

# PTCH1 pathway network model in diffuse-type gastric cancer and epithelial mesenchymal transition

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## Abstract

Patched 1 (PTCH1) gene plays an important role in the Hedgehog signalling in cancer. To reveal the role of PTCH1 and the network in epithelial mesenchymal transition (EMT), gene expression and molecular network of the PTCH1 was investigated in mesenchymal stem cells (MSCs) and diffuse-type gastric cancer (GC). The PTCH1 gene expression was up-regulated in diffuse-type GC compared to MSCs. PTCH1 network model was generated with the gene expression profiling of the molecules related to PTCH1 and EMT. The signalling and molecular network of PTCH1 was analyzed using several databases, including cBioPortal for Cancer Genomics, Kyoto Encyclopedia of Genes and Genomes (KEGG), BioGRID, VaProS and Ingenuity Pathways Analysis (IPA) databases. The PTCH1 model network contains cancer-related genes such as cadherin 1 (CDH1), catenin beta 1 (CTNNB1) and transforming growth factor beta receptor 3 (TGFB3). The results revealed a PTCH1 pathway network model in cancer and stem cells.

## Introduction

The Patched (PTCH) protein is the receptor for Sonic Hedgehog (Shh) and functions in tumorigenesis and the development of embryonic structures through inhibiting cancer-related pathways by the inhibition of the signalling of the seven-transmembrane protein Smoothed (SMO) [1]. It has been shown that the binding of Shh to Patched 1 (PTCH1) prevents its inhibition of SMO [1]. SMO, glioma-associated oncogene 1 (GLI1), and forkhead box M1 (FOXM1), which are implicated in tumour formation, are up-regulated in cervical cancer tissues and are correlated with the clinical stage of tumours, whereas the expression of Shh, PTCH1 and GLI1 proteins is correlated with the pathological grade [2]. Internalization and degradation of PTCH1 is regulated by Itchy homolog (Itch), E3 ubiquitin-protein ligase, and the C-terminal domain of PTCH1, leading to Gli activation and cell death [3]. The Sonic Hedgehog pathway consists of SHH, PTCH1, SMO, Gli1, Gli2, Gli3 and regulates the transcription of Gli1, PTCH1, Cyclin D1, c-Myc and Bcl-2 [4]. The Hedgehog (Hh) signalling pathway and p53-mediated DNA repair mechanisms are dysregulated in human cancer, and this dysregulation is associated with the PTCH1 homolog, PTCH53, activated by p53 [5]. The expression of Hh signalling pathway molecules in articular cartilage during adjuvant-induced arthritis is up-regulated, which provides justification for the clinical implication of Hh signalling [6]. The micro-RNA miRNA-206 was found to be down-regulated in the Shh subgroup of medulloblastoma in analysis of *Smo*<sup>+/+</sup> transgenic mice and *Ptch1*<sup>+/-</sup> knockout mice, which suggests the role of miRNAs in tumour suppression [7].

Epithelial-mesenchymal transition (EMT) is the phenotypic shift from epithelial phenotype into mesenchymal phenotype of the cells, which contributes to the cancer metastasis and malignancy. EMT occurs in cancer development and the EMT contributes into cancer stem cell (CSC) maintenance [8]. To investigate the role of EMT in

Shh pathways in cancer, we compared gene expression of PTCH1 in mesenchymal stem cells (MSCs) and diffuse-type gastric cancer (GC). MSCs are stem cells with mesenchymal features and diffuse-type GC exhibits more mesenchymal-like features compared to intestinal-type GC. It may be possible to reveal the EMT-related molecular networks by comparing MSCs and diffuse-type GC. We have previously shown the involvement of catenin beta 1 (CTNNB1) in diffuse-type GC compared to MSCs [9]. During the process of database analysis with cBioPortal for Cancer Genomics, PTCH1 was found to be co-altered with CTNNB1 in stomach adenocarcinoma. It is known that tissue-specific accumulation of genetic mutations in human adult stem cells is an important factor for disease prognosis [10]. In this article, we focused on the regulation and role of PTCH1 in stem cells and cancer.

## Materials and methods

### Gene expression analysis of MSCs and GC

The gene expression in MSCs (6 early- and 6 late- stage cultures, n = 12) and diffuse-type GC (n = 5) was analysed with GeneChip<sup>®</sup> Human Genome U133 Plus 2.0 microarray (Affymetrix, Santa Clara, California, USA), as previously described [9,11,12]. Briefly, total RNA purified from the cells were biotinylated and hybridized to the microarray. The signal intensity in each gene was analysed and

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compared between MSCs and diffuse-type GC. The microarray data for MSCs and diffuse-type GC 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 [9,11,12].

### Cell culture

Human MSCs from bone marrow (Lonza, Walkersville, MD, USA) were cultured in MSC growth medium (MSCGM; Lonza #PT-3001; MSC basal medium supplemented with mesenchymal cell growth supplement, L-glutamine and penicillin/streptomycin) at 37°C in a CO<sub>2</sub> (5%) incubator. Cells were passaged according to the manufacturer's protocol; however a slight modification was made to use trypsin-EDTA solution (Lonza #CC-3232). Lot numbers of the human MSC batches were as follows: #4F1127, #4F0312, #5F0138, #4F1560, #4F0591 and #4F0760. Informed consent was obtained for Poietics human MSC systems (Lonza) [12]. Passage numbers of MSC cultures were #4 and #22 for #4F1127, #4 and #28 for #4F0312, #4 and #24 for #5F0138, #5 and #28 for #4F1560, #4 and #28 for #4F0591, and #4 and #28 for #4F0760.

### Diffuse-type GC tissues

Diffuse-type GC tissues were originally provided by the National Cancer Centre Hospital after obtaining written informed consent from each patient and approval from the National Cancer Centre Institutional Review Board. All cancer specimens were reviewed and classified histopathologically according to the Japanese Classification of Gastric Cancer. Following surgical extraction, tissue specimens were immediately frozen with liquid nitrogen and stored at -80°C until microarray analysis [9,11]. Existing, publicly available data were analysed in the article.

### Analysis with cBioPortal for cancer genomics

The cancer genomics data analysis related to CTNNB1 was performed using cBioPortal for Cancer Genomics (<http://www.cbioportal.org>) [13,14]. The term "PTCH1" was searched in the cBioPortal for Cancer Genomics, and the cross-cancer alteration summary for PTCH1 was obtained. Studies of stomach adenocarcinoma (TCGA, Nature 2014) were further analysed for pathway network analysis [15].

### Gene ontology analysis of PTCH1

Gene Ontology of PTCH1 was analysed using several databases, including EMBL-EBI (<http://www.ebi.ac.uk/QuickGO/>), AmiGO 2 (<http://amigo.geneontology.org/amigo/landing>) and the Gene Ontology Consortium (<http://geneontology.org/>).

### Pathway network analysis of PTCH1 and related genes

Pathway network analysis was performed using the databases VaProS (<http://pford.info/vapro/>), KEGG (<http://www.genome.jp/kegg/>), cBioPortal for Cancer Genomics (<http://www.cbioportal.org/>), and Cytoscape (<http://www.cytoscape.org/>). Molecular interactions were analysed using the BioGRID (<http://www.thebiogrid.org/>) database [16]. Molecule localization was analyzed using The Human Protein Atlas (<http://www.proteinatlas.org/>) [17] and UniProt (<http://www.uniprot.org/>) [18]. The NextBio (<http://www.nextbio.com/b/nextbio.nb>) was used for the exploration of the gene expression in gastric cancer [19].

### Network generation

The list of PTCH1 and EMT-related genes was uploaded in the Ingenuity Pathway Analysis (IPA, <https://analysis.ingenuity.com/pa/launch.jsp>). Each identifier was mapped to its corresponding object in Ingenuity's Knowledge Base [20].

### Statistical analysis

The data were expressed as the mean ± SE. Student's t-test was performed in Microsoft Excel (Microsoft, Redmond, WA, USA).  $p < 0.001$  ( $n = 12$  in MSCs,  $n = 5$  in diffuse-type GC) was considered as statistically significant.

### Results

#### Gene expression of PTCH1 and EMT-related genes in MSCs and diffuse-type GC

The gene expression of PTCH1 was up-regulated in diffuse-type GC compared to MSCs as shown in figure 1. The gene expression of PTCH1 was stably low after each passage of MSCs, whereas the gene expression of PTCH1 in diffuse-type GC was up-regulated. Notably, in one of the diffuse-type GC samples, PTCH1 gene expression was not altered. Gene expression of vimentin (VIM), an EMT-related gene, was up-regulated in one probe set and down-regulated in another probe set in diffuse-type GC compared to MSCs [11]. On the other hand, gene expression of twist family bHLH transcription factor 1 (TWIST1) and snail family transcriptional repressor 2 (SNAI2) were down-regulated in diffuse-type GC compared to MSCs [9,21]. Other EMT-related genes, TWIST2, SNAI1 or actin, alpha 1, skeletal muscle (ACTA1) had low signal intensity. Gene expression of GLI family zinc finger 3 (GLI3) was not changed between MSCs and diffuse-type GC. From the immunohistochemistry data in the Human Protein Atlas, E-cadherin (CDH1), of which gene expression was down-regulated in MSCs compared to diffuse-type GC, was less expressed in progressed cancer [11].

#### Gene ontology of PTCH1

According to KEGG, the gene ontology of PTCH1 is as follows: cAMP signalling pathway, Hedgehog signalling pathway, axon guidance, pathways in cancer, proteoglycans in cancer, and basal cell carcinoma. Genes in which the GO terms include regulation of EMT (GO:0010717) have been analysed with AmiGO 2 and are shown in table 1 (<http://amigo.geneontology.org/amigo/term/GO:0010717>).

