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*Research article*

# Molecular pathway network of EFNA1 in cancer and mesenchymal stem cells

**Shihori Tanabe[1,*], Kazuhiko Aoyagi[2], Hiroshi Yokozaki[3] and Hiroki Sasaki[4]**

[1] Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, Kawasaki, Japan
[2] Department of Clinical Genomics, National Cancer Center Research Institute, Tokyo, Japan
[3] Department of Pathology, Kobe University of Graduate School of Medicine, Kobe, Japan
[4] Department of Translational Oncology, National Cancer Center Research Institute, Tokyo, Japan

*** Correspondence:** Email: stanabe@nihs.go.jp; Phone: +81442706686; Fax: +81442706703.

**Abstract:** Abundant molecules are dynamically activated in cancer and stem cells. To investigate the role of ephrin A1 (EFNA1) in cancer and stem cell signaling pathways, we analyzed the gene expression and molecular network of EFNA1 in mesenchymal stem cells (MSCs) and diffuse-type gastric cancer (GC). Diffuse-type GC has more mesenchymal-like feature and malignant characteristics compared to intestinal-type GC. The signaling and molecular network of EFNA1 in cancer and stem cells were analyzed using several databases, including cBioPortal for Cancer Genomics, Kyoto Encyclopedia of Genes and Genomes (KEGG). The gene expression of EFNA1 was up-regulated in diffuse-type GC compared to MSCs. The molecular pathway network of EFNA1 includes cadherin 1 (CDH1), catenin beta 1 (CTNNB1), ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1) (RAC1), EPH receptor A5 (EPHA5), and the KRAS proto-oncogene, GTPase (KRAS). We summarized molecular pathway network of EFNA1 in cancer and stem cells. The results revealed a network model for EFNA1 in cancer and stem cells.

**Keywords:** epithelial-mesenchymal transition; gastric cancer; gene expression; mesenchymal stem cell; EFNA1; signaling pathway

**Abbreviations:** CDH1: cadherin 1; CTNNB1: catenin beta 1; EFNA1: ephrin A1; EMT: epithelial-mesenchymal transition; EPHA5: EPH receptor A5; GC: gastric cancer; KEGG: Kyoto Encyclopedia of Genes and Genomes; KRAS: KRAS proto-oncogene; MSC: mesenchymal stem

cell; RAC1: ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)

## 1. Introduction

The signaling network moves dynamically in stem cells and cancer. An alteration in the gene expression of molecules drives the signaling network pattern transition. The molecular pattern changes upon cellular stimuli or cellular phenotype transition. Among the abundant molecule-related cancer and stem cell signaling pathways, the membrane protein is an entrance for the intracellular pathway cascade. Ephrin A1 (EFNA1) encodes a member of the ephrins (Eph receptor interacting proteins), which functions as a ligand for the Eph (erythropoietin-producing hepatocellular carcinoma) receptor protein-tyrosine kinase family [1–4]. EFNA1 binds to the EPHA2, EPHA4, EPHA5, EPHA6 and EPHA7 receptors *in vitro* and selectively binds to EPHA4 but not EPHA7 in the lysates of rat striatal tissue [5–9].

EPH/ephrin signaling is involved in many cellular functions such as cell proliferation and cell cycle progression, and it is suggested to play a role even in cancer stem cells (CSCs) [2]. EFNA1 is up-regulated in melanoma progression [10]. The EFNA1 and EPHA2 axis is implicated in gastric cancer (GC) [11]. EPHA2 expression is a poor prognostic marker in stage II/III colorectal cancer [5]. The EPHA2 and EFNA1 signaling system leads to an increase in the migration and invasion of solid tumors [12]. In contrast, EPHA1 is suggested to be a tumor suppressor [13]. Ephrins and EPHAs are induced in stem cells and regulate myogenic progression [14]. We have previously demonstrated that the epithelial-mesenchymal transition (EMT) network includes EFNA1 [15]. Since EPH/ephrin signaling is important for cancer, we investigated the EFNA1 gene expression and network pathway related to EMT in diffuse-type GC and mesenchymal stem cells (MSCs). EMT plays a role in cancer metastasis and malignancy, which is a critical phenotype of cancer stem cells (CSCs). Diffuse-type GC exhibits the EMT-like feature compared to the intestinal-type GC, which may contribute to CSC phenotype. We investigated the gene expression profiling in MSCs and diffuse-type GC, since the regulated genes in diffuse-type GC may contain the molecules related to malignancy or CSCs. To elucidate the EFNA1 role in EMT mechanism associated with cancer and stem cells, we compared EFNA1 gene expression in MSCs and diffuse-type GC.

