Wnt*/Frizzled signaling in Ewing sarcoma. *Pediatr Blood Cancer* 43: 243-249, 2004.

41. Blanc E, Le Roux G, Bénard J and Raguénez G: Low expression of *Wnt5a* gene is associated with high-risk neuroblastoma. *Oncogene* 24: 1277-1283, 2005.
42. Bonifas JM, Pennypacker S, Chuang PT, *et al*: Activation of expression of Hedgehog target genes in basal cell carcinomas. *J Invest Dermatol* 116: 739-742, 2001.
43. Haider AS, Peters SB, Kaporis H, *et al*: Genomic analysis defines a cancer-specific gene expression signature for human SCC. *J Invest Dermatol* 126: 869-881, 2006.
44. Khan NI, Bradstock KF and Bendall LJ: Activation of Wnt/ β -catenin pathway mediates growth and survival in B-cell progenitor ALL. *Br J Haematol* 138: 338-348, 2007.
45. Roman-Gomez J, Jimenez-Velasco A, Cordeu L, *et al*: *WNT5A* is inactivated by aberrant methylation in ALL. *Eur J Cancer* 43: 2736-2746, 2007.
46. Katoh M and Katoh M: STAT3-induced WNT5A signaling loop in embryonic stem cells, adult normal tissues, chronic persistent inflammation, rheumatoid arthritis and cancer. *Int J Mol Med* 19: 273-278, 2007.
47. Fujio Y, Matsuda T, Ohshima Y, *et al*: Signals through gp130 upregulate *Wnt5a* and contribute to cell adhesion in cardiac myocytes. *FEBS Lett* 573: 202-206, 2004.
48. McCall KD, Harii N, Lewis CJ, *et al*: High basal levels of functional TLR3 and noncanonical *Wnt5a* are expressed in papillary thyroid cancer and are coordinately decreased by phenylmethimazole together with cell proliferation and migration. *Endocrinology* 148: 4226-4237, 2007.
49. Katoh Y and Katoh M: Conserved POU-binding site linked to SP1-binding site within *FZD5* promoter. *Int J Oncol* 30: 751-755, 2007.
50. Katoh M and Katoh M: Comparative integromics on *FZD7* orthologs. *Int J Mol Med* 19: 529-533, 2007.
51. Katoh Y and Katoh M: Comparative genomics on *PROM1* gene encoding stem cell marker CD133. *Int J Mol Med* 19: 967-970, 2007.
52. Katoh M and Katoh M: Conserved POU/OCT- and GATA-binding sites in 5'-flanking promoter region of mammalian *WNT8B* orthologs. *Int J Oncol* 30: 1273-1277, 2007.
53. Katoh M and Katoh M: Comparative integromics on non-canonical WNT or planar cell polarity signaling molecules. *Int J Mol Med* 20: 405-409, 2007.
54. Katoh Y and Katoh Y: Integrative genomic analyses on *GLI2*. *Int J Oncol* 33: 881-886, 2008.
55. Clevers H: At the crossroads of inflammation and cancer. *Cell* 118: 671-674, 2004.
56. Karin M: NF- κ B in cancer development and progression. *Nature* 441: 431-436, 2006.
57. Katoh M: Dysregulation of stem cell signaling network due to germline mutation, SNP, *Helicobacter pylori* infection, epigenetic change, and genetic alteration in gastric cancer. *Cancer Biol Ther* 6: 832-839, 2007.
58. Hayden MS and Ghosh S: Shared principles in NF- κ B signaling. *Cell* 132: 344-362, 2008.
59. Mantovani A, Allavena P, Sica A and Balkwill F: Cancer-related inflammation. *Nature* 454: 436-444, 2008.
60. Blumenthal A, Ehlers S, Lauber J, *et al*: The Wingless homolog WNT5A and its receptor Frizzled-5 regulate inflammatory responses of human mononuclear cells induced by microbial stimulation. *Blood* 108: 965-973, 2006.
61. Beachy PA, Karhadkar SS and Berman DM: Tissue repair and stem cell renewal in carcinogenesis. *Nature* 432: 324-331, 2004.
62. Hooper JF and Scott MP: Communicating with Hedgehogs. *Nature Rev Mol Cell Biol* 6: 306-317, 2005.
63. Katoh Y and Katoh M: Hedgehog signaling, epithelial-to-mesenchymal transition, and miRNA. *Int J Mol Med* 22: 271-275, 2008.
64. Berman DM, Karhadkar SS, Maitra A, *et al*: Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumors. *Nature* 425: 846-851, 2003.
65. Katoh Y and Katoh M: Hedgehog signaling in gastric cancer. *Cancer Biol Ther* 4: 1050-1054, 2005.
66. Epstein EH: Basal cell carcinomas: attack of the Hedgehog. *Nat Rev Cancer* 8: 743-754, 2008.
67. Bierie B and Moses HL: Tumour microenvironment: TGF β : the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer* 6: 506-520, 2006.
68. Massagué J: TGF β in cancer. *Cell* 134: 215-230, 2008.
69. Roarty K and Serra R: *Wnt5a* is required for proper mammary gland development and TGF- β -mediated inhibition of ductal growth. *Development* 134: 3929-3939, 2007.
70. Ripka S, König A, Buchholz M, *et al*: WNT5A - target of CUTL1 and potent modulator of tumor cell migration and invasion in pancreatic cancer. *Carcinogenesis* 28: 1178-1187, 2007.
71. Artavanis-Tsakonas S, Rand MD and Lake RJ: Notch signaling: cell fate control and signal integration in development. *Science* 284: 770-776, 1999.
72. Radtke F and Raj K: The role of Notch in tumorigenesis: oncogene or tumor suppressor? *Nat Rev Cancer* 3: 765-767, 2003.
73. Katoh M and Katoh M: Notch signaling in gastrointestinal tract. *Int J Oncol* 30: 247-251, 2007.
74. Koyanagi M, Bushoven P, Iwasaki M, Urbich C, Zeiher AM and Dimmeler S: Notch signaling contributes to the expression of cardiac markers in human circulating progenitor cells. *Circ Res* 101: 1139-1145, 2007.
75. Katoh M: WNT signaling in stem cell biology and regenerative medicine. *Curr Drug Targets* 9: 565-570, 2008.
76. Bailey J, Singh PK and Hollingsworth MA: Cancer metastasis facilitated by developmental pathways: Sonic hedgehog, Notch, and bone morphogenetic proteins. *J Cell Biochem* 102: 829-839, 2007.
77. Katoh M: Cancer genomics and genetics of *FGFR2*. *Int J Oncol* 33: 233-237, 2008.
78. Katoh M: Demand for user-friendly 'omics' web service utilizing next-generation supercomputer in the sequencing era. *Mol Cancer Genet* 1: 1-2, 2008.