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ORIGINAL ARTICLE

Basic Study

Gene expression and pathway analysis of *CTNNB1* in cancer and stem cells

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Informed consent statement: Written informed consent from each patient were obtained.

Conflict-of-interest statement: To the best of the authors' knowledge, no conflict of interest exists.

Data sharing statement: The microarray data for mesenchymal stem cells and diffuse-type gastric cancer are available to the public in NCBI's Gene Expression Omnibus (GEO) database and are accessible via GEO Series accession number GSE7888 and GSE42252, respectively.

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Abstract

AIM

To investigate β-catenin (CTNNB1) signaling in cancer and stem cells, the gene expression and pathway were analyzed using bioinformatics.

METHODS

The expression of the catenin β 1 (*CTNNB1*) gene, which codes for β-catenin, was analyzed in mesenchymal stem cells (MSCs) and gastric cancer (GC) cells. Beta-catenin signaling and the mutation of related proteins were also analyzed using the cBioPortal for Cancer Genomics and HOMology modeling of Complex Structure (HOMCOS) databases.

RESULTS

The expression of the *CTNNB1* gene was up-regulated in GC cells compared to MSCs. The expression of EPH receptor A8 (EPHA8), synovial sarcoma translocation chromosome 18 (SS18), interactor of little elongation

complex ELL subunit 1 (ICE1), patched 1 (PTCH1), mutS homolog 3 (MSH3) and caspase recruitment domain family member 11 (CARD11) were also shown to be altered in GC cells in the cBioPortal for Cancer Genomics analysis. 3D complex structures were reported for E-cadherin 1 (CDH1), lymphoid enhancer binding factor 1 (LEF1), transcription factor 7 like 2 (TCF7L2) and adenomatous polyposis coli protein (APC) with β-catenin.

CONCLUSION

The results indicate that the epithelial-mesenchymal transition (EMT)-related gene *CTNNB1* plays an important role in the regulation of stem cell pluripotency and cancer signaling.

Key words: β-catenin; CTNNB1; Epithelial-mesenchymal transition; Mesenchymal stem cell; Stem cell

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Core tip: β-catenin signaling consists of several pathway cascades, such as those that are involved in pluripotent stem cell generation and cancer. Several genes, including *EPHA8*, *SS18*, *ICE1*, *PTCH1*, *MSH3* and *CARD11*, are mutated along with *CTNNB1*. The expression of the *CTNNB1*, *CDH1*, *MYC*, *LEF1* and *TCF7L2* genes, which are related to the *CTNNB1* network, is up-regulated in diffuse-type GC cells compared to MSCs. 3D complex structures for β-catenin (CTNB1_HUMAN) with LEF_MOUSE and TF7L2_HUMAN were found using the HOMCOS database. The EMT-related gene *CTNNB1* plays an important role in pluripotent stem cell signaling and cancer signaling.

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INTRODUCTION

Changes in the phenotypes of cancer and stem cells are related to changes in gene expression and protein signaling. This study aims to reveal the β-catenin (*CTNNB1*) regulation in diffuse-type gastric cancer (GC) cells and mesenchymal stem cells (MSCs). Wnt/β-catenin signaling is necessary for epithelial-mesenchymal transitions (EMT)[1]. Stem cell division is strongly correlated with cancer risk, and this highlights the importance of molecular signaling in stem cells and cancer cells[2]. Epigenetics and stem cell functions are regulated by several exogenous stimuli, including cell-cell and cell-matrix interactions[3]. To ensure the safety of therapeutic stem cell applications in terms of stem cell modification, an understanding of the regulation of the stem cells and their niche is necessary[4]. In the case of bone metastasis, the tissue-specific stromal response for prostate cancer can be identified by a molecular signature for which a novel mechanism has been revealed in hematopoietic and prostate epithelial stem cell niches[5].

Cancer stem cell (CSC) maintenance requires hypoxia-inducible factor (HIF)-α transcription factors and the inhibitor of DNA binding 2 (ID2)[6]. The down-regulated expression of ID2 is associated with a poor prognosis in hepatocellular carcinoma[7].

Because the compendium of gene expression, chromosomal copy number and sequencing data from human cancer cell lines, which is called the Cancer Cell Line Encyclopedia (CCLE), has revealed that genomic data are capable of predicting anti-cancer drug sensitivity, molecular and network analyses should be carried out[8]. It has been reported that cadherin 1 (*CDH1*) is up-regulated in diffuse-type GC cells compared to MSCs[9]. However, *CDH2* was down-regulated in diffuse-type GC cells compared to MSCs; this provides a useful indicator - the ratio of *CDH2* to *CDH1* expression - to distinguish the mesenchymal and epithelial phenotypes of the cells[9]. It has been reported that catenin β 1 (*CTNNB1*) is mutated in hepatocellular carcinoma[10,11]. To further elucidate the EMT phenotype and the molecules that are involved in β-catenin signaling in cancer, the *CTNNB1* network and the β-catenin binding partners have been investigated in this report using bioinformatics tools such as microarray analysis and databases.

MATERIALS AND METHODS

Gene expression analysis of MSCs and diffuse-type GC cells

Gene expression in MSCs (n = 12) and diffuse-type GC cells (n = 5) was analyzed using Human Genome U133 Plus 2.0 microarrays, as previously described[9,12]. In brief, total RNA was purified from the cells, biotinylated and hybridized to microarrays. The signal intensity of each gene transcript was analyzed and compared between MSCs and diffuse-type GC cells. The microarray data for MSCs and diffuse-type GC cells are available to the public in NCBI's Gene Expression Omnibus (GEO) database and are accessible *via* GEO Series accession number GSE7888 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7888) and GSE42252 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE42252), respectively[9,12].