## 2. Materials and methods

### *2.1. Cell cultures of MSCs and diffuse-type GC samples*

The human bone marrow MSCs were commercially available (Lonza, Walkersville, MD, USA) and 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 ℃ in a $CO_2$ (5%) incubator as previously described [15]. The diffuse-type GC tissues were provided by the National Cancer Center Hospital in Japan after obtaining written informed consent from each patient and approval from the National Cancer Center Institutional Review Board, and total RNA was obtained from the frozen sample [16].

### *2.2. Gene expression analysis of MSCs and diffuse-type GC*

The gene expression in MSCs (n = 12) and diffuse-type GC (n = 5) was analyzed with GeneChip® Human Genome U133 Plus 2.0 microarray (Affymetrix, Santa Clara, California, USA), as previously described [15–17]. Briefly, total RNA purified from the cells were biotinylated and hybridized to the microarray. The signal intensity in each gene was analyzed and 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 [15–17].

### *2.3. Molecular Pathway Network of EFNA1*

The cancer genomics data analysis related to *EFNA1* was performed using the cBioPortal for Cancer Genomics (http://www.cbioportal.org) [18,19]. The term "EFNA1" was searched in the cBioPortal for Cancer Genomics, and the cross-cancer alteration summary for *EFNA1* was obtained. The network pathway analysis of EFNA1 using the cBioPortal for Cancer Genomics (http://www.cbioportal.org) showed the cross-cancer alteration and EFNA1-associated network. The EFNA1 molecular network was analyzed in stomach adenocarcinoma (TCGA, Nature 2014, tumor samples with sequencing and CNA data, 287 samples/1 gene) [20].

### *2.4. Gene Ontology analysis of EFNA1*

Gene Ontology of *EFNA1* was analyzed using several databases, including the EMBL-EBI (http://www.ebi.ac.uk/QuickGO/), AmiGO 2 (http://amigo.geneontology.org/amigo/landing) and the Gene Ontology Consortium (http://geneontology.org/).

### *2.5. Pathway network analysis of EFNA1 and the related genes*

Pathway network analysis was performed using the databases VaProS (http://pford.info/vapros/), Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/), cBioPortal for Cancer Genomics (http://www.cbioportal.org), and Cytoscape (http://www.cytoscape.org/). The localization of molecules was analyzed using The Human Protein Atlas (http://www.proteinatlas.org/) [21] and UniProt (http://www.uniprot.org/). Molecular interactions were analyzed using the BioGRID (http://www.thebiogrid.org) database.

### *2.6. Statistical analysis*

The data were expressed as the mean ± SE. For the statistics, Student's *t*-test in each probe sets was performed in Microsoft® Excel®. $p < 0.05$, 0.01 or 0.001 (n = 12 in MSCs, n = 5 in GC) was considered as statistically significant.