#### Pathway network analysis of PTCH1 and EMT-related genes

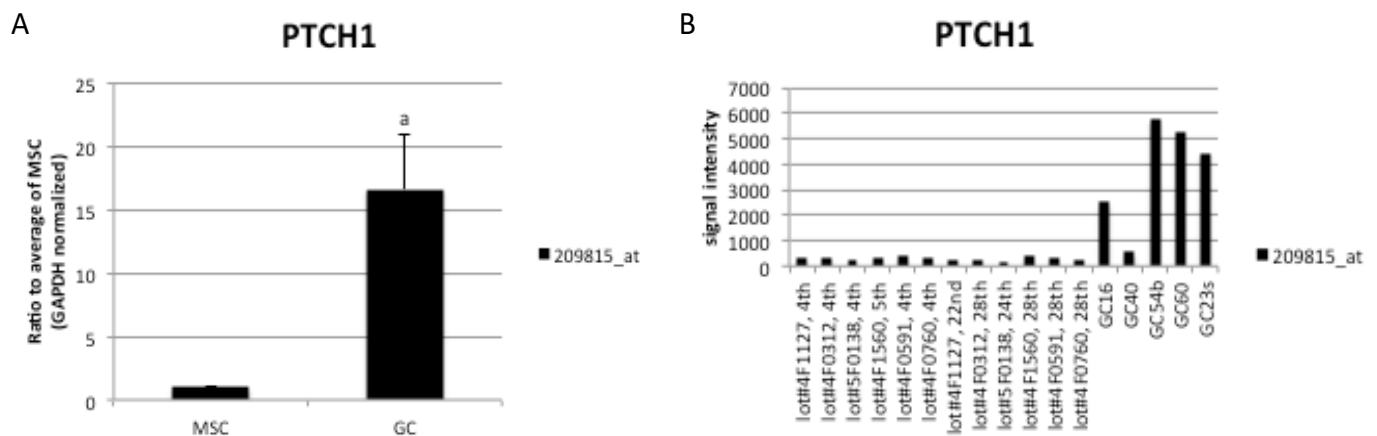
According to the analysis of cBioPortal for Cancer Genomics, PTCH1 interacts with GLI3 in the network generated using stomach adenocarcinoma samples (TCGA, Nature 2014) (Figure 2) [15]. Next, the network was generated with PTCH1 and EMT-related genes. Information and the gene expression of 89 input genes, including PTCH1, from cBioPortal for Cancer Genomics are listed in table 2. The network contained 139 nodes and included the 89 query genes, and the most frequently altered neighbour genes (out of a total of 3060) in cBioPortal. The network includes 66 core genes originally queried and neighbouring genes. From the analysis of molecular interactions in VaProS, PTCH1 was found to be associated with the TGFB2 network. PTCH1 is also associated with TGFB3 and TGFB2 in the VaProS molecular network.

Among the genes in the network generated with cBioPortal, the queried core genes that are connected with other core genes were

**Table 1.** Molecules related to the regulation of EMT (AmiGO2, *Homo sapiens*)

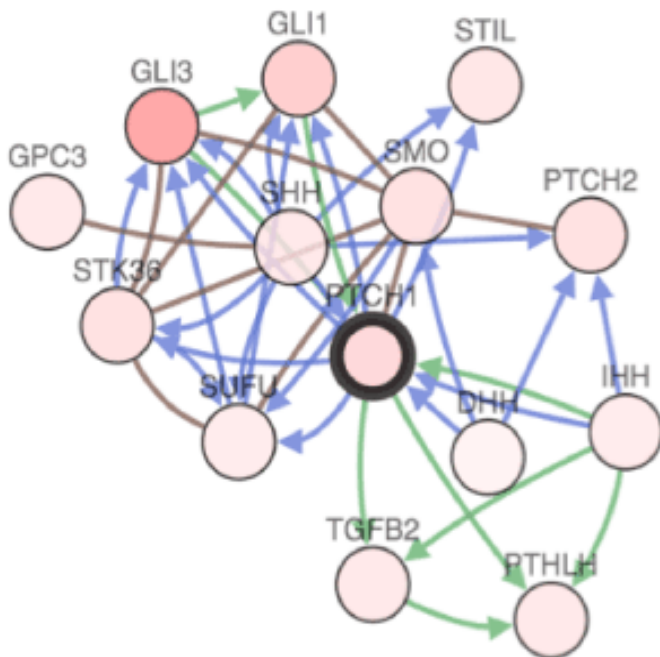
| Gene Symbol | Gene/ product name  | Contributor | Reference      |
|-------------|---|-------------|----------------|
| ACVR1       | Activin receptor type-1   | BHF-UCL     | GO_REF:0000024 |
| ADIPOR1     | Adiponectin receptor protein 1  | BHF-UCL     | PMID:23776679  |
| AGER        | Advanced glycosylation end product-specific receptor                    | Ensembl     | GO_REF:0000019 |
| ALX1        | ALX homeobox protein 1  | UniProt     | PMID:23288509  |
| AXIN2       | Axin-2  | BHF-UCL     | PMID:17072303  |
| BAMBI       | BMP and activin membrane-bound inhibitor homolog                        | BHF-UCL     | PMID:19328798  |
| BCL9L       | B-cell CLL/lymphoma 9-like protein                                      | BHF-UCL     | PMID:19328798  |
| BMP2        | Bone morphogenetic protein 2  | BHF-UCL     | PMID:20890042  |
| BMP5        | Bone morphogenetic protein 5  | BHF-UCL     | PMID:21319131  |
| CLASP1      | CLIP-associating protein 1  | UniProt     | PMID:23940118  |
| CLASP2      | CLIP-associating protein 2  | UniProt     | PMID:23940118  |
| COL1A1      | Collagen type 1 alpha-1 chain   | UniProt     | PMID:20018240  |
| CRB2        | Protein crumbs homolog 2  | UniProt     | GO_REF:0000024 |
| CTNNB1      | Catenin beta-1  | MGI         | PMID:23983127  |
| DAB2        | Disabled homolog 2  | UniProt     | PMID:15734730  |
| DAB2IP      | Disabled homolog 2-interacting protein                                  | BHF-UCL     | PMID:20080667  |
| DACT3       | Dapper homolog 3  | Ensembl     | GO_REF:0000019 |
| DAG1        | Dystroglycan  | UniProt     | PMID:23940118  |
| EFNA1       | Ephrin-A1   | BHF-UCL     | GO_REF:0000024 |
| ELL3        | RNA polymerase II elongation factor ELL3                                | UniProt     | GO_REF:0000024 |
| ENG         | Endoglin  | BHF-UCL     | GO_REF:0000024 |
| EPB41L5     | Band 4.1-like protein 5   | Ensembl     | GO_REF:0000019 |
| EPHA3       | Ephrin type-A receptor 3  | UniProt     | GO_REF:0000024 |
| EZH2        | Histone-lysine N-methyltransferase EZH2                                 | UniProt     | PMID:20154697  |
| FAM195B     | Mapk-regulated corepressor-interacting protein 1                        | UniProt     | PMID:25728771  |
| FOXA1       | Hepatocyte nuclear factor 3-alpha                                       | BHF-UCL     | PMID:20160041  |
| FOXA2       | Hepatocyte nuclear factor 3-beta  | BHF-UCL     | PMID:20160041  |
| FUZ         | Protein fuzzy homolog   | UniProt     | GO_REF:0000024 |
| GCNT2       | N-acetylglucosaminide beta-1,6-N-acetylglucosaminyl-transferase         | UniProt     | PMID:21750175  |
| GLIPR2      | Golgi-associated plant pathogenesis-related protein 1                   | UniProt     | PMID:23516513  |
| GREM1       | Gremlin-1   | UniProt     | PMID:16816361  |
| HDAC2       | Histone deacetylase 2   | Ensembl     | GO_REF:0000019 |
| HPN         | Serine protease hepsin  | UniProt     | PMID:19843851  |
| LDLRAD4     | Low-density lipoprotein receptor class A domain-containing protein 4    | UniProt     | PMID:24627487  |
| LEF1        | Lymphoid enhancer-binding factor 1                                      | UniProt     | PMID:20018240  |
| MAD2L2      | Mitotic spindle assembly checkpoint protein MAD2B                       | BHF-UCL     | PMID:19443654  |
| NKX2-1      | Homeobox protein Nkx-2.1  | UniProt     | PMID:19293183  |
| NOTCH1      | Neurogenic locus notch homolog protein 1                                | BHF-UCL     | PMID:17984306  |
| OLFM1       | Noelin  | AgBase      | GO_REF:0000024 |
| OVOL2       | Transcription factor Ovo-like 2   | UniProt     | GO_REF:0000024 |
| PBLD        | Phenazine biosynthesis-like domain-containing protein                   | UniProt     | PMID:23687415  |
| PHLDB1      | Pleckstrin homology-like domain family B member 1                       | UniProt     | PMID:23940118  |
| PHLDB2      | Pleckstrin homology-like domain family B member 2                       | UniProt     | PMID:23940118  |
| POFUT2      | GDP-fucose protein O-fucosyltransferase 2                               | Ensembl     | GO_REF:0000019 |
| PPP2CA      | Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform | BHF-UCL     | PMID:20080667  |
| RGCC        | Regulator of cell cycle RGCC  | BHF-UCL     | PMID:19158077  |
| SDCBP       | Syntenin-1  | UniProt     | PMID:25893292  |
| SDHAF2      | Succinate dehydrogenase assembly factor 2, mitochondrial                | MGI         | PMID:23983127  |
| SERPINB3    | Serpin B3   | UniProt     | PMID:20527027  |
| SFRP1       | Secreted frizzled-related protein 1                                     | UniProt     | PMID:19095296  |
| SFRP2       | Secreted frizzled-related protein 2                                     | UniProt     | PMID:19095296  |
| SMAD2       | Mothers against decapentaplegic homolog 2                               | BHF-UCL     | GO_REF:0000024 |
| SMAD3       | Mothers against decapentaplegic homolog 3                               | BHF-UCL     | PMID:18505915  |
| SMAD4       | Mothers against decapentaplegic homolog 4                               | BHF-UCL     | GO_REF:0000024 |
| SMAD7       | Mothers against decapentaplegic homolog 7                               | BHF-UCL     | PMID:18762808  |
| SNAI1       | Zinc finger protein SNAI1   | BHF-UCL     | PMID:17072303  |
| STRAP       | Serine-threonine kinase receptor-associated protein                     | UniProt     | PMID:23687415  |
| TBX5        | T-box transcription factor TBX5   | DFLAT       | PMID:20299672  |
| TCF7L2      | Transcription factor 7-like 2   | BHF-UCL     | PMID:17072303  |
| TGFB1       | Transforming growth factor beta-1                                       | BHF-UCL     | PMID:21319131  |

|                    |  |         |                |
|--------------------|--|---------|----------------|
| TGFB11             | Transforming growth factor beta-1-induced transcript 1 protein | BHF-UCL | PMID:18762808  |
| TGFB2              | Transforming growth factor beta-2                              | BHF-UCL | PMID:17999987  |
| TGFB3              | Transforming growth factor beta-3                              | BHF-UCL | GO_REF:0000024 |
| TGFBR1             | Receptor protein serine/threonine kinase                       | AgBase  | GO_REF:0000024 |
| TGFBR2             | TGF-beta receptor type-2                                       | BHF-UCL | PMID:26459119  |
| TRIM62             | E3 ubiquitin-protein ligase TRIM62                             | Ensembl | GO_REF:0000019 |
| TWIST1             | Twist-related protein 1  | BHF-UCL | PMID:18297062  |
| URS0000170CF4_9606 | Homo sapiens (human) hsa-miR-221-3p.                           | BHF-UCL | PMID:21673316  |
| URS000039ED8D_9606 | Homo sapiens (human) hsa-miR-21-5p.                            | BHF-UCL | PMID:24887517  |
| VASN               | Vasorin  | UniProt | PMID:21170088  |
| WWTR1              | WW domain-containing transcription regulator protein 1         | UniProt | PMID:18227151  |
| ZNF703             | Zinc finger protein 703  | UniProt | GO_REF:0000024 |



**Figure 1.** Gene expression of PTCH1 in MSCs and diffuse-type GC

A: Gene expression of PTCH1 was up-regulated in diffuse-type GC compared to MSCs ( $p < 0.001$  in Student's  $t$ -test,  $n = 12$  in MSCs,  $n = 5$  in diffuse-type GC). The ratio to the average of microarray signal intensity in MSCs was calculated. B: Signal intensity in each sample of MSCs and diffuse-type GC are shown.



**Figure 2.** PTCH1 network in stomach adenocarcinoma (cBioPortal for Cancer Genomics)  
A PTCH1 model network was generated for stomach adenocarcinoma with cBioPortal for Cancer Genomics with query of PTCH1.

extracted to generate the model network for PTCH1 and EMT-related genes, as shown in figure 3. In figure 3, the up-regulated (UR) genes in diffuse-type GC compared to MSCs are shown in red, whereas down-regulated (DR) genes are shown in light purple (Fold change (FC)  $> 3$ ,  $p < 0.01$ ). The genes in which FC of gene expression is between 2 and 3 and  $p$  value is less than 0.01 are highlighted with beige. UR genes in gastric cancer in the NextBio database are shown in an orange gradient, whereas DR genes are shown in green gradient. The genes in which expression was not altered in diffuse-type GC and MSCs are shown in light blue. A total of 60 genes were used to generate the PTCH1 model network.

In the model network generated from the pathway analysis shown in figure 3, notch 1 (NOTCH1), CTNBN1, stratifin (SFN), AKT serine/threonine kinase 2 (AKT2), secreted frizzled related protein 2 (SFRP2), and claudin 1 (CLDN1) were UR in diffuse-type GC compared to MSCs. F11 receptor (F11R) and the Jun proto-oncogene AP-1 transcription factor subunit (JUN) were up-regulated in diffuse-type GC compared to MSCs, although these genes are shown as DR in gastric cancer based on the NextBio database. The gene expression of integrin subunit alpha 5 (ITGA5) was DR in diffuse-type GC compared to MSCs, although it is shown to be UR in gastric cancer based on NextBio database. We have previously shown that the gene expression of cadherin 1 (CDH1) was UR, and CDH2 and fibronectin 1 (FN1) were DR in diffuse-type GC compared to MSCs [11]. Gene expression of Janus kinase 1 (JAK1), a signal transducer and activator of transcription 1 (STAT1) and STAT2 in the JAK-STAT pathway, was not altered in diffuse-type GC and



**Table 2.** Genes queried for network analysis of cBioPortal for Cancer Genomics and IPA

| Gene symbol | Full name   |
|-------------|---|
| AKT1        | v-akt murine thymoma viral oncogene homolog 1   |
| AKT2        | v-akt murine thymoma viral oncogene homolog 2   |
| AKT3        | v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)                 |
| ALCAM       | activated leukocyte cell adhesion molecule  |
| AXIN2       | axin 2  |
| BAMBI       | BMP and activin membrane bound inhibitor  |
| BMI1        | BMI1 proto-oncogene, polycomb ring finger   |
| BMP2        | bone morphogenetic protein 2  |
| CDH1        | cadherin 1  |
| CDH2        | cadherin 2  |
| CLDN1       | claudin 1   |
| COL1A1      | collagen, type I, alpha 1   |
| CTNNB1      | catenin (cadherin-associated protein), beta 1, 88kDa                                    |
| CXADR       | coxsackie virus and adenovirus receptor   |
| DAB2        | Dab, mitogen-responsive phosphoprotein, homolog 2 (Drosophila)                          |
| EFNA1       | ephrin-A1   |
| ELL3        | elongation factor RNA polymerase II-like 3  |
| EPB41L5     | erythrocyte membrane protein band 4.1 like 5  |
| EPCAM       | epithelial cell adhesion molecule   |
| EPHA3       | EPH receptor A3   |
| EZH2        | enhancer of zeste homolog 2 (Drosophila)  |
| F11R        | F11 receptor  |
| FAM101B     | family with sequence similarity 101, member B   |
| FLNA        | filamin A, alpha  |
| FN1         | fibronectin 1   |
| FOXF2       | forkhead box F2   |
| GJB2        | gap junction protein, beta 2, 26kDa   |
| GREM1       | gremlin 1, DAN family BMP antagonist  |
| GSK3B       | glycogen synthase kinase 3 beta   |
| HEY1        | hairy/enhancer-of-split related with YRPW motif 1                                       |
| HEYL        | hairy/enhancer-of-split related with YRPW motif-like                                    |
| HGF         | hepatocyte growth factor (hepapoietin A; scatter factor)                                |
| HIF1A       | hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) |
| HMGA2       | high mobility group AT-hook 2   |
| HNRNPAB     | heterogeneous nuclear ribonucleoprotein A/B   |
| ICAM3       | intercellular adhesion molecule 3   |
| ITGA5       | integrin, alpha 5 (fibronectin receptor, alpha polypeptide)                             |
| JAK1        | Janus kinase 1  |
| JUN         | jun proto-oncogene  |
| KRT8        | keratin 8   |
| LEF1        | lymphoid enhancer-binding factor 1  |
| LOXL2       | lysyl oxidase-like 2  |
| LOXL3       | lysyl oxidase-like 3  |
| MAD2L2      | MAD2 mitotic arrest deficient-like 2 (yeast)  |
| NANOG       | Nanog homeobox  |
| NCAM1       | neural cell adhesion molecule 1   |
| NFATC1      | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1               |
| NOTCH1      | notch 1   |
| OCLN        | occludin  |
| PDCD1       | programmed cell death 1   |
| PECAM1      | platelet/endothelial cell adhesion molecule 1   |
| PINK1       | PTEN induced putative kinase 1  |
| POFUT2      | protein O-fucosyltransferase 2  |
| PPP2CA      | protein phosphatase 2, catalytic subunit, alpha isozyme                                 |
| PRRX1       | paired related homeobox 1   |
| PTCH1       | patched 1   |
| RBPJ        | recombination signal binding protein for immunoglobulin kappa J region                  |

|         |  |
|---------|--|
| RGCC    | regulator of cell cycle                                    |
| RTN4    | reticulon 4  |
| S100A4  | S100 calcium binding protein A4                            |
| SFN     | stratifin  |
| SFRP1   | secreted frizzled-related protein 1                        |
| SFRP2   | secreted frizzled-related protein 2                        |
| SMAD2   | SMAD family member 2                                       |
| SMAD3   | SMAD family member 3                                       |
| SMAD4   | SMAD family member 4                                       |
| SMAD7   | SMAD family member 7                                       |
| SNAI2   | snail family zinc finger 2                                 |
| SOX2    | SRY (sex determining region Y)-box 2                       |
| SOX9    | SRY (sex determining region Y)-box 9                       |
| SP1     | Sp1 transcription factor                                   |
| STAT1   | signal transducer and activator of transcription 1, 91kDa  |
| STAT2   | signal transducer and activator of transcription 2, 113kDa |
| TCF7L2  | transcription factor 7-like 2 (T-cell specific, HMG-box)   |
| TGFB1   | transforming growth factor, beta 1                         |
| TGFB111 | transforming growth factor beta 1 induced transcript 1     |
| TGFB2   | transforming growth factor, beta 2                         |
| TGFB3   | transforming growth factor, beta 3                         |
| TGFBR1  | transforming growth factor, beta receptor 1                |
| TGFBR3  | transforming growth factor, beta receptor 3                |
| TJP1    | tight junction protein 1                                   |
| TRIM28  | tripartite motif containing 28                             |
| TWIST1  | twist basic helix-loop-helix transcription factor 1        |
| VIM     | vimentin   |
| WNT5A   | wingless-type MMTV integration site family, member 5A      |
| WWTR1   | WW domain containing transcription regulator 1             |
| ZEB1    | zinc finger E-box binding homeobox 1                       |
| ZEB2    | zinc finger E-box binding homeobox 2                       |
| ZNF703  | zinc finger protein 703                                    |

MSCs. BMI1 proto-oncogene, polycomb ring finger (BMI1), CDH2, high mobility group AT-hook 2 (HMGA2), neural cell adhesion molecule 1 (NCAM1) and VIM were included in network generated with cBioPortal for Cancer Genomics, which resulted in generation of PTCH1 model network without these genes due to the linkage was through neighbor genes not with query genes.

### Network generated with EMT-related genes in Ingenuity Pathway Analysis (IPA)

The 89 genes including PTCH1 and EMT-related genes were used for generating networks in IPA (Table 2). The networks generate with IPA included 11 networks related to cellular movement or cellular development. The most significant network is shown in figure 4. The molecules in the network includes activated leukocyte cell adhesion molecule (ALCAM), BMP and activin membrane bound inhibitor (BAMBI), CDH2, collagen type I alpha 1 chain (COL1A1), ephrin A1 (EFNA1), HMGA2, ITGA5, mitotic arrest deficient 2 like 2 (MAD2L2), platelet and endothelial cell adhesion molecule 1 (PECAM1), SFRP2, SMAD family member 4 (SMAD4), SMAD7, SNAI2, transforming growth factor beta receptor 1 (TGFBR1), TGFBR3, TWIST1, WW domain containing transcription regulator 1 (WWTR1). The function of the network was associated with cardiovascular system development and function, and cellular movement.

### PTCH1 interactors identified by BioGRID

Table 3 shows the gene ontology of 88 interactors with PTCH1 analysed with the BioGRID database [16]. PTCH1 had 94 total unique

**Table 3.** Gene Ontology (GO) analysis of 88 interactors of PTCH1 (BioGRID)

| Gene Symbol | Gene Title   | GO biological process term  |
|-------------|--|---|
| ABCA2       | ATP-binding cassette, sub-family A (ABC1), member 2              | ATP catabolic process / regulation of transcription from RNA polymerase II promoter / lipid metabolic process / transport / metabolic process / regulation of intracellular cholesterol transport / response to drug / cholesterol homeostasis / response to steroid hormone / transmembrane transport  |
| ADAM17      | ADAM metallopeptidase domain 17                                  | response to hypoxia / positive regulation of protein phosphorylation / neutrophil mediated immunity / germinal center formation / positive regulation of leukocyte chemotaxis / proteolysis / membrane protein ectodomain proteolysis / apoptotic process / cell adhesion / epidermal growth factor receptor signaling pathway / Notch signaling pathway / Notch receptor processing / positive regulation of cell proliferation / positive regulation of T cell chemotaxis / extracellular matrix disassembly / B cell differentiation / extracellular matrix organization / positive regulation of cell growth / positive regulation of cell migration / positive regulation of transforming growth factor beta receptor signaling pathway / negative regulation of transforming growth factor beta receptor signaling pathway / collagen catabolic process / membrane protein intracellular domain proteolysis / positive regulation of cyclin-dependent protein serine/threonine kinase activity involved in G <sub>1</sub> /S transition of mitotic cell cycle / response to lipopolysaccharide / negative regulation of interleukin-8 production / positive regulation of chemokine production / regulation of mast cell apoptotic process / T cell differentiation in thymus / cell adhesion mediated by integrin / wound healing, spreading of epidermal cells / epidermal growth factor-activated receptor transactivation by G-protein coupled receptor signaling pathway / response to drug / positive regulation of epidermal growth factor-activated receptor activity / neurotrophin TRK receptor signaling pathway / spleen development / cell motility / PMA-inducible membrane protein ectodomain proteolysis / positive regulation of cellular component movement / response to high density lipoprotein particle / JAK-STAT cascade involved in growth hormone signaling pathway / apoptotic signaling pathway |
| ARSK        | arylsulfatase family, member K                                   | sphingolipid metabolic process / glycosphingolipid metabolic process / metabolic process / post-translational protein modification / cellular protein metabolic process / small molecule metabolic process  |
| ASPH        | aspartate beta-hydroxylase                                       | detection of calcium ion / muscle contraction / pattern specification process / negative regulation of cell proliferation / positive regulation of calcium ion transport into cytosol / regulation of cell communication by electrical coupling / regulation of release of sequestered calcium ion into cytosol by sarcoplasmic reticulum / regulation of cardiac muscle contraction by regulation of the release of sequestered calcium ion / peptidyl-amino acid modification / regulation of inositol 1,4,5-trisphosphate-sensitive calcium-release channel activity / regulation of protein stability / activation of store-operated calcium channel activity / response to ATP / limb morphogenesis / peptidyl-aspartic acid hydroxylation / positive regulation of proteolysis / positive regulation of transcription, DNA-templated / oxidation-reduction process / palate development / regulation of ryanodine-sensitive calcium-release channel activity / face morphogenesis / calcium ion transmembrane transport / cellular response to calcium ion / positive regulation of intracellular protein transport / activation of cysteine-type endopeptidase activity / regulation of protein depolymerization   |
| ATF6B       | activating transcription factor 6 beta                           | transcription, DNA-templated / regulation of transcription, DNA-templated / response to unfolded protein / signal transduction  |
| B3GALTL     | beta 1,3-galactosyltransferase-like                              | carbohydrate metabolic process / fucose metabolic process / protein glycosylation   |
| B4GALT1     | UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1 | epithelial cell development / acute inflammatory response / carbohydrate metabolic process / lactose biosynthetic process / galactose metabolic process / protein glycosylation / protein N-linked glycosylation / cell adhesion / Notch signaling pathway / single fertilization / binding of sperm to zona pellucida / penetration of zona pellucida / negative regulation of cell proliferation / glycoprotein biosynthetic process / oligosaccharide biosynthetic process / keratan sulfate biosynthetic process / protein N-linked glycosylation via asparagine / extracellular matrix organization / glycosaminoglycan metabolic process / mammary gland development / multicellular organism reproduction / wound healing / regulation of cell proliferation / keratan sulfate metabolic process / positive regulation of apoptotic process / post-translational protein modification / cellular protein metabolic process / small molecule metabolic process / development of secondary sexual characteristics / branching morphogenesis of an epithelial tube / leukocyte migration / regulation of cellular component movement / regulation of acrosome reaction / positive regulation of epithelial cell proliferation involved in wound healing / angiogenesis involved in wound healing / positive regulation of apoptotic process involved in mammary gland involution  |
| B4GALT3     | UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 3 | carbohydrate metabolic process / protein glycosylation / keratan sulfate biosynthetic process / protein N-linked glycosylation via asparagine / glycosaminoglycan metabolic process / keratan sulfate metabolic process / post-translational protein modification / cellular protein metabolic process / small molecule metabolic process   |
| B4GALT7     | xylosylprotein beta 1,4-galactosyltransferase, polypeptide 7     | carbohydrate metabolic process / glycosaminoglycan biosynthetic process / proteoglycan metabolic process / cellular protein modification process / protein glycosylation / protein N-linked glycosylation / glycosaminoglycan metabolic process / chondroitin sulfate metabolic process / extracellular fibril organization / small molecule metabolic process / negative regulation of fibroblast proliferation  |
| CACNA2D1    | calcium channel, voltage-dependent, alpha 2/delta subunit 1      | transport / ion transport / calcium ion transport / regulation of ion transmembrane transport / regulation of calcium ion transport / calcium ion transmembrane transport   |
| CACNA2D2    | calcium channel, voltage-dependent, alpha 2/delta subunit 2      | energy reserve metabolic process / transport / ion transport / calcium ion transport / neuromuscular junction development / regulation of ion transmembrane transport / regulation of multicellular organism growth / small molecule metabolic process / positive regulation of organ growth / muscle fiber development / regulation of insulin secretion / rhythmic synaptic transmission / calcium ion transmembrane transport  |
| CASP9       | caspase 9, apoptosis-related cysteine peptidase                  | proteolysis / apoptotic process / activation of cysteine-type endopeptidase activity involved in apoptotic process / cellular response to DNA damage stimulus / epidermal growth factor receptor signaling pathway / aging / fibroblast growth factor receptor signaling pathway / intrinsic apoptotic signaling pathway in response to DNA damage / activation of cysteine-type endopeptidase activity involved in apoptotic process by cytochrome c / response to UV / response to organic cyclic compound / platelet formation / response to cobalt ion / response to estradiol / response to lipopolysaccharide / glial cell apoptotic process / cellular response to UV / Fc-epsilon receptor signaling pathway / signal transduction in response to DNA damage / regulation of apoptotic process / positive regulation of apoptotic process / positive regulation of neuron apoptotic process / innate immune response / response to antibiotic / neurotrophin TRK receptor signaling pathway / phosphatidylinositol-mediated signaling / cellular response to organic cyclic compound / cellular response to dexamethasone stimulus / intrinsic apoptotic signaling pathway / regulation of response to DNA damage stimulus  |

|         |  |   |
|---------|--|---|
| CAV1    | caveolin 1, caveolae protein, 22kDa                          | negative regulation of transcription from RNA polymerase II promoter / MAPK cascade / inactivation of MAPK activity / angiogenesis / vasculogenesis / response to hypoxia / negative regulation of endothelial cell proliferation / negative regulation of cytokine-mediated signaling pathway / regulation of the force of heart contraction / response to ischemia / regulation of the force of heart contraction by chemical signal / triglyceride metabolic process / calcium ion transport / cellular calcium ion homeostasis / regulation of smooth muscle contraction / skeletal muscle tissue development / lactation / blood coagulation / protein localization / negative regulation of cell proliferation / cellular response to starvation / negative regulation of signal transduction / positive regulation of calcium ion transport into cytosol / positive regulation of peptidase activity / viral process / vesicle organization / modulation by virus of host morphology or physiology / regulation of fatty acid metabolic process / lipid storage / regulation of blood coagulation / cholesterol transport / negative regulation of BMP signaling pathway / negative regulation of epithelial cell differentiation / mammary gland development / T cell costimulation / negative regulation of protein ubiquitination / negative regulation of protein binding / maintenance of protein location in cell / response to progesterone / negative regulation of peptidyl-serine phosphorylation / positive regulation of peptidyl-serine phosphorylation / nitric oxide homeostasis / vasoconstriction / negative regulation of tyrosine phosphorylation of Stat5 protein / cholesterol homeostasis / negative regulation of MAP kinase activity / negative regulation of MAPK cascade / response to estrogen / small molecule metabolic process / negative regulation of nitric oxide biosynthetic process / positive regulation of vasoconstriction / nitric oxide metabolic process / negative regulation of JAK-STAT cascade / negative regulation of pinocytosis / positive regulation of metalloenzyme activity / leukocyte migration / regulation of nitric-oxide synthase activity / protein oligomerization / protein homooligomerization / cytosolic calcium ion homeostasis / response to calcium ion / membrane depolarization / regulation of peptidase activity / calcium ion homeostasis / mammary gland involution / caveola assembly / cellular response to hyperoxia / caveolin-mediated endocytosis / negative regulation of canonical Wnt signaling pathway / positive regulation of canonical Wnt signaling pathway / apoptotic signaling pathway / receptor internalization involved in canonical Wnt signaling pathway / negative regulation of anoikis / positive regulation of extrinsic apoptotic signaling pathway / positive regulation of intrinsic apoptotic signaling pathway   |
| CCPG1   | cell cycle progression 1                                     | cell cycle / positive regulation of cell proliferation / positive regulation of cell cycle / positive regulation of transcription from RNA polymerase II promoter / regulation of Rho guanyl-nucleotide exchange factor activity  |
| CHPF    | chondroitin polymerizing factor                              | carbohydrate metabolic process / metabolic process / glycosaminoglycan metabolic process / chondroitin sulfate metabolic process / chondroitin sulfate biosynthetic process / small molecule metabolic process  |
| CHST12  | carbohydrate (chondroitin 4) sulfotransferase 12             | carbohydrate metabolic process / carbohydrate biosynthetic process / glycosaminoglycan metabolic process / chondroitin sulfate metabolic process / chondroitin sulfate biosynthetic process / dermatan sulfate biosynthetic process / small molecule metabolic process  |
| CHST14  | carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 14 | carbohydrate metabolic process / carbohydrate biosynthetic process / glycosaminoglycan metabolic process / chondroitin sulfate metabolic process / dermatan sulfate biosynthetic process / small molecule metabolic process / dermatan sulfate proteoglycan metabolic process   |
| CHSY1   | chondroitin sulfate synthase 1                               | carbohydrate metabolic process / metabolic process / glycosaminoglycan metabolic process / chondroitin sulfate metabolic process / chondroitin sulfate biosynthetic process / negative regulation of ossification / response to nutrient levels / small molecule metabolic process  |
| CNNM1   | cyclin M1  | transport / ion transport   |
| CNNM4   | cyclin M4  | transport / ion transport / visual perception / biomineral tissue development / response to stimulus  |
| CNTNAP3 | contactin associated protein-like 3                          | cell adhesion / cell recognition  |
| COCH    | cochlin  | sensory perception of sound / regulation of cell shape  |
| COL18A1 | collagen, type XVIII, alpha 1                                | angiogenesis / endothelial cell morphogenesis / cell adhesion / visual perception / positive regulation of cell proliferation / negative regulation of cell proliferation / organ morphogenesis / extracellular matrix disassembly / extracellular matrix organization / positive regulation of cell migration / collagen catabolic process / response to drug / positive regulation of apoptotic process / response to hydrostatic pressure / positive regulation of endothelial cell apoptotic process  |
| DDX19B  | DEAD (Asp-Glu-Ala-Asp) box polypeptide 19B                   | mRNA export from nucleus / transport / metabolic process / protein transport / mRNA transport   |
| DHFRL1  | dihydrofolate reductase-like 1                               | glycine biosynthetic process / one-carbon metabolic process / nucleotide biosynthetic process / thymidine biosynthetic process / tetrahydrofolate metabolic process / tetrahydrofolate biosynthetic process / oxidation-reduction process   |
| EGFR    | epidermal growth factor receptor                             | MAPK cascade / activation of MAPKK activity / cell morphogenesis / ossification / liver development / embryonic placenta development / positive regulation of protein phosphorylation / hair follicle development / polysaccharide metabolic process / translation / protein phosphorylation / response to stress / response to osmotic stress / response to oxidative stress / signal transduction / cell surface receptor signaling pathway / transmembrane receptor protein tyrosine kinase signaling pathway / epidermal growth factor receptor signaling pathway / activation of phospholipase C activity / multicellular organismal development / axon guidance / salivary gland morphogenesis / learning or memory / circadian rhythm / cell proliferation / positive regulation of cell proliferation / fibroblast growth factor receptor signaling pathway / epidermis development / magnesium ion homeostasis / response to organic cyclic compound / diterpenoid metabolic process / phosphorylation / single organismal cell-cell adhesion / peptidyl-tyrosine phosphorylation / alkanesulfonate metabolic process / cerebral cortex cell migration / signal transduction by phosphorylation / lung development / positive regulation of cell migration / positive regulation of cyclin-dependent protein serine/threonine kinase activity involved in G1/S transition of mitotic cell cycle / response to estradiol / positive regulation of superoxide anion generation / response to cobalamin / response to hydroxyisoflavone / response to lipid / positive regulation of catenin import into nucleus / cellular response to drug / Fc-epsilon receptor signaling pathway / negative regulation of epidermal growth factor receptor signaling pathway / regulation of cell proliferation / negative regulation of protein catabolic process / positive regulation of phosphorylation / ovulation cycle / hydrogen peroxide metabolic process / activation of phospholipase A2 activity by calcium-mediated signaling / negative regulation of apoptotic process / positive regulation of MAP kinase activity / tongue development / innate immune response / positive regulation of nitric oxide biosynthetic process / positive regulation of DNA repair / positive regulation of DNA replication / positive regulation of vasoconstriction / positive regulation of vasodilation / negative regulation of mitotic cell cycle / positive regulation of transcription from RNA polymerase II promoter / protein autophosphorylation / neurotrophin TRK receptor signaling pathway / phosphatidylinositol-mediated signaling / astrocyte activation / positive regulation of fibroblast proliferation / digestive tract morphogenesis / positive regulation of smooth muscle cell proliferation / neuron projection morphogenesis / positive regulation of epithelial cell proliferation / positive regulation of inflammatory response / regulation of peptidyl-tyrosine phosphorylation / regulation of nitric-oxide synthase activity / protein insertion into membrane / response to calcium ion / positive regulation of protein kinase B signaling / positive regulation of synaptic transmission, glutamatergic / morphogenesis of an epithelial fold / response to UV-A / positive regulation of ERK1 and ERK2 cascade / cellular response to mechanical stimulus / cellular response to growth factor stimulus / cellular response to epidermal growth factor stimulus / cellular response to estradiol stimulus / cellular response to dexamethasone stimulus |

|        |  |   |
|--------|--|---|
| ENDOG  | endonuclease G   | in utero embryonic development / apoptotic DNA fragmentation / DNA recombination / response to tumor necrosis factor / positive regulation of apoptotic process / response to antibiotic  |
| ENPP1  | ectonucleotide pyrophosphatase/phosphodiesterase 1                   | generation of precursor metabolites and energy / ATP catabolic process / vitamin metabolic process / water-soluble vitamin metabolic process / riboflavin metabolic process / phosphate-containing compound metabolic process / receptor-mediated endocytosis / immune response / metabolic process / nucleoside triphosphate catabolic process / negative regulation of ossification / negative regulation of cell growth / regulation of bone mineralization / inorganic diphosphate transport / cellular phosphate ion homeostasis / sequestering of triglyceride / biomineral tissue development / negative regulation of protein autophosphorylation / cellular response to insulin stimulus / small molecule metabolic process / negative regulation of fat cell differentiation / negative regulation of glycogen biosynthetic process / negative regulation of glucose import / negative regulation of insulin receptor signaling pathway / bone remodeling / 3'-phosphoadenosine 5'-phosphosulfate metabolic process / nucleic acid phosphodiester bond hydrolysis   |
| EXT1   | exostosin glycosyltransferase 1                                      | skeletal system development / ossification / carbohydrate metabolic process / glycosaminoglycan biosynthetic process / protein glycosylation / signal transduction / gastrulation / axon guidance / brain development / endoderm development / mesoderm development / heparan sulfate proteoglycan biosynthetic process / polysaccharide chain biosynthetic process / olfactory bulb development / glycosaminoglycan metabolic process / cellular polysaccharide biosynthetic process / small molecule metabolic process / embryonic skeletal joint development   |
| FHL2   | four and a half LIM domains 2  | negative regulation of transcription from RNA polymerase II promoter / osteoblast differentiation / transcription, DNA-templated / regulation of transcription, DNA-templated / regulation of transcription from RNA polymerase II promoter / response to hormone / androgen receptor signaling pathway / negative regulation of apoptotic process / cellular lipid metabolic process / small molecule metabolic process / negative regulation of transcription, DNA-templated / positive regulation of transcription, DNA-templated / atrial cardiac muscle cell development / ventricular cardiac muscle cell development / heart trabecula formation   |
| FUT11  | fucosyltransferase 11 (alpha (1,3) fucosyltransferase)               | protein glycosylation / fucosylation  |
| GALNT7 | polypeptide N-acetylgalactosaminyltransferase 7                      | carbohydrate metabolic process / protein glycosylation / protein O-linked glycosylation / metabolic process / O-glycan processing / post-translational protein modification / cellular protein metabolic process  |
| GCNT1  | glucosaminyl (N-acetyl) transferase 1, core 2                        | protein glycosylation / metabolic process / glycoprotein biosynthetic process / O-glycan processing / response to insulin / post-translational protein modification / cellular protein metabolic process / tissue morphogenesis / leukocyte tethering or rolling / cell adhesion molecule production / kidney morphogenesis   |
| HLA-A  | major histocompatibility complex, class I, A                         | positive regulation of T cell mediated cytotoxicity / immune system process / antigen processing and presentation of peptide antigen via MHC class I / antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent / antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-independent / antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, TAP-independent / immune response / viral process / detection of bacterium / cytokine-mediated signaling pathway / antigen processing and presentation / positive regulation of interferon-gamma production / protection from natural killer cell mediated cytotoxicity / antigen processing and presentation of exogenous peptide antigen via MHC class I / regulation of defense response to virus by virus / regulation of immune response / interferon-gamma-mediated signaling pathway / type I interferon signaling pathway / positive regulation of memory T cell activation  |
| HLA-C  | major histocompatibility complex, class I, C                         | cilium or flagellum-dependent cell motility / positive regulation of T cell mediated cytotoxicity / immune system process / antigen processing and presentation of peptide antigen via MHC class I / antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent / antigen processing and presentation of exogenous peptide antigen via MHC class I via ER pathway, TAP-independent / immune response / microtubule-based movement / signal transduction / metabolic process / viral process / modulation by virus of host morphology or physiology / cytokine-mediated signaling pathway / antigen processing and presentation / antigen processing and presentation of exogenous peptide antigen via MHC class I / innate immune response / regulation of immune response / interferon-gamma-mediated signaling pathway / type I interferon signaling pathway   |
| HS6ST2 | heparan sulfate 6-O-sulfotransferase 2                               | carbohydrate metabolic process / glycosaminoglycan biosynthetic process / metabolic process / glycosaminoglycan metabolic process / small molecule metabolic process  |
| HSPA5  | heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)        | cell morphogenesis / platelet degranulation / ATP catabolic process / ER overload response / activation of signaling protein activity involved in unfolded protein response / blood coagulation / cerebellum structural organization / cerebellar Purkinje cell layer development / substantia nigra development / platelet activation / positive regulation of cell migration / ER-associated ubiquitin-dependent protein catabolic process / negative regulation of transforming growth factor beta receptor signaling pathway / endoplasmic reticulum unfolded protein response / positive regulation of protein ubiquitination / maintenance of protein localization in endoplasmic reticulum / cellular response to glucose starvation / negative regulation of apoptotic process / cellular protein metabolic process / proteolysis involved in cellular protein catabolic process / regulation of protein folding in endoplasmic reticulum / cellular response to antibiotic / cellular response to interleukin-4  |
| IMPAD1 | inositol monophosphatase domain containing 1                         | skeletal system development / endochondral ossification / chondrocyte development / inositol biosynthetic process / post-embryonic development / dephosphorylation / chondroitin sulfate metabolic process / embryonic digit morphogenesis / phosphatidylinositol phosphorylation   |
| ITCH   | itchy E3 ubiquitin protein ligase                                    | protein polyubiquitination / regulation of cell growth / immune system process / positive regulation of T cell anergy / ubiquitin-dependent protein catabolic process / apoptotic process / inflammatory response / Notch signaling pathway / metabolic process / viral process / protein ubiquitination / modulation by virus of host morphology or physiology / negative regulation of NF-kappaB transcription factor activity / negative regulation of type I interferon production / protein K29-linked ubiquitination / nucleotide-binding domain, leucine rich repeat containing receptor signaling pathway / protein ubiquitination involved in ubiquitin-dependent protein catabolic process / negative regulation of apoptotic process / innate immune response / positive regulation of protein catabolic process / negative regulation of JNK cascade / negative regulation of alpha-beta T cell proliferation / viral entry into host cell / negative regulation of defense response to virus / defense response to virus / nucleotide-binding oligomerization domain containing signaling pathway / protein K63-linked ubiquitination / protein K48-linked ubiquitination / regulation of protein deubiquitination |
| ITGA4  | integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor) | blood vessel remodeling / cell adhesion / heterophilic cell-cell adhesion / leukocyte cell-cell adhesion / integrin-mediated signaling pathway / heart development / blood coagulation / cell migration / B cell differentiation / extracellular matrix organization / regulation of immune response / leukocyte migration / face development / chorio-allantoic fusion / negative regulation of protein homodimerization activity  |



|          |   |  |
|----------|---|--|
| ITGA6    | integrin, alpha 6   | cell-substrate junction assembly / cell adhesion / cell-matrix adhesion / integrin-mediated signaling pathway / blood coagulation / positive regulation of cell-substrate adhesion / single organismal cell-cell adhesion / positive regulation of cell-cell adhesion / extracellular matrix organization / hemidesmosome assembly / cell-substrate adhesion / cellular response to extracellular stimulus / cell adhesion mediated by integrin / cell junction assembly / positive regulation of phosphorylation / odontogenesis of dentin-containing tooth / positive regulation of apoptotic process / positive regulation of transcription from RNA polymerase II promoter / filopodium assembly / brown fat cell differentiation / leukocyte migration / cellular response to organic cyclic compound / negative regulation of extrinsic apoptotic signaling pathway  |
| ITGA7    | integrin, alpha 7   | cell adhesion / cell-matrix adhesion / integrin-mediated signaling pathway / muscle organ development / skeletal muscle tissue development / regulation of cell shape / cell migration / extracellular matrix organization / blood vessel morphogenesis  |
| ITGA8    | integrin, alpha 8   | metanephros development / cell adhesion / cell-matrix adhesion / integrin-mediated signaling pathway / multicellular organismal development / nervous system development / brain development / memory / single organismal cell-cell adhesion / cell projection organization / cell differentiation / extracellular matrix organization / positive regulation of transforming growth factor beta receptor signaling pathway / inner ear morphogenesis / establishment of protein localization / smooth muscle tissue development / positive regulation of transcription from RNA polymerase II promoter involved in smooth muscle cell differentiation  |
| KIAA1161 | KIAA1161  | carbohydrate metabolic process / metabolic process / positive regulation of insulin-like growth factor receptor signaling pathway / skeletal muscle fiber development / positive regulation of protein kinase B signaling  |
| LRFN3    | leucine rich repeat and fibronectin type III domain containing 3                                    | cell adhesion  |
| MAN2A1   | mannosidase, alpha, class 2A, member 1  | in utero embryonic development / liver development / carbohydrate metabolic process / mannose metabolic process / protein glycosylation / N-glycan processing / mitochondrion organization / vacuole organization / respiratory gaseous exchange / metabolic process / protein N-linked glycosylation via asparagine / post-translational protein modification / cellular protein metabolic process / lung alveolus development / positive regulation of neurogenesis / retina morphogenesis in camera-type eye  |
| MGAT1    | mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase                         | in utero embryonic development / carbohydrate metabolic process / UDP-N-acetylglucosamine catabolic process / protein glycosylation / protein N-linked glycosylation via asparagine / post-translational protein modification / cellular protein metabolic process   |
| MGAT5    | mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetylglucosaminyltransferase                         | protein glycosylation / protein N-linked glycosylation / protein N-linked glycosylation via asparagine / post-translational protein modification / cellular protein metabolic process  |
| MOXD1    | monooxygenase, DBH-like 1   | catecholamine metabolic process / oxidation-reduction process  |
| MUCL1    | mucin-like 1  | O-glycan processing / post-translational protein modification / cellular protein metabolic process   |
| NDST2    | N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 2   | carbohydrate metabolic process / glycosaminoglycan biosynthetic process / metabolic process / heparan sulfate proteoglycan biosynthetic process / glycosaminoglycan metabolic process / heparin biosynthetic process / small molecule metabolic process  |
| NEDD4L   | neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein ligase | negative regulation of transcription from RNA polymerase II promoter / negative regulation of systemic arterial blood pressure / regulation of membrane depolarization / transcription, DNA-templated / transcription initiation from RNA polymerase II promoter / protein monoubiquitination / sodium ion transport / cellular sodium ion homeostasis / transforming growth factor beta receptor signaling pathway / excretion / metabolic process / response to salt stress / response to metal ion / gene expression / positive regulation of sodium ion transport / negative regulation of sodium ion transport / viral process / protein ubiquitination / modulation by virus of host morphology or physiology / viral life cycle / water homeostasis / negative regulation of transforming growth factor beta receptor signaling pathway / ion transmembrane transport / regulation of ion transmembrane transport / regulation of protein catabolic process / regulation of membrane potential / protein ubiquitination involved in ubiquitin-dependent protein catabolic process / proteasome-mediated ubiquitin-dependent protein catabolic process / ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway / positive regulation of protein catabolic process / positive regulation of endocytosis / transmembrane transport / regulation of membrane repolarization / protein K48-linked ubiquitination / ventricular cardiac muscle cell action potential / regulation of potassium ion transmembrane transporter activity / negative regulation of potassium ion transmembrane transporter activity / negative regulation of potassium ion transmembrane transport / negative regulation of sodium ion transmembrane transport / negative regulation of protein localization to cell surface / negative regulation of sodium ion transmembrane transporter activity / regulation of tight junction assembly / positive regulation of cation channel activity / positive regulation of caveolin-mediated endocytosis |
| NEDD4    | neural precursor cell expressed, developmentally down-regulated 4, E3 ubiquitin protein ligase      | negative regulation of transcription from RNA polymerase II promoter / adaptive immune response / outflow tract morphogenesis / endocardial cushion development / protein monoubiquitination / protein targeting to lysosome / lysosomal transport / nervous system development / neuromuscular junction development / metabolic process / negative regulation of sodium ion transport / negative regulation of transcription from RNA polymerase II promoter in response to UV-induced DNA damage / positive regulation of phosphatidylinositol 3-kinase signaling / viral process / protein ubiquitination / modulation by virus of host morphology or physiology / transmission of virus / cytokine-mediated signaling pathway / negative regulation of vascular endothelial growth factor receptor signaling pathway / neuron projection development / receptor internalization / receptor catabolic process / cellular response to UV / regulation of ion transmembrane transport / T cell activation / regulation of membrane potential / protein ubiquitination involved in ubiquitin-dependent protein catabolic process / glucocorticoid receptor signaling pathway / ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway / development involved in symbiotic interaction / positive regulation of protein catabolic process / positive regulation of nucleocytoplasmic transport / blood vessel morphogenesis / regulation of dendrite morphogenesis / regulation of synapse organization / progesterone receptor signaling pathway / response to calcium ion / protein K63-linked ubiquitination / regulation of potassium ion transmembrane transporter activity   |
| NETO2    | neuropilin (NRP) and tolloid (TLL)-like 2   | regulation of kainate selective glutamate receptor activity  |
| PAM      | peptidylglycine alpha-amidating monooxygenase   | peptide amidation / response to hypoxia / long-chain fatty acid metabolic process / regulation of transcription from RNA polymerase II promoter / cellular protein modification process / peptide metabolic process / mitotic chromosome condensation / central nervous system development / heart development / lactation / metabolic process / response to pH / toxin metabolic process / protein amidation / protein metabolic process / ovulation cycle process / response to estradiol / regulation of actin cytoskeleton organization / odontogenesis / response to drug / response to copper ion / regulation of protein secretion / protein homo-oligomerization / response to glucocorticoid / oxidation-reduction process / maternal process involved in female pregnancy / limb development   |