Diffuse-type GC tissues

Diffuse-type GC tissues were originally provided by the National Cancer Center Hospital after obtaining written informed consent from each patient and approval by National Cancer Center Institutional Review Board. All cancer specimens were reviewed and classified histopathologically according to the Japanese Classification of Gastric Cancer. Tissue specimens were immediately frozen with liquid nitrogen after surgical extraction, and stored at -80 ℃ until microarray analysis[9,13]. The existing data

Table 1 3D complex structures of β-catenin (CTNNB1) and interacting proteins

pdb_id	β-catenin (CTNNB1)			Proteins that interact with β-catenin					
	ChainID	Length	UniProtID	Molecule	ChainID	Length	UniProtID	Contact protein name	Regulation of gene expression in GC cells compared to MSCs
1th1	B	513	CTNB1_HUMAN	APC	D	54	APC_HUMAN	Adenomatous polyposis coli protein	Not changed/-
1qz7	A	524	CTNB1_HUMAN	AXIN1	B	17	AXN_XENLA	Axin-1	-
3sl9	B	165	CTNB1_HUMAN	BCL9	D	23	BCL9_HUMAN	B-cell CLL/ lymphoma 9 protein	-
1i7w	C	509	CTNB1_MOUSE	CDH1	D	60	CADH1_MOUSE	Cadherin-1	Up-regulated
1m1e	A	512	CTNB1_MOUSE	CTNNBIP1	B	65	CNBP1_HUMAN	Beta-catenin-interacting protein 1	-
3oux	A	503	CTNB1_MOUSE	LEF1	B	47	LEF1_MOUSE	Lymphoid enhancer-binding factor 1	Up-regulated
3tx7	A	504	CTNB1_HUMAN	NR5A2	B	218	NR5A2_HUMAN	Nuclear receptor subfamily 5 group A member 2	-
1g3j	A	439	CTNB1_HUMAN	TCF7L1	B	34	T7L1A_XENLA	Transcription factor 7-like 1-A	-
1jdh	A	508	CTNB1_HUMAN	TCF7L2	B	38	TF7L2_HUMAN	Transcription factor 7-like 2	Up-regulated
1dow	B	32	CTNB1_MOUSE	CTNNA1	A	205	CTNA1_MOUSE	Catenin alpha-1	Not changed/-
4ons	D	56	CTNB1_MOUSE	CTNNA2	C	230	CTNA2_MOUSE	Catenin alpha-2	-

already available to the public were analyzed in the article.

Analysis of cancer genomics using cBioPortal

The cancer genomics data analysis was performed relative to *CTNNB1* using the cBioPortal for Cancer Genomics (http://www.cbioportal.org)[14,15]. The term "CTNNB1" was searched in the cBioPortal for Cancer Genomics database, and a cross-cancer alteration summary was obtained for *CTNNB1*. A study on stomach adenocarcinoma was further analyzed for enrichments[16]. Genes with mutations that were enriched in samples that contained altered *CTNNB1* were selected in the cBioPortal for cancer genomics for further study.

3D complex structures

3D complex structures were searched in the HOMology modeling of COmplex Structure (HOMCOS) database (http://homcos.pdbj.org) using the search engine that was provided by the VaProS server (http://pford.info/vapros)[17]. The UniProtID "CTNB_HUMAN" was input as the query for the "searching contact molecule" field of the HOMCOS. Only close homologues (sequence identity > 95%) were selected. The complex structures that were found were superimposed using the MATRAS program[18].

Statistical analysis

The data were expressed as the mean ± SE. For the statistics, Student's *t* test was used. $P < 0.01$ was considered as statistically significant.

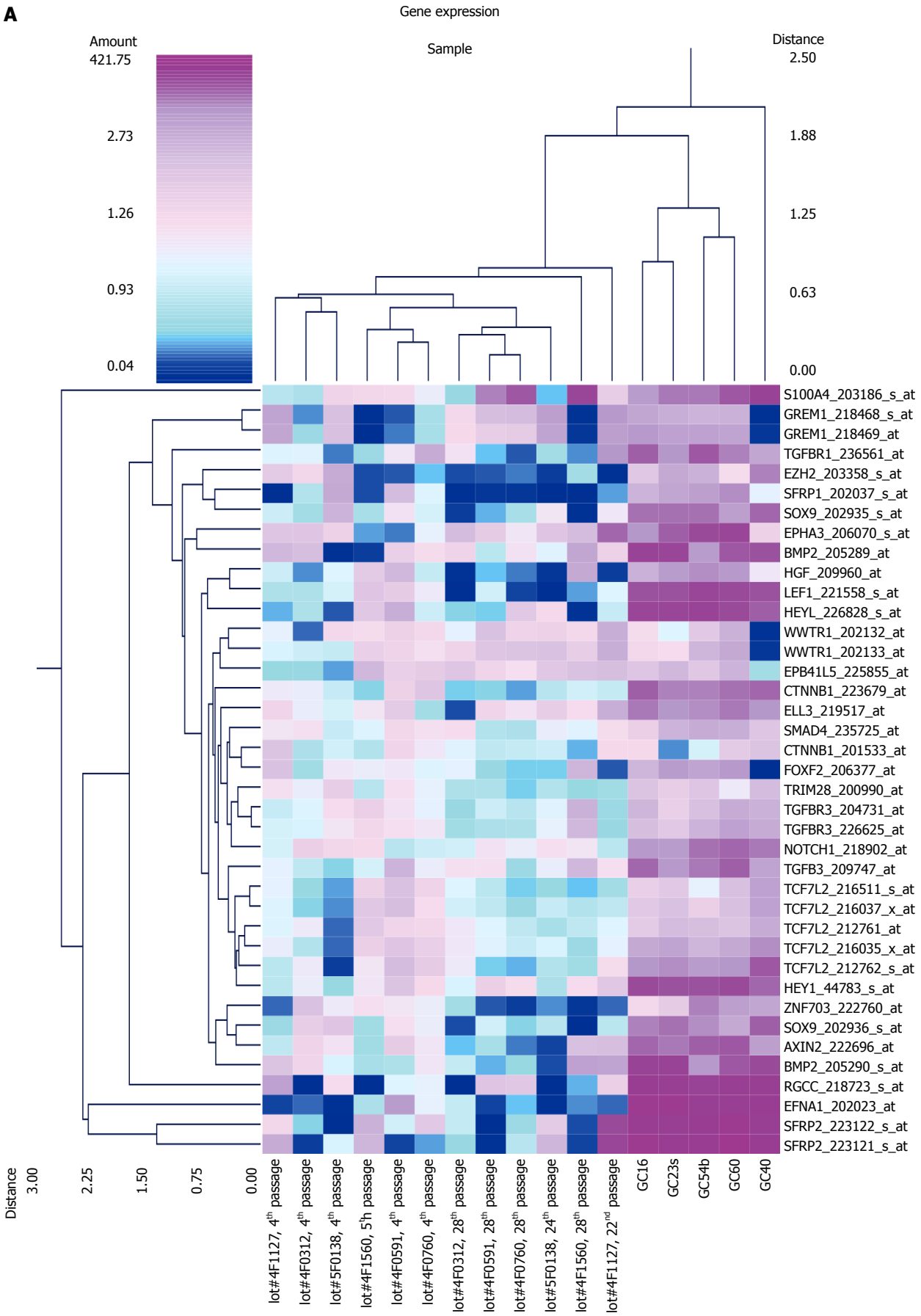
RESULTS

Expression of EMT-related genes in MSCs and diffuse-type GC cells

The expression of EMT-related genes in MSCs and diffuse-type GC cells is shown in Figure 1. The genes for which probe sets included the "EMT" term in the Gene Ontology (GO) Biological Process field were selected as EMT-related genes. The average signal intensity for early-stage MSCs, late-stage MSCs, or GC cells was greater than 500. Panel A shows the results of a cluster analysis of 39 probe sets that were up-regulated in diffuse-type GC cells compared to early-stage MSCs (n = 6 in early-stage MSCs, n = 6 in late-stage MSCs, n = 5 in GC). Panel B shows the results of a cluster analysis of 46 probe sets that were down-regulated in diffuse-type GC cells compared to early-stage MSCs (n = 6 in early-stage MSCs, n = 6 in late-stage MSCs, n = 5 in GC). To evaluate *CTNNB1* expression in cancer and stem cells, the expression of the *CTNNB1* gene was compared in MSCs and diffuse-type GC cells, and the results indicate that *CTNNB1* is up-regulated in GC cells (Figure 2). One of the probe sets was up-regulated more than 8-fold over its expression level in MSCs, whereas the other probe sets showed no increases in expression in GC cells compared to MSCs.

3D complex structures of β-catenin

To verify and explore protein-protein interactions with β-catenin, 3D complex structures of β-catenin were found using the HOMCOS database (http://homcos.pdbj.org)[17] and are summarized in Table 1. Figure 3 shows the superimposed 3D structure of the complex. Most of the proteins bind to the inner concave surface of the armadillo repeat region of β-catenin by using their 40-60 residue length extended peptides [adenomatous polyposis coli protein (APC), E-cadherin 1 (CDH1), catenin beta interacting protein 1 (CTNNBIP1), lymphoid enhancer binding factor 1 (LEF1), transcription factor 7 like 1 (TCF7L1) and transcription factor 7 like 2 (TCF7L2)].


A
Gene expression
Sample
Amount
421.75
2.73
1.26
0.93
0.04
Distance
2.50
1.88
1.25
0.63
0.00
S100A4_203186_s_at
GREM1_218468_s_at
GREM1_218469_at
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EPHA3_206070_s_at
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HEYL_226828_s_at
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WWTR1_202133_at
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CTNNB1_223679_at
ELL3_219517_at
SMAD4_235725_at
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FOXF2_206377_at
TRIM28_200990_at
TGFBR3_204731_at
TGFBR3_226625_at
NOTCH1_218902_at
TGFB3_209747_at
TCF7L2_216511_s_at
TCF7L2_216037_x_at
TCF7L2_212761_at
TCF7L2_216035_x_at
TCF7L2_212762_s_at
HEY1_44783_s_at
ZNF703_222760_at
SOX9_202936_s_at
AXIN2_222696_at
BMP2_205290_s_at
RGCC_218723_s_at
EFNA1_202023_at
SFRP2_223122_s_at
SFRP2_223121_s_at
Distance
3.00
2.25
1.50
0.75
0.00
lot#4F1127, 4th passage
lot#4F0312, 4th passage
lot#5F0138, 4th passage
lot#4F1560, 5h passage
lot#4F0591, 4th passage
lot#4F0760, 4th passage
lot#4F0312, 28th passage
lot#4F0591, 28th passage
lot#4F0760, 28th passage
lot#5F0138, 24th passage
lot#4F1560, 28th passage
lot#4F1127, 22nd passage
GC16
GC23s
GC54b
GC60
GC40