## 3. Results

### *3.1. Gene expression of EFNA1 in MSCs and diffuse-type GC*

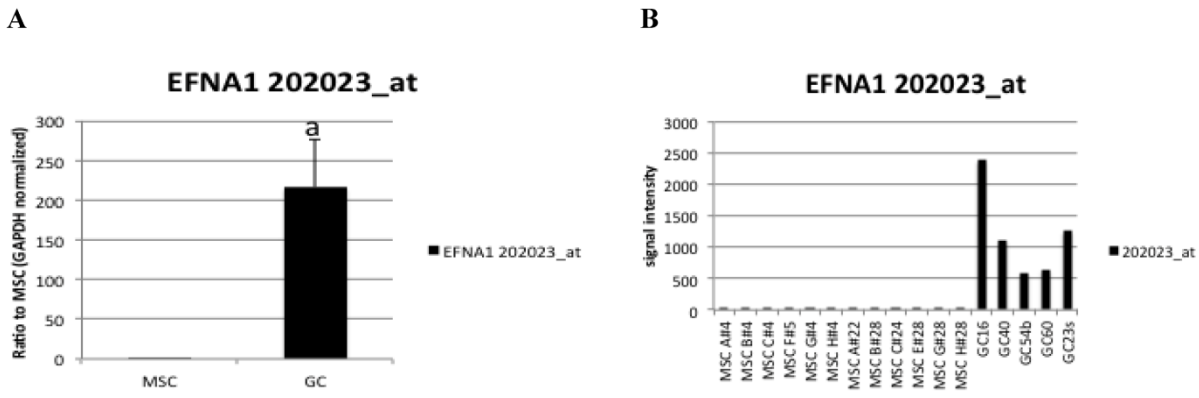


**Figure 1.** Gene expression of EFNA1 in MSCs and diffuse-type GC.

A: Microarray analysis revealed that gene expression of EFNA1 was up-regulated in diffuse-type GC compared to MSCs ([a] $p < 0.001$ in Student's *t*-test, n = 12 in MSCs, n = 5 in diffuse-type GC). B: The signal intensity in each samples of MSCs and diffuse-type GC are shown.

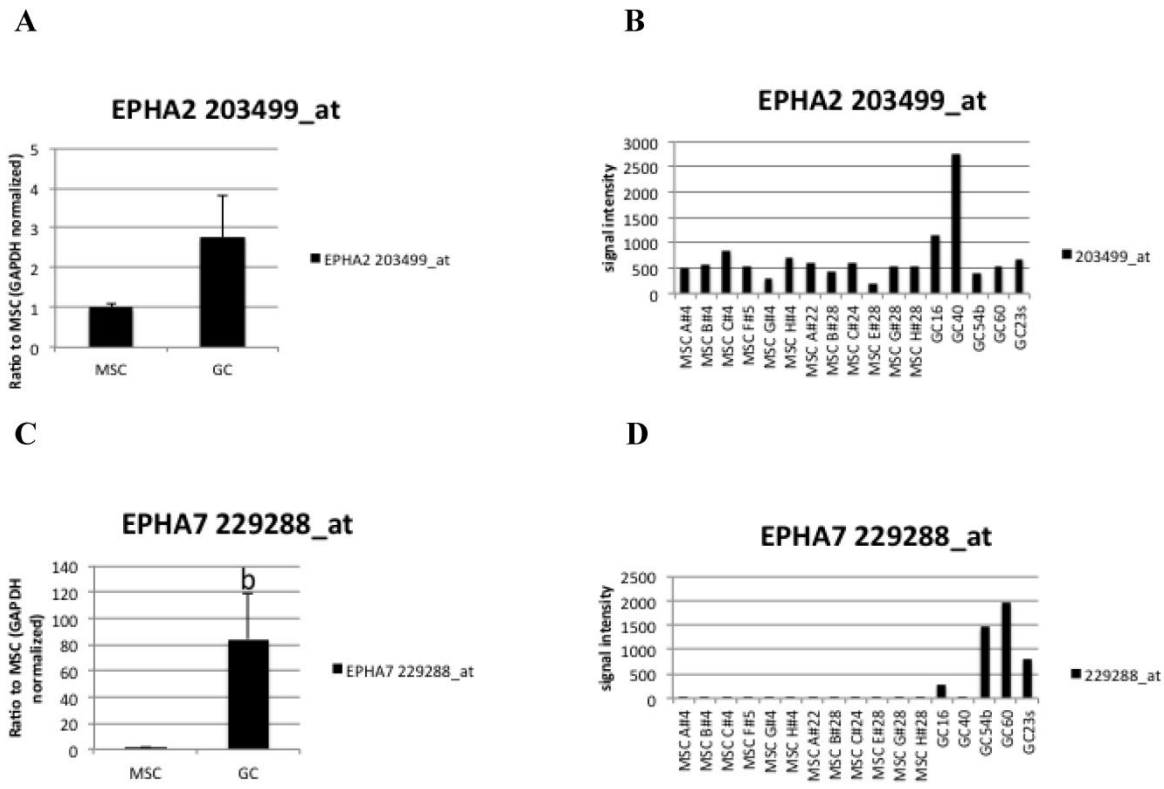


**Figure 2.** Gene expression of EPHAs in MSCs and diffuse-type GC.

A: EPHA2 gene expression in MSCs and diffuse-type GC is shown. B: EPHA2 gene expression in each sample of MSCs and diffuse-type GC is shown. C: EPHA7 gene expression in MSCs and diffuse-type GC is shown. D: EPHA7 gene expression in each sample of MSCs and diffuse-type GC is shown ([b] $p < 0.01$ in Student's *t*-test, n = 12 in MSCs, n = 5 in diffuse-type GC).