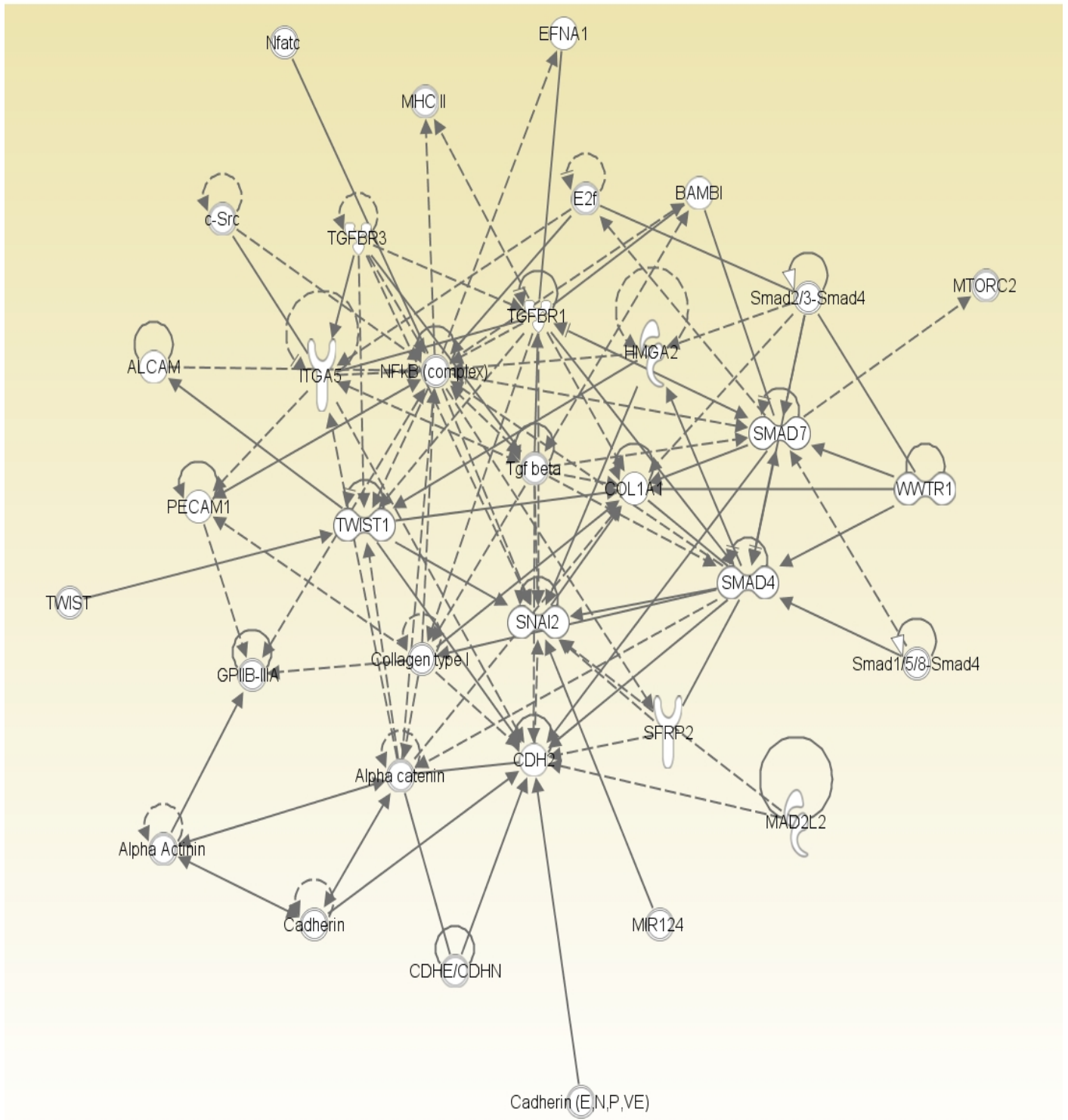
|         |  |  |
|---------|--|--|
| PDF     | peptide deformylase, mitochondrial   | co-translational protein modification / translation / positive regulation of cell proliferation / peptidyl-methionine modification / N-terminal protein amino acid modification  |
| PLXNA1  | plexin A1  | signal transduction / multicellular organismal development / axon guidance / regulation of smooth muscle cell migration / positive regulation of GTPase activity / regulation of small GTPase mediated signal transduction / dichotomous subdivision of terminal units involved in salivary gland branching / semaphorin-plexin signaling pathway  |
| PLXNB2  | plexin B2  | neural tube closure / regulation of protein phosphorylation / signal transduction / multicellular organismal development / neuroblast proliferation / brain development / regulation of cell shape / regulation of Rho GTPase activity / positive regulation of GTPase activity / positive regulation of axonogenesis / regulation of small GTPase mediated signal transduction / semaphorin-plexin signaling pathway / regulation of neuron migration   |
| POMGNT2 | protein O-linked mannose N-acetylglucosaminyltransferase 2 (beta 1,4-)   | protein glycosylation / protein O-linked glycosylation   |
| POMT2   | protein-O-mannosyltransferase 2  | protein glycosylation / protein O-linked glycosylation / protein O-linked mannosylation / mannosylation  |
| PXYLP1  | 2-phosphoxylose phosphatase 1  | metabolic process / dephosphorylation  |
| RNF130  | ring finger protein 130  | apoptotic process / programmed cell death / protein ubiquitination   |
| SEMA4F  | sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4F | cell-cell signaling / multicellular organismal development / nervous system development / axon guidance / cell differentiation / negative regulation of axon extension / retinal ganglion cell axon guidance   |
| SEMA6A  | sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A                                  | neuron migration / apoptotic process / cytoskeleton organization / cell surface receptor signaling pathway / multicellular organismal development / nervous system development / axon guidance / organ morphogenesis / cell differentiation / centrosome localization / semaphorin-plexin signaling pathway / positive regulation of neuron migration  |
| SLIT2   | slit homolog 2 (Drosophila)  | metanephros development / ureteric bud development / in utero embryonic development / negative regulation of protein phosphorylation / cell migration involved in sprouting angiogenesis / negative regulation of leukocyte chemotaxis / chemotaxis / multicellular organismal development / nervous system development / axonogenesis / axon guidance / motor neuron axon guidance / negative regulation of cell proliferation / negative regulation of lamellipodium assembly / negative regulation of endothelial cell migration / negative regulation of gene expression / negative regulation of smooth muscle cell migration / single organismal cell-cell adhesion / olfactory bulb development / chemorepulsion involved in embryonic olfactory bulb interneuron precursor migration / chemorepulsion involved in postnatal olfactory bulb interneuron migration / corticospinal neuron axon guidance through spinal cord / telencephalon cell migration / cell differentiation / negative regulation of cell growth / negative regulation of cell migration / negative regulation of actin filament polymerization / retinal ganglion cell axon guidance / cellular response to hormone stimulus / dorsal/ventral axon guidance / Roundabout signaling pathway / positive regulation of apoptotic process / negative regulation of catalytic activity / negative regulation of vascular permeability / branching morphogenesis of an epithelial tube / neuron projection morphogenesis / axon extension involved in axon guidance / positive regulation of axonogenesis / negative chemotaxis / induction of negative chemotaxis / negative regulation of small GTPase mediated signal transduction / response to cortisol / mammary gland duct morphogenesis / mammary duct terminal end bud growth / apoptotic process involved in luteolysis / negative regulation of chemokine-mediated signaling pathway / cellular response to heparin / negative regulation of smooth muscle cell chemotaxis / negative regulation of mononuclear cell migration / negative regulation of neutrophil chemotaxis / negative regulation of monocyte chemotaxis / negative regulation of retinal ganglion cell axon guidance / negative regulation of cellular response to growth factor stimulus   |
| SMO     | smoothened, frizzled class receptor  | negative regulation of transcription from RNA polymerase II promoter / ossification / vasculogenesis / osteoblast differentiation / in utero embryonic development / cell fate specification / neural crest cell migration / heart looping / positive regulation of neuroblast proliferation / positive regulation of mesenchymal cell proliferation / heart morphogenesis / determination of left/right asymmetry in lateral mesoderm / type B pancreatic cell development / signal transduction / cell surface receptor signaling pathway / G-protein coupled receptor signaling pathway / smoothened signaling pathway / positive regulation of hh target transcription factor activity / multicellular organismal development / spermatogenesis / determination of left/right symmetry / ventral midline determination / pattern specification process / central nervous system development / midgut development / positive regulation of cell proliferation / gonad development / dorsal/ventral pattern formation / cardioblast differentiation / regulation of gene expression / positive regulation of gene expression / negative regulation of gene expression / floor plate formation / facial nerve development / cerebellar cortex morphogenesis / thalamus development / dorsal/ventral neural tube patterning / smoothened signaling pathway involved in ventral spinal cord patterning / smoothened signaling pathway involved in regulation of cerebellar granule cell precursor cell proliferation / central nervous system neuron differentiation / adenohypophysis development / negative regulation of epithelial cell differentiation / exocrine pancreas development / hair follicle morphogenesis / embryonic camera-type eye development / neuron projection regeneration / otolith morphogenesis / protein localization to nucleus / multicellular organism growth / positive regulation of protein import into nucleus / odontogenesis of dentin-containing tooth / muscle cell fate commitment / negative regulation of apoptotic process / negative regulation of DNA binding / positive regulation of smoothened signaling pathway / positive regulation of transcription from RNA polymerase II promoter / cell development / embryonic digestive tract morphogenesis / digestive tract development / developmental growth / neuron fate commitment / embryonic neurocranium morphogenesis / embryonic viscerocranium morphogenesis / semicircular canal morphogenesis / axon extension involved in axon guidance / forebrain morphogenesis / positive regulation of epithelial cell proliferation / protein stabilization / negative regulation of hair follicle development / canonical Wnt signaling pathway / detection of cell density by contact stimulus involved in contact inhibition / atrial septum morphogenesis / mammary gland epithelial cell differentiation / epithelial-mesenchymal cell signaling / ciliary receptor clustering involved in smoothened signaling pathway / somite development / pancreas morphogenesis / cellular response to cholesterol / renal system development / mesenchymal to epithelial transition involved in metanephric renal vesicle formation / positive regulation of branching involved in ureteric bud morphogenesis / regulation of stem cell maintenance / regulation of heart morphogenesis |
| SMURF1  | SMAD specific E3 ubiquitin protein ligase 1  | protein polyubiquitination / protein export from nucleus / transforming growth factor beta receptor signaling pathway / ectoderm development / metabolic process / protein ubiquitination / cell differentiation / negative regulation of ossification / BMP signaling pathway / negative regulation of transforming growth factor beta receptor signaling pathway / negative regulation of BMP signaling pathway / ubiquitin-dependent SMAD protein catabolic process / receptor catabolic process / protein localization to cell surface / protein ubiquitination involved in ubiquitin-dependent protein catabolic process / proteasome-mediated ubiquitin-dependent protein catabolic process  |

|            |   |  |
|------------|---|--|
| SMURF2     | SMAD specific E3 ubiquitin protein ligase 2   | negative regulation of transcription from RNA polymerase II promoter / transcription, DNA-templated / transcription initiation from RNA polymerase II promoter / ubiquitin-dependent protein catabolic process / transforming growth factor beta receptor signaling pathway / metabolic process / gene expression / protein ubiquitination / regulation of transforming growth factor beta receptor signaling pathway / BMP signaling pathway / negative regulation of transforming growth factor beta receptor signaling pathway / ubiquitin-dependent SMAD protein catabolic process / protein ubiquitination involved in ubiquitin-dependent protein catabolic process / negative regulation of transcription, DNA-templated  |
| SPPL2B     | signal peptide peptidase like 2B  | proteolysis / membrane protein ectodomain proteolysis / membrane protein intracellular domain proteolysis / regulation of immune response  |
| ST3GAL4    | ST3 beta-galactoside alpha-2,3-sialyltransferase 4  | carbohydrate metabolic process / protein glycosylation / O-glycan processing / keratan sulfate biosynthetic process / protein N-linked glycosylation via asparagine / glycosaminoglycan metabolic process / keratan sulfate metabolic process / post-translational protein modification / cellular protein metabolic process / small molecule metabolic process / cognition / sialylation  |
| ST3GAL6    | ST3 beta-galactoside alpha-2,3-sialyltransferase 6  | carbohydrate metabolic process / cellular protein modification process / protein glycosylation / glycolipid metabolic process / oligosaccharide metabolic process / keratan sulfate biosynthetic process / glycosaminoglycan metabolic process / keratan sulfate metabolic process / small molecule metabolic process / cellular response to interleukin-6 / sialylation   |
| ST6GALNAC3 | ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 3 | protein glycosylation / glycosylceramide metabolic process / sialylation   |
| ST8SIA4    | ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4  | ganglioside biosynthetic process / cellular protein modification process / protein glycosylation / N-glycan processing / nervous system development / axon guidance / oligosaccharide metabolic process / sialylation  |
| SULF2      | sulfatase 2   | kidney development / chondrocyte development / glomerular filtration / metabolic process / positive regulation vascular endothelial growth factor production / esophagus smooth muscle contraction / positive regulation of Wnt signaling pathway / heparan sulfate proteoglycan metabolic process / glomerular basement membrane development / glial cell-derived neurotrophic factor receptor signaling pathway / negative regulation of fibroblast growth factor receptor signaling pathway / embryonic skeletal system development / cartilage development / bone development / innervation  |
| TGFB3      | transforming growth factor, beta receptor III   | blood vessel development / response to hypoxia / in utero embryonic development / blastocyst development / epithelial to mesenchymal transition / liver development / heart morphogenesis / protein complex assembly / immune response / transforming growth factor beta receptor signaling pathway / transforming growth factor beta receptor complex assembly / negative regulation of epithelial cell migration / negative regulation of epithelial to mesenchymal transition / cell growth / cell migration / BMP signaling pathway / positive regulation of transforming growth factor beta receptor signaling pathway / negative regulation of transforming growth factor beta receptor signaling pathway / organ regeneration / response to follicle-stimulating hormone / response to prostaglandin E / response to luteinizing hormone / intracellular signal transduction / regulation of protein binding / negative regulation of epithelial cell proliferation / positive regulation of NF-kappaB transcription factor activity / negative regulation of cellular component movement / ventricular cardiac muscle tissue morphogenesis / palate development / cardiac muscle cell proliferation / definitive hemopoiesis / cardiac epithelial to mesenchymal transition / definitive erythrocyte differentiation / heart trabecula formation / pathway-restricted SMAD protein phosphorylation |
| TMEM2      | transmembrane protein 2   | multicellular organismal development   |
| TMEM131    | transmembrane protein 131   | ---  |
| TMEM132A   | transmembrane protein 132A  | ---  |
| TMEM160    | transmembrane protein 160   | ---  |
| TOR1AIP1   | torsin A interacting protein 1  | positive regulation of ATPase activity / protein localization to nucleus / nuclear membrane organization   |
| TPST2      | tyrosylprotein sulfotransferase 2   | peptidyl-tyrosine sulfation / fusion of sperm to egg plasma membrane / prevention of polyspermy  |
| TTC17      | tetratricopeptide repeat domain 17  | cell projection organization / actin filament polymerization / cilium organization   |
| TXNDC15    | thioredoxin domain containing 15  | cell redox homeostasis   |
| UGT3A2     | UDP glycosyltransferase family 3 member A2  | cellular response to genesterin / flavonoid biosynthetic process / flavonoid glucuronidation   |
| UGT8       | UDP glycosyltransferase 8   | protein localization to paranode region of axon / carbohydrate metabolic process / lipid metabolic process / sphingolipid metabolic process / galactosylceramide biosynthetic process / cytoskeleton organization / central nervous system development / peripheral nervous system development / axon cargo transport / metabolic process / lipid glycosylation / paranodal junction assembly / neuron projection morphogenesis  |
| WWP1       | WW domain containing E3 ubiquitin protein ligase 1  | signal transduction / central nervous system development / metabolic process / viral process / protein ubiquitination / modulation by virus of host morphology or physiology / ion transmembrane transport / protein ubiquitination involved in ubiquitin-dependent protein catabolic process / proteasome-mediated ubiquitin-dependent protein catabolic process / negative regulation of transcription, DNA-templated / viral entry into host cell / transmembrane transport   |
| WWP2       | WW domain containing E3 ubiquitin protein ligase 2  | negative regulation of transcription from RNA polymerase II promoter / cellular protein modification process / negative regulation of gene expression / viral process / protein ubiquitination / modulation by virus of host morphology or physiology / negative regulation of transporter activity / regulation of ion transmembrane transport / regulation of membrane potential / protein ubiquitination involved in ubiquitin-dependent protein catabolic process / proteasome-mediated ubiquitin-dependent protein catabolic process / negative regulation of sequence-specific DNA binding transcription factor activity / negative regulation of transcription, DNA-templated / viral entry into host cell / negative regulation of protein transport / protein autoubiquitination / protein K63-linked ubiquitination / regulation of potassium ion transmembrane transporter activity   |
| YAP1       | Yes-associated protein 1  | vasculogenesis / embryonic heart tube morphogenesis / regulation of transcription, DNA-templated / transcription from RNA polymerase II promoter / transcription initiation from RNA polymerase II promoter / cellular response to DNA damage stimulus / cell proliferation / positive regulation of cell proliferation / gene expression / regulation of keratinocyte proliferation / keratinocyte differentiation / negative regulation of epithelial cell differentiation / notochord development / somatic stem cell maintenance / hippo signaling / regulation of cell proliferation / negative regulation of apoptotic process / positive regulation of transcription from RNA polymerase II promoter / positive regulation of organ growth / paraxial mesoderm development / lateral mesoderm development / contact inhibition / cellular response to gamma radiation / regulation of stem cell proliferation / positive regulation of canonical Wnt signaling pathway / negative regulation of extrinsic apoptotic signaling pathway   |





Path Designer Network 1



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**Figure 4.** Network generated by Ingenuity Pathway Analysis (IPA)

A network generated with PTCH1 and EMT-related genes included ALCAM, BAMBI, CDH2, COL1A1, EFNA1, HMG2, ITGA5, MAD2L2, PECAM1, SFRP2, SMAD4, SMAD7, SNAI2, TGFB1, TGFB3, TWIST1 and WWTR1. It was associated with cardiovascular system development and function, and cellular movement.

lesions in GC [33]. VIM was queried for network analysis in cBioPortal for Cancer Genomics, which resulted in PTCH1 model network generation including VIM.

The metastasis was induced by stemness promotion by HER2, WNT4 or RANKL [34]. The activation of migration was observed in migration/sphere-formation assays [34]. The high-throughput 3D screening system for EMT inhibitors has revealed that TGF-beta receptor I inhibitor SB431542 suppressed the EMT-related phenotypic changes such as mRNA level alteration of CDH1, CDH2, VIM and zinc finger E-box binding homeobox 1 (ZEB1) [35].

A database, such as the National Health and Nutritional Examination Survey (NHANES), which allows the correlation analysis of gene expression, cancer types, and cancer stages, and patient demographics and phenotypes, may be useful to understand cancer mechanism and improve public health [36].

In conclusion, the Hedgehog receptor PTCH1 gene expression was found to be up-regulated in diffuse-type GC compared to MSCs in microarray analysis. PTCH1 and EMT-related molecular network was generated, which may reveal the EMT mechanism in diffuse-type GC and stem cells.

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