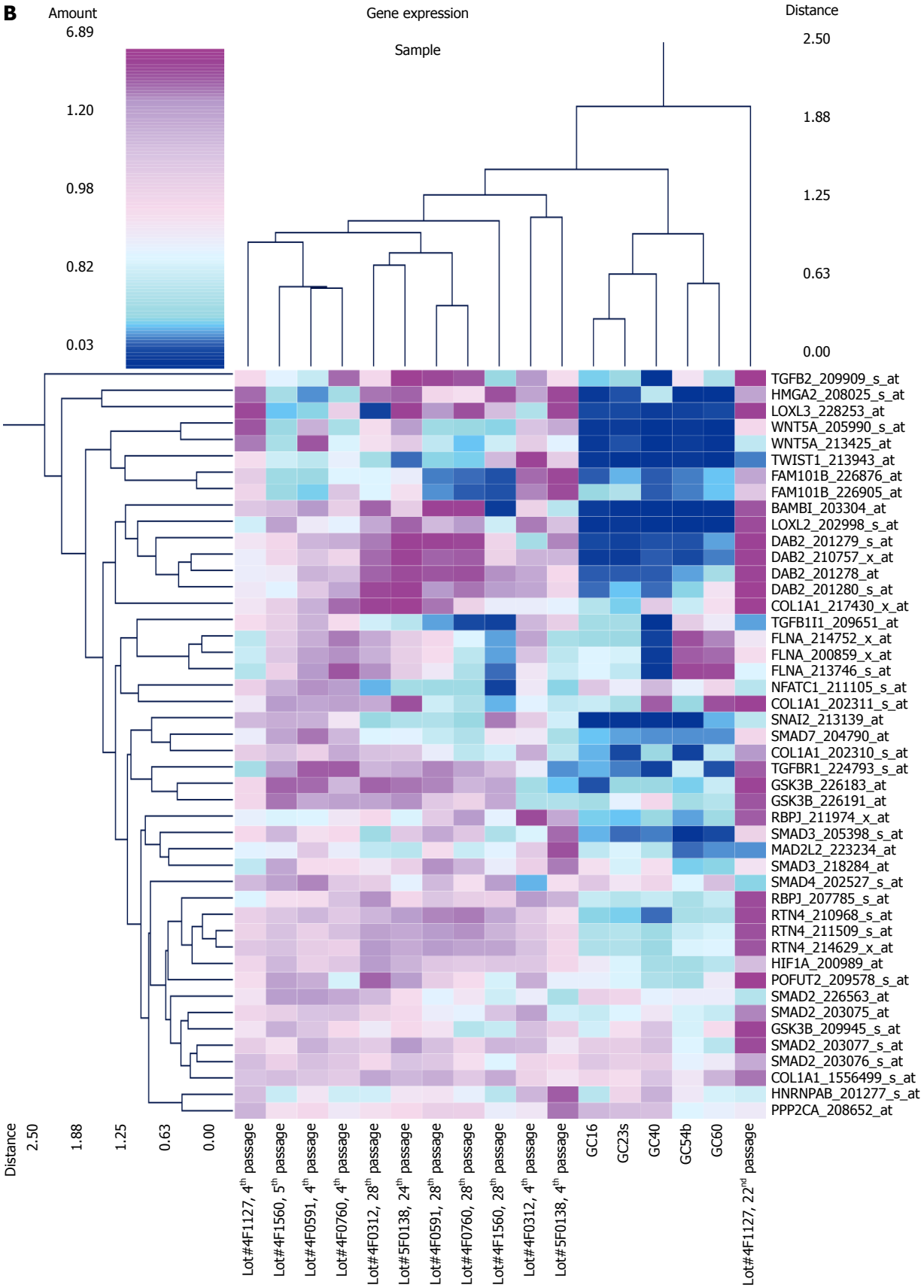

Figure 1 Expression of epithelial-mesenchymal transition-related genes in mesenchymal stem cells and diffuse-type gastric cancer cells. Cluster analysis of gene expression in mesenchymal stem cells (MSCs) and diffuse-type gastric cancer (GC) cells. A: The result of the cluster analysis of 39 probe sets that were up-regulated in diffuse-type GC cells compared to early-stage MSCs (n = 6 in early-stage MSCs, n = 6 in late-stage MSCs, n = 5 in GC); B: The result of the cluster analysis of 46 probe sets that were down-regulated in diffuse-type GC cells compared to early-stage MSCs (n = 6 in early-stage MSCs, n = 6 in late-stage MSCs, n = 5 in GC). The probe sets with epithelial to mesenchymal transition in the Gene Ontology Biological Process were selected (the average signal intensity in early-stage MSCs, late-stage MSCs, or GC cells is greater than 500).

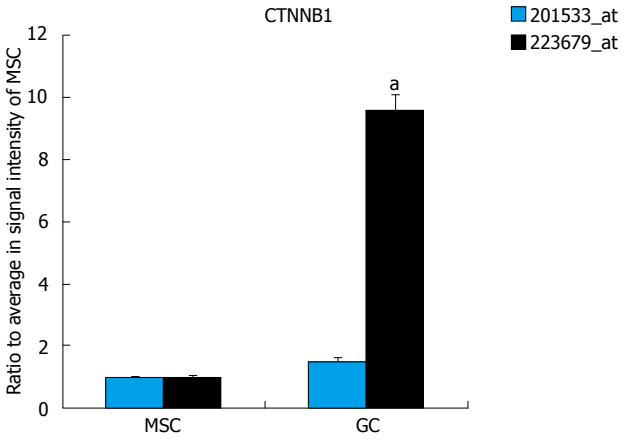

Figure 2 *CTNNB1* expression in mesenchymal stem cells and diffuse-type gastric cancer cells. *CTNNB1* gene expression was up-regulated in GC cells compared to MSCs. The signal intensity of probe set ID 223679_at was up-regulated more than 8-fold in GC cells compared to MSCs, whereas the signal intensity in probe set ID 201533_at was unchanged (n = 12 in MSC, n = 5 in GC, $^{a}P < 0.01$ in t test). GC: Gastric cancer; MSCs: Mesenchymal stem cells.

CDH1 and B-cell CLL/lymphoma 9 (BCL9) bind to the N-terminal region of the repeat that has the alpha-helical peptides. The nuclear receptor subfamily 5 group A member 2 (NR5A2) ligand binding domain binds to the middle of the armadillo repeat region. Of these binding factors, the transcription of the *CDH1*, *LEF1* and *TCF7L2* genes was up-regulated in GC cells (Table 1). It has been reported that a small molecule antagonist of the β-catenin/T-cell transcription factor 4 [TCF4; official name is transcription factor 7 like 2 (TCF7L2)] interaction inhibits self-renewal of CSCs and suppresses tumorigenesis[19]. The 3D complex structures of β-catenin and TCF7L2 are available[20,21]. The complex structure of NR5A2 has also been reported[22]. NR5A2 (or liver receptor homolog-1; LRH1) is a member of the nuclear hormone receptor family of transcription factors that play essential roles in development, metabolism, and cancer and are implicated in Wnt/β-catenin signaling[22]. NR5A2 is essential for the early development and maintenance of pluripotent mouse embryonic stem (ES) cells[22,23]. Network models for CTNNB1, the Wnt signaling pathway, Hippo signaling pathway and adherens junction signaling in cancer are shown in Figure 4. CTNNB1 binds to CDH1 near the cellular membrane or to TCF to transcribe anti-apoptotic or pro-proliferation genes, such as SRY-box 2 (*SOX2*) or v-myc avian myelocytomatosis viral oncogene homolog (*MYC*) (Figure 4). Wnt stimulation prevents glycogen synthase kinase 3 beta (GSK3β) from phosphorylating CTNNB1 and leads to CTNNB1 translocation into the nucleus to induce transcription. The 3D complex structure (PDB code: 1m1e) clearly shows how CDH1 binds to CTNNB1 in the mouse model.

CTNNB1 pathway (Kyoto Encyclopedia of Genes and Genomes)

CTNNB1 is listed in 21 pathways in Kyoto Encyclopedia of Genes and Genomes (KEGG), including the Rap1 signaling pathway, Wnt signaling pathway, Hippo signaling pathway, focal adhesion regulation, adherens junction regulation, tight junction regulation, signaling pathways that regulate the pluripotency of stem cells, leukocyte transendothelial migration, melanogenesis, the thyroid hormone signaling pathway; bacterial invasion of epithelial cells, pathogenic *Escherichia coli* infection, HTLV-I infection, and various cancer pathways. The following conditions use the aforementioned pathways and are also thus implicated: Proteoglycans in cancer, colorectal cancer, endometrial cancer, prostate cancer, thyroid cancer, basal cell carcinoma, and arrhythmogenic right ventricular cardiomyopathy (ARVC) (http://www.genome.jp/dbget-bin/www_bget?hsa:1499). The inhibition of GSK3β kinase activates β-catenin, which stimulates endoderm induction *via* the degradation of Tcf7l1 and forkhead box A2 (FoxA2) expression[24]. Wnt signaling induces intracellular β-catenin signaling *via* GSK3β kinase inhibition and dephosphorylation of β-catenin[25-28]. The inhibition of β-catenin decreases proliferation and induces apoptosis in the mantle cell lymphoma cell line[29]. Noncanonical Wnt signaling is activated in circulating tumor cells from the prostate that are anti-androgen-resistant[30].