The gene expression of EFNA1 was up-regulated in diffuse-type GC compared to MSCs (Figure 1). The gene expression of EPHA2, a receptor for EFNA1, was up-regulated in some samples of diffuse-type GC compared to MSCs (Figure 2A, B). The gene expression of EPHA7 was differentially up-regulated in diffuse-type GC compared to MSCs (Figure 2C, D). The data are expressed as the means ± SE. In case significantly different, *p* value calculated with Student's t-test is shown in Figure legends.

### *3.2. Gene Ontology of EFNA1*

According to the KEGG pathway, pathways of EFNA1 are the Ras signaling pathway, Rap1 signaling pathway, PI3K-Akt signaling pathway and axon guidance. The Gene Ontology (GO) of EFNA1 includes cell migration, cell-cell signaling, ephrin receptor binding, ephrin receptor signaling pathway, and negative regulation of EMT (Table 1) (http://amigo.geneontology.org/amigo/gene_product/UniProtKB:P20827).

**Table 1.** Gene Ontology of EFNA1 (AmiGO2, *Homo sapiens*).

| GO class (direct) | Evidence |
|---|---|
| anchored component of plasma membrane | IBA |
| angiogenesis | IBA |
| aortic valve morphogenesis | ISS |
| axon guidance | IBA |
| cell migration | IDA |
| cell-cell signaling | TAS |
| endocardial cushion to mesenchymal transition involved in heart valve formation | ISS |
| ephrin receptor binding | IBA, IPI |
| ephrin receptor signaling pathway | IBA, IDA, IGI, TAS |
| extracellular region | IEA |
| integral component of plasma membrane | TAS |
| mitral valve morphogenesis | ISS |
| negative regulation of dendritic spine morphogenesis | ISS |
| negative regulation of epithelial to mesenchymal transition | ISS |
| negative regulation of MAPK cascade | IEA |
| negative regulation of proteolysis involved in cellular protein catabolic process | IGI |
| negative regulation of thymocyte apoptotic process | IEA |
| negative regulation of transcription by RNA polymerase II | ISS |
| notochord formation | IEA |
| plasma membrane | IBA, NAS, TAS |
| positive regulation of amyloid-beta formation | IDA |
| positive regulation of aspartic-type endopeptidase activity involved in amyloid precursor protein catabolic process | IGI |
| positive regulation of MAPK cascade | IEA |
| positive regulation of peptidyl-tyrosine phosphorylation | IDA |
| positive regulation of protein phosphorylation | IGI |

*Continued on next page*

| GO class (direct) | Evidence |
|---|---|
| positive regulation of protein tyrosine kinase activity | IGI |
| protein binding | IPI |
| protein stabilization | IGI |
| regulation of angiogenesis | IEA |
| regulation of axonogenesis | IEA |
| regulation of blood vessel endothelial cell migration | IEA |
| regulation of cell adhesion mediated by integrin | IDA |
| regulation of peptidyl-tyrosine phosphorylation | ISS |
| signaling receptor binding | TAS |
| substrate adhesion-dependent cell spreading | IDA |

IBA: Inferred from Biological Ancestry, IDA: Inferred from Direct Assay, IEA: Inferred from Electronic Annotation, IGI: Inferred from Genetic Interaction, IPI: Inferred from Physical Interaction, ISS: Inferred from Sequence or structural Similarity, NAS: Non-traceable Author Statement, TAS: Traceable Author Statement.

### *3.3. Enrichment analysis of EFNA1*

The enrichment analysis of EFNA1 using the cBioPortal for Cancer Genomics revealed mRNA expression alteration of SCARNA2 and ZFAND1 in EFNA1-altered cases and EFNA1-unaltered cases in stomach adenocarcinoma (TCGA, Nature 2014) (Table 2) [20].

**Table 2.** mRNA expression alteration in EFNA1-altered and unaltered cases (cBioPortal).

| Gene | Cytoband | Mean mRNA expression (Altered) | Mean mRNA expression (Unaltered) | Standard deviation of mRNA expression (Altered) | Standard deviation of mRNA expression (Unaltered) | *p*-Value | *q*-Value |
|---|---|---|---|---|---|---|---|
| SCARNA2 | 1q13.1 | 3.7 | 2.44 | 0.26 | 1.43 | 5.65E−09 | 8.17E−05 |
| ZFAND1 | 8q21.13 | 3.46 | 4.11 | 0.19 | 0.68 | 6.17E−06 | 0.0446 |
| NGEF | 2q37 | 2.62 | 1.25 | 0.44 | 1.66 | 1.75E−05 | 0.0845 |
| PTAFR | 1p35−p34.3 | 2.13 | 2.54 | 0.15 | 1.05 | 4.18E−05 | 0.151 |
| STK17A | 7p13 | 2.54 | 2.91 | 0.14 | 0.54 | 9.73E−05 | 0.282 |
| CHI3L1 | 1q32.1 | 1.63 | 2.83 | 0.49 | 2.09 | 1.22E−04 | 0.295 |
| CEP120 | 5q23.2 | 1.62 | 2.25 | 0.25 | 0.6 | 2.99E−04 | 0.552 |
| TPD52L1 | 6q22−q23 | 2.38 | 0.92 | 0.64 | 2.11 | 3.48E−04 | 0.552 |
| ZNF232 | 17p13.2 | 0.95 | 1.4 | 0.2 | 0.62 | 3.82E−04 | 0.552 |
| IGSF9 | 1q22−q23 | 2.97 | 1.53 | 0.64 | 1.86 | 4.08E−04 | 0.552 |
| CDH13 | 16q23.3 | 0.98 | 1.93 | 0.42 | 1.06 | 4.66E−04 | 0.552 |
| RMDN1 | 8q21.3 | 3.15 | 3.56 | 0.19 | 0.56 | 5.06E−04 | 0.552 |
| PAPD4 | 5q14.1 | 3.07 | 3.6 | 0.23 | 0.48 | 5.95E−04 | 0.552 |

*Continued on next page*

| Gene | Cytoband | Mean mRNA expression (Altered) | Mean mRNA expression (Unaltered) | Standard deviation of mRNA expression (Altered) | Standard deviation of mRNA expression (Unaltered) | *p*-Value | *q*-Value |
|---|---|---|---|---|---|---|---|
| GPR35 | 2q37.3 | 4.76 | 4.08 | 0.33 | 1.42 | 6.08E−04 | 0.552 |
| C1ORF54 | 1q21.2 | 2.06 | 3.07 | 0.45 | 0.9 | 6.71E−04 | 0.552 |
| CSNK1G3 | 5q23 | 2.43 | 2.89 | 0.21 | 0.53 | 6.74E−04 | 0.552 |
| FGF11 | 17p13.1 | −1.56 | −0.48 | 0.51 | 1.33 | 7.10E−04 | 0.552 |
| CAMK2N2 | 3q27.1 | −1.45 | −0.32 | 0.55 | 1.84 | 7.19E−04 | 0.552 |
| CSNK1A1 | 5q32 | 4.36 | 4.8 | 0.2 | 0.4 | 7.51E−04 | 0.552 |
| CREBL2 | 12p13 | 3.29 | 3.83 | 0.25 | 0.57 | 7.74E−04 | 0.552 |
| CORO2A | 9q22.3 | 3.66 | 2.87 | 0.38 | 1.21 | 8.06E−04 | 0.552 |
| UGT8 | 4q26 | 3.44 | 2.26 | 0.59 | 2.02 | 8.62E−04 | 0.552 |
| ZSCAN32 | 16p13.3 | 1.29 | 1.88 | 0.27 | 0.44 | 9.04E−04 | 0.552 |
| DNAJC22 | 12q13.12 | 3.23 | 2.4 | 0.42 | 1.71 | 9.16E−04 | 0.552 |

### *3.4. Model Network of EFNA1*

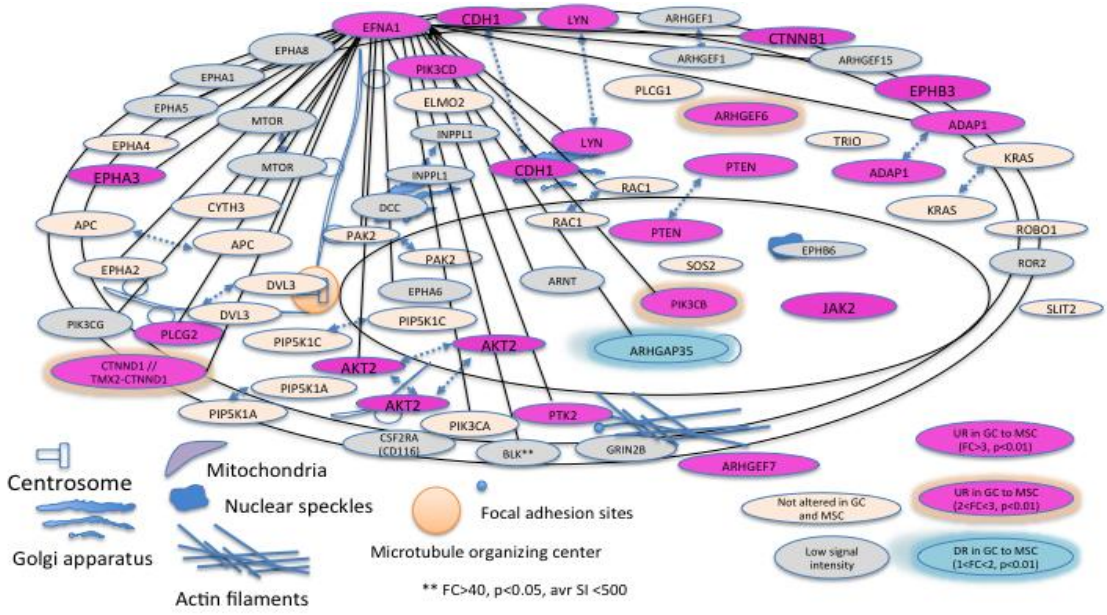


**Figure 3.** The EFNA1 model network.

The EFNA1 model network is shown. The localization of molecules was analyzed with The Human Protein Atlas (http://www.proteinatlas.org/) and UniProt (http://www.uniprot.org/).

A model network of EFNA1 is shown in Figure 3. Primary localization information was based on the Cell Atlas in the Human Protein Atlas where available, and secondary localization information was based on subcellular location information in the UniProt database. Among the genes in the

network generated with cBioPortal, the up-regulated (UR) genes in GC compared to MSCs are shown in pink, whereas down-regulated (DR) genes are shown in blue. Genes showing a fold change (FC) greater than 3 with a *p* value less than 0.01 are shown without highlight. The genes in which the FC of gene expression is between 2 and 3 and the *p* value is less than 0.01 are highlighted in light beige.

### *3.5. EFNA1 interaction analysis*

The interaction of EFNA1 was analyzed using the BioGRID database (Table 3). The interactors of EFNA1 included pro-platelet basic protein (PPBP), eukaryotic translation elongation factor 1 gamma (EEF1G), lysine acetyltransferase 5 (KAT5), EPH receptor A4 (EPHA4), EPH receptor A3 (EPHA3), and X-ray repair cross complementing 6 (XRCC6). EFNA1 had 7 published interactions and 6 interactors [22–25].

**Table 3.** Interactors of EFNA1 analyzed with BIOGRID database.

| Official Symbol | Entrez Gene | Pubmed ID |
|---|---|---|
| PPBP | 5473 | 16169070, 21900206 |
| EEF1G | 1937 | 16169070 |
| KAT5 | 10524 | 16169070 |
| EPHA4 | 2043 | 10366629 |
| EPHA3 | 2042 | 9195962 |
| XRCC6 | 2547 | 21900206 |

### *3.6. Network pathway of CSCs*

To reveal the molecular network in CSCs, 30 genes involved in CSCs were queried in the cBioPortal for Cancer Genomics, and the network was analyzed in stomach adenocarcinoma (TCGA, Nature 2014, tumor samples with sequencing and CNA data, 287 samples/30 genes) [20] (Figure 4A, B). The network contained 80 nodes, including 30 queried genes and the 50 most frequently altered neighbor genes (out of a total of 131 genes). In the network shown in Figure 4A, 16 core genes are marked with bold circles, and a total of 61 genes are mapped. The gene expression and localization information of core genes and adjacent genes are summarized in Figure 4B and Table 4. The node colored in pink indicates UR genes, and the node colored in blue indicates DR genes.

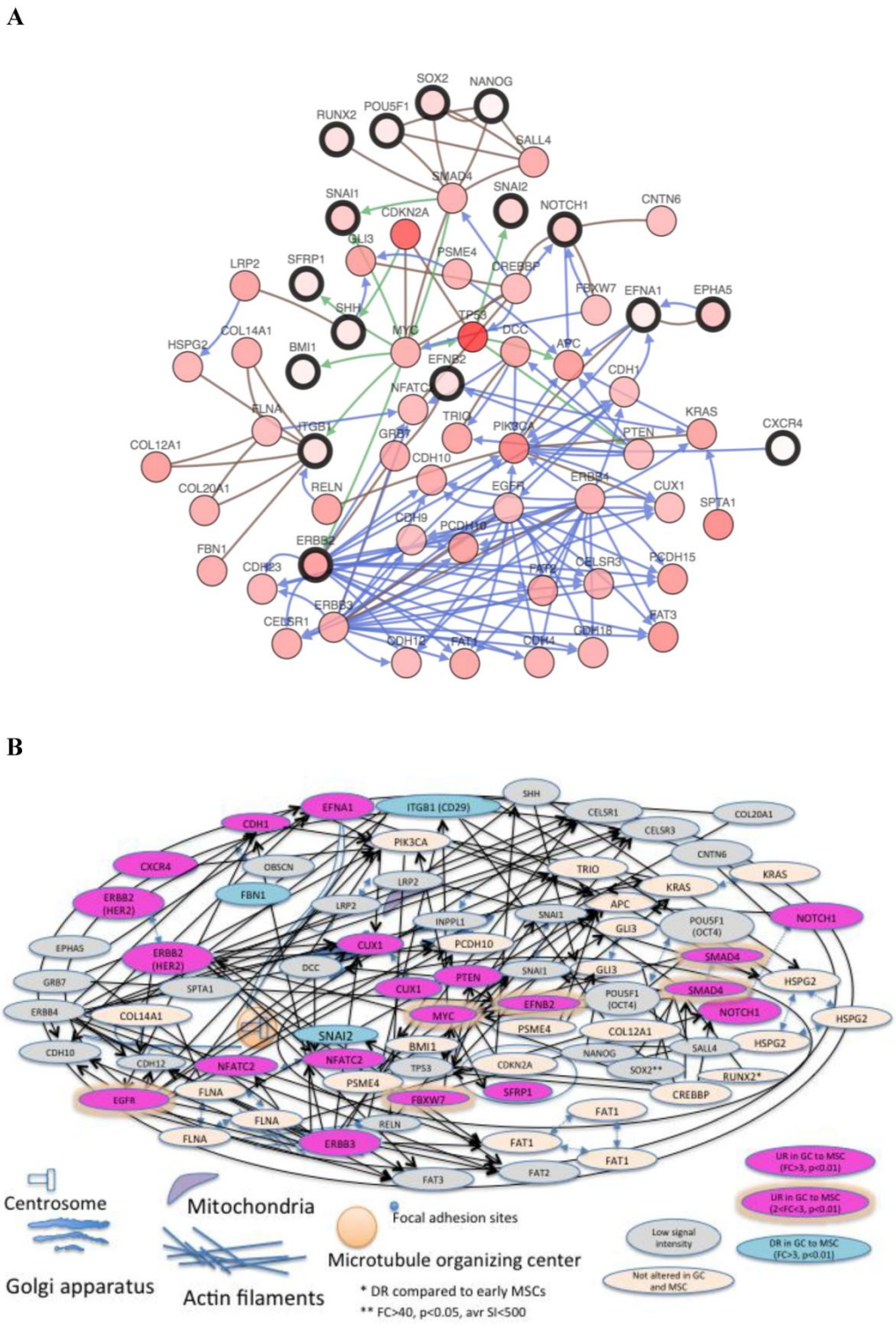


**Figure 4.** The cancer stem cell model network.

A: The cancer stem cell (CSC) model network was generated using the cBioPortal for Cancer Genomics. A total of 30 genes involved in CSCs were queried in the cBioPortal Cancer Genