Basic Study

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

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Abstract

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

METHODS
The expression of the catenin ß 1 (CTNNB1) gene, which codes for ß-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 TCF7L2_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\(^10\). 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 °C until microarray analysis\(^9,13\). The existing data
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://homicos.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://homicos.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)].

| pdb_id | β-catenin (CTNNB1) | Proteins that interact with β-catenin | 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/ - |
| 1qP7   | A 524 CTNB1_HUMAN | AXIN1 B 17 AXN_XENLA Axin-1 | - |
| 3s9    | B 165 CTNB1_HUMAN | BCL9 D 23 BCL9_HUMAN B-cell CLL/lymphoma 9 protein | - |
| 17w    | C 509 CTNB1_MOUSE | CDH1 D 60 CADH1_MOUSE Cadherin-1 Beta-catenin-interacting protein 1 | Up-regulated - |
| 1m1e   | A 512 CTNB1_MOUSE | CTNNBIP1 B 65 CNBP1_HUMAN | - |
| 3oux   | A 503 CTNB1_MOUSE | LEF1 B 47 LEF1_MOUSE Lymphoid enhancer-binding factor 1 | Up-regulated |
| 3ex7   | A 504 CTNB1_HUMAN | NR5A2 B 218 NR5A2_HUMAN Nuclear receptor subfamily 5 group A member 2 | - |
| 1q3l   | A 439 CTNB1_HUMAN | TCF7L1 B 34 T7L1A_XENLA Transcription factor 7-like 1-A | - |
| 1jdh   | A 508 CTNB1_HUMAN | TCF7L2 B 38 T7L2_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 | - |
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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).
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 compared to MSCs, whereas the signal intensity in probe set ID 201533_at was unchanged (P<0.01 in t-test) (Figure 4). Wnt stimulation prevents glycogen 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).

The 3D complex structures of CTNNB1, the 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 (29). Wnt signaling induces intracellular β-catenin signaling via GSK3β kinase inhibition and dephosphorylation of β-catenin (29–31). The inhibition of β-catenin decreases proliferation and induces apoptosis in the mantle cell lymphoma cell line (30). Noncanonical Wnt signaling is activated in circulating tumor cells from the prostate that are anti-androgen-resistant (30).

**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).

**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.

**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 (26). 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 (26). 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 frame 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-kB 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...
The pluripotentcy pathway should be investigated to reveal TCF7L2 bind to factors. 3D complex structures show that CDH1, LEF1 and mechanisms with the binding of different transcription which suggests that TCF7L2, share high mobility group (HMG)-box domains, TCNBN1 alteration is shown in Figure 5A. Of with the CTNNB1 alteration is shown in Figure 5B. Of

### 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 |
|-------------|------------------------------------|------------|---------------------|-----------|-------------|--------------------------|
| EPHA8       | EPH1 receptor A8                    | 1p36.12    | 29.17%              | 2.28%     | 3.68        | 1.4E-05                  |
| SS18        | Synovial sarcoma translocation      | 18q11.2    | 16.67%              | 0.00%     | > 10        | 3.8E-05                  |
| ICE1        | Interactor of little elongator      | 5p15.32    | 33.33%              | 4.56%     | 2.87        | 4.7E-05                  |
| PTCH1       | Patched 1                           | 9q22.3     | 29.17%              | 3.42%     | 3.09        | 8.16E-05                 |
| MSH3        | MSH homolog 3                       | 5q14.1     | 20.83%              | 1.14%     | 4.19        | 1.28E-04                 |
| 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 // multilocular organismal development // nervous system development // axon guidance // phosphohydrolysis // 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 II 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 ureretic bud morphogenesis // neural tube formation // neural tube closure // heart morphogenesis // signal transduction // smoothed signaling pathway // smoothed signaling pathway // regulation of mitotic cell cycle // pattern specification process // brain development // negative regulation of cell proliferation // epithend development // regulation of smoothed signaling pathway // response to mechanical stimulus // organ morphogenesis // dorsal/ventral pattern formation // response to chorate // 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 smoothed signaling pathway // negative regulation of smoothed 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 // smoothed signaling pathway involved in dorsal/ventral neural tube patterning // cell differentiation involved in kidney development // somite development // cellular response to cholesterol // cellular response to cholestrol // 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 pluripotency 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
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
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, TCF7L2 and ERBB2 genes

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].
was up-regulated in GC cells compared to MSCs, whereas the expression of the 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-β-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[49].

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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