Mutations in *CTNNB1* and related genes (cBioPortal: Stomach adenocarcinoma)

The Cancer Genome Atlas Research Network project has indicated that there is a characteristic molecular signature for ras homolog family member A (*RHOA*) mutations in diffuse type stomach adenocarcinoma[16]. Two-hundred and ninety-five primary gastric adenocarcinomas have been investigated, and mutations in *RHOA* have been enriched in genomically stable subtype, diffuse-type GC cells[16]. The analysis with cBioPortal showed that *CTNNB1* was altered in 24 (8%) of 287 cases/patients in stomach adenocarcinoma: 4 amplifications, 2 deep deletions, 12 missense mutations, 5 truncating mutations and 1 inframe mutation. Several gene mutations occurred concurrently with *CTNNB1* alterations in stomach adenocarcinoma (Table 2). The development of mutations in EPH receptor A8 (*EPHA8*), synovial sarcoma translocation chromosome 18 (*SS18*), interactor of little elongator complex ELL subunit 1 (*ICE1*), patched 1 (*PTCH1*), mutS homolog 3 (*MSH3*) and caspase recruitment domain family member 11 (*CARD11*) occurred alongside the *CTNNB1* alterations (Table 2). Of the mutated genes, *PTCH1* expression was up-regulated in GC cells compared to MSCs (Table 2). The GO of the mutated genes is shown in Table 3. EPHA8 possesses kinase activity, SS18 is involved in cell morphogenesis, ICE1 may play a role in positive regulation of intracellular protein transport, PTCH1 is involved in morphogenesis and cell growth, MSH3 is involved in mismatch repair, and CARD11 regulates B cell proliferation, apoptosis and NF-κB signaling, according to GO biological process (Table 3). GO biological process terms in Table 3 are based on Affymetrix annotation (http://www.affymetrix.com/estore/) and gene information in NCBI (http://www.ncbi.nlm.nih.gov/).

β-catenin signaling model

Several β-catenin-binding proteins, such as LEF1 or

Table 2 Genes mutated along with the *CTNNB1* alteration

Gene symbol	Gene title	Cytoband	Mutation percentage		Log ratio	P-value	Ratio of GC cells to MSCs
			In altered group	In unaltered group			
EPHA8	EPH receptor A8	1p36.12	29.17%	2.28%	3.68	1.45E-05	Signal intensity is low
SS18	Synovial sarcoma translocation Chromosome 18	18q11.2	16.67%	0.00%	> 10	3.84E-05	0.6 1.4
ICE1	Interactor of little elongator complex ELL subunit 1	5p15.32	33.33%	4.56%	2.87	4.74E-05	1.5
PTCH1	Patched 1	9q22.3	29.17%	3.42%	3.09	8.16E-05	16.6
MSH3	MutS homolog 3	5q14.1	20.83%	1.14%	4.19	1.28E-04	Signal intensity is low
CARD11	Caspase recruitment domain family, member 11	7p22	29.17%	4.18%	2.8	2.03E-04	Signal intensity is low

Table 3 Gene ontology of mutated genes along with *CTNNB1* alteration

Gene symbol	Gene ontology biological process
EPHA8	Protein phosphorylation // substrate-dependent cell migration // cell adhesion // transmembrane receptor protein tyrosine kinase signaling pathway // multicellular organismal development // nervous system development // axon guidance // phosphorylation // neuron remodeling // peptidyl-tyrosine phosphorylation // regulation of cell adhesion // neuron projection development // regulation of cell adhesion mediated by integrin // positive regulation of MAPK cascade // positive regulation of phosphatidylinositol 3-kinase activity // protein autophosphorylation // ephrin receptor signaling pathway
SS18	Microtubule cytoskeleton organization // cell morphogenesis // transcription, DNA-templated // regulation of transcription, DNA-templated // cytoskeleton organization // response to drug // positive regulation of transcription from RNA polymerase II promoter // ephrin receptor signaling pathway
ICE1	Positive regulation of intracellular protein transport // positive regulation of protein complex assembly // positive regulation of transcription from RNA polymerase III promoter // snRNA transcription from RNA polymerase II promoter // snRNA transcription from RNA polymerase III promoter
PTCH1	Negative regulation of transcription from RNA polymerase II promoter // branching involved in ureteric bud morphogenesis // neural tube formation // neural tube closure // heart morphogenesis // signal transduction // smoothened signaling pathway // smoothened signaling pathway // regulation of mitotic cell cycle // pattern specification process // brain development // negative regulation of cell proliferation // epidermis development // regulation of smoothened signaling pathway // response to mechanical stimulus // organ morphogenesis // dorsal/ventral pattern formation // response to chlorate // positive regulation of cholesterol efflux // response to organic cyclic compound // protein processing // spinal cord motor neuron differentiation // neural tube patterning // dorsal/ventral neural tube patterning // neural plate axis specification // embryonic limb morphogenesis // mammary gland development // response to estradiol // response to retinoic acid // regulation of protein localization // limb morphogenesis // hindlimb morphogenesis // regulation of growth // negative regulation of multicellular organism growth // regulation of cell proliferation // response to drug // glucose homeostasis // negative regulation of sequence-specific DNA binding transcription factor activity // keratinocyte proliferation // negative regulation of osteoblast differentiation // negative regulation of smoothened signaling pathway // negative regulation of smoothened signaling pathway // negative regulation of epithelial cell proliferation // negative regulation of cell division // pharyngeal system development // mammary gland duct morphogenesis // mammary gland epithelial cell differentiation // smoothened signaling pathway involved in dorsal/ventral neural tube patterning // cell differentiation involved in kidney development // somite development // cellular response to cholesterol // cellular response to cholesterol // renal system development // cell proliferation involved in metanephros development // protein targeting to plasma membrane
MSH3	Meiotic mismatch repair // ATP catabolic process // DNA repair // mismatch repair // cellular response to DNA damage stimulus // reciprocal meiotic recombination // somatic recombination of immunoglobulin gene segments // maintenance of DNA repeat elements // negative regulation of DNA recombination // positive regulation of helicase activity
CARD11	Positive regulation of cytokine production // signal transduction // positive regulation of B cell proliferation // T cell costimulation // Fc-epsilon receptor signaling pathway // positive regulation of T cell proliferation // regulation of apoptotic process // positive regulation of I-kappaB kinase/NF-kappaB signaling // thymic T cell selection // positive regulation of interleukin-2 biosynthetic process // innate immune response // regulation of B cell differentiation // regulation of T cell differentiation // nucleotide phosphorylation // regulation of immune response // T cell receptor signaling pathway // positive regulation of T cell activation // positive regulation of NF-kappaB transcription factor activity

TCF7L2, share high mobility group (HMG)-box domains, which suggests that β-catenin signaling switches mechanisms with the binding of different transcription factors. 3D complex structures show that CDH1, LEF1 and TCF7L2 bind to β-catenin. The role of β-catenin signaling in the pluripotentcy pathway should be investigated to reveal its mechanism in cancer and stem cells. The Wnt pathway is located upstream, and TCF, downstream of CTNNB1 in the cascade[31]. The merged network model of the β-catenin signaling network and CDH1, together with molecules in the 3D complex structures and genes mutated along with the *CTNNB1* alteration is shown in Figure 5A. The merged network model of the *CTNNB1*, *Wnt*, and *TCF* signaling networks and *CDH1*, together with molecules in the 3D complex structures and genes mutated along with the *CTNNB1* alteration is shown in Figure 5B. Of

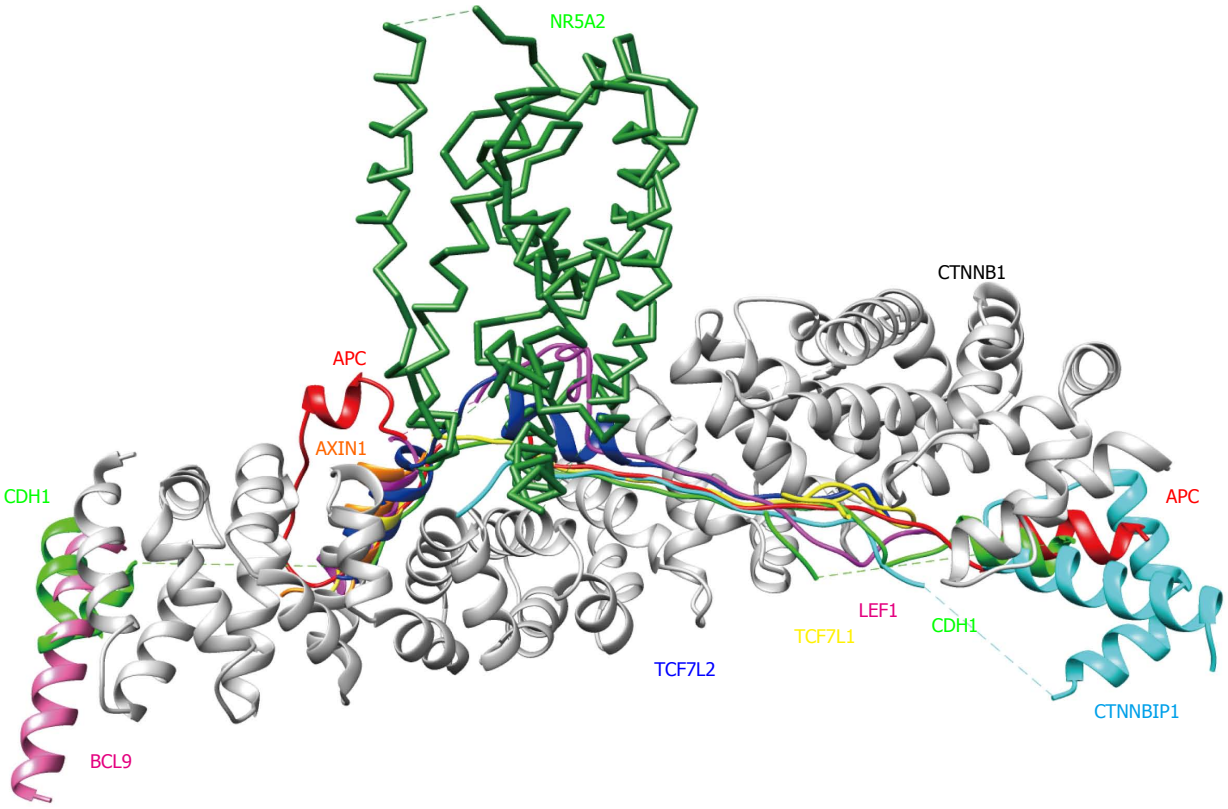

Figure 3 3D structures of β-catenin (CTNNB1) binding proteins. Each CTNNB1 complex structure was superimposed onto the CTNNB1 structure in a complex with APC (PDB code: 1th1), using the program MATRAS (From Ref. [18]). Colors and PDB codes are summarized as follows: White: CTNNB1 (CTNB1_HUMAN, 1th1); red: APC (APC_HUMAN, 1th1); orange: AXIN1 (AXN_XELNA, 1qz7); hot pink: BCL9 (BCL9_HUMAN, 3sl9); green: CDH1 (CADH1_MOUSE. 1i7w); cyan: CTNNBIP1 (CNBP1_HUMAN, 1m1e); magenta: LEF1 (LEF1_MOUSE, 3oux); forest green: NR5A2 (NR5A2_HUMAN, 3tx7); yellow: TCF7L1 (T7L1A_XENLA, 1g3j); blue: TCF7L2 (TF7L2_HUMAN, 1jdh).

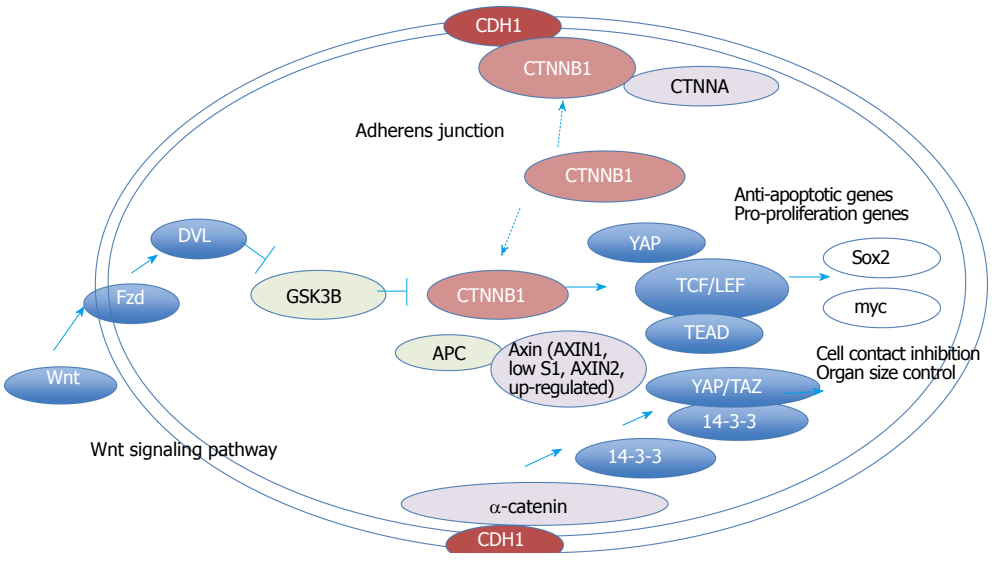

Figure 4 Network model for CTNNB1. The molecular network model for CTNNB1 signaling is shown. The extracted networks for pathways in cancer, Hippo signaling pathway and the Wnt signaling pathway (KEGG) were merged and are shown in a molecular network model. Wnt signaling and adherens junction molecules cross-talk *via* CTNNB1. Activated CTNNB1 induces the transcription of anti-apoptosis genes and pro-proliferation genes.

the common genes, EPHA8, SS18 and PTCH1 interact with phosphatidylinositol-4,5-bisphosphate-3-kinase catalytic subunit gamma (PIK3CG), SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4), and GLI family zinc finger 1 (GLI1), respectively, whereas CARD11, ICE1, MSH3 have no known interactions with molecules in the CTNNB1 network. The networks for stomach

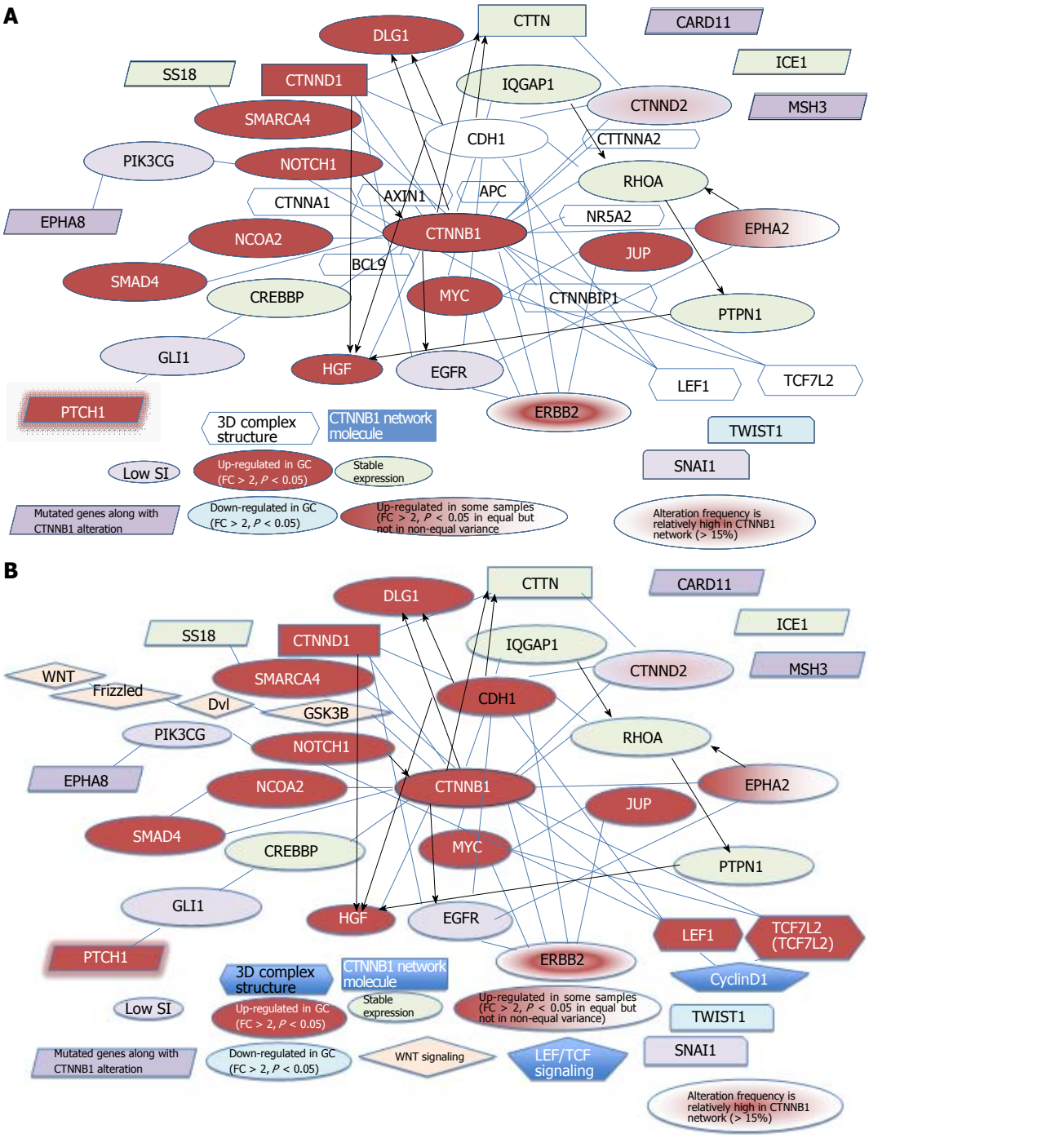

Figure 5 Network model for *CTNNB1* and related genes. A: Network model for *CTNNB1* and genes mutated along with *CTNNB1*. The networks of extracted *CTNNB1* with other mutated genes plus that of extracted *CTNNB1* alone are shown (cBioPortal-oriented, Stomach Adenocarcinoma)[16]; B: Network model for *CTNNB1* and 6 mutated genes. Wnt and LEF/TCF signaling are merged in *CTNNB1* signaling (cBioPortal-oriented, Stomach Adenocarcinoma)[16].

adenocarcinoma that were generated using cBioPortal for Cancer Genomics for CTNNB1 alone and for CTNNB1 with the 6 genes that are mutated along with the *CTNNB1* alteration have been partially merged in Figure 5. Catenin delta 2 (*CTNND2*) and erb-b2 receptor tyrosine kinase 2 (*ERBB2*) showed a relatively high frequency of mutation (> 15% in 287 tumor samples) in the analysis using cBioPortal for Cancer Genomics of the *CTNNB1* network in stomach adenocarcinoma (TCGA, Nature 2014)[16]. The genes that were up-regulated in GC cells compared to MSCs are shown in red, whereas the down-regulated genes are shown in light blue (Fold change > 2, $P < 0.05$, $n = 12$ in MSCs, $n = 5$ in GC; the average signal intensity of MSCs or GC cells is greater than 500). The expression of the *CTNNB1*, *CDH1*, notch1 (*NOTCH1*), hepatocyte growth factor (*HGF*), *PTCH1*, discs large homolog 1, scribble cell polarity complex component (*DLG1*), *LEF1*, *CTNND1*, *SMARCA4*, nuclear receptor coactivator 2 (*NCOA2*), SMAD family member 4 (*SMAD4*), *MYC*, junction plakoglobin (*JUP*), *TCF7L2* and *ERBB2* genes

was up-regulated in GC cells compared to MSCs, whereas the expression of twist family bHLH transcription factor 1 (*TWIST1*) was down-regulated in GC cells compared to MSCs. The expression of *EPHA2* was up-regulated in some GC samples. The expression of the IQ motif-containing GTPase activating protein 1 (*IQGAP1*), *SS18*, *ICE1*, cortactin (*CTTN*), *RHOA*, CREB binding protein (*CREBBP*) and protein tyrosine phosphatase, non-receptor (*PTPN1*) genes was not altered in MSCs and GC cells. The expression of the *EPHA8*, *PIK3CG*, *CARD11*, *MSH3*, *GLI1*, epidermal growth factor receptor (*EGFR*), snail family zinc finger 1 (*SNAI1*) and *CTNND2* genes was not examined due to a low signal intensity. The alteration frequencies of *CTNND2* and *ERBB2* are relatively high in the CTNNB1 network (> 15%), according to the cBioPortal for Cancer Genomics. Interestingly, *IQGAP2* was up-regulated in GC cells compared to MSCs.

DISCUSSION

In summary, the *CTNNB1* gene expression was up-regulated in diffuse-type GC compared to MSC. The various molecules are regulated with *CTNNB1*, which suggests the *CTNNB1* signaling network in cancer and stem cells. EMT-related genes have been reported to be induced by transforming growth factor (TGF)-β or epidermal growth factor (EGF), and genes in the Wnt signaling pathway are mutated in non-small cell lung cancer[32-34]. The expression of β-catenin was up-regulated in the TGF-β1-induced EMT model and was inhibited by cucurbitacin B treatment[32]. Solid tumors induce hypoxia, leading to HIF-1α protein regulation of molecules that are involved in angiogenesis, erythropoiesis, metabolism, cell survival and cell proliferation[35]. *SNAI2* and *TWIST1* were down-regulated in GC cells compared to MSCs, whereas *SNAI1* expression was not detected because of low signal intensity[9,36,37]. Because SNAI and TWIST are associated with EMT, the regulation of their expression is important for understanding EMT mechanisms. Although 3D complex structures of SNAI2 and TWIST1 with β-catenin are not available, some indirect β-catenin signaling cascade may be involved in the SNAI2 and TWIST1 pathway[38,39]. TGFβ is also an important factor in EMT[40]. TGFβ regulates osteoblast differentiation, whereas calycosin-7-O-β-D-glucopyranoside-induced osteoblast differentiation is regulated *via* the bone morphogenetic protein (BMP) and Wnt/β-catenin-signaling pathway[41]. The TGFβ-induced nuclear translocation of β-catenin has been reported to be one of the key factors that activates the EMT program[42-45]. Wnt/β-catenin is regulated in stem cells, and Wnt target genes are controlled by the TCF/β-catenin complex[46].

In gastrointestinal cancer, somatic mutations that provoke an immune response have been found in tumor-infiltrating lymphocytes, which may be very specific to the individual and are targets for cancer immunotherapy[47]. KRAS-mutation-specific T cells, as well as personalized mutation-specific T cells, have been identified, and these may be useful in the future for individual cancer immunotherapeutics[47]. It has been reported that *Helicobacter pylori* up-regulates Nanog and Oct4 expression *via* Wnt/β-catenin signaling[48]. Wnt/β-catenin signaling and the phosphorylation of β-catenin may be involved in stemness in gastric cancer[48].

In conclusion, CTNNB1 plays an important role in the regulation of stem cell pluripotency and cancer signaling. For future direction, precise analyses of Wnt signaling, Notch signaling, and Ephrin signaling are needed to reveal the entire picture of β-catenin signaling in cancer and stem cells. RHO mutations, and regulator of G-protein signaling, with network analysis tools, such as Cytoscape, must be investigated for a greater understanding of this process.

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COMMENTS

Background

β-catenin signaling is essential in pluripotent stem cells and cancer. It is also involved in the epithelial-mesenchymal transitions (EMT). CTNNB1 is activated by Wnt, and the binding of CTNNB1 to transcription factors leads to pluripotent gene regulation.

Research frontiers

The regulation of pluripotency and proliferation is important for elucidating the mechanism of cell phenotype transitions. The EMT mechanism should be investigated to better understand cancer resistance to therapeutics.

Innovations and breakthroughs

The 3D complex structures of β-catenin and related molecules were studied using molecular networks, which is an innovation in the field. The mutated genes that were altered along with *CTNNB1* in stomach adenocarcinoma samples were also investigated.

Applications

These results may affect the study of the pluripotency mechanism and potential therapeutic predictions of gastric cancer. The genes in the molecular network that are related to *CTNNB1* may be the targets of predictive medicine for cancer and disease using pluripotent cells.

Terminology

EMT is a cellular phenotype of a transition from an epithelial to a mesenchymal cell type. EMT is regulated in cancer metastasis and malignancy, and it is related to the acquisition of resistance in cancer cells to therapeutics. It is important to understand the EMT mechanism to understand the mechanisms of cancer resistance.

Peer-review

In general, the manuscript is interesting not only for scientific reasons, but also due to its potential clinical relevance, since it provides some light about the

relationships between stem and cancer cells.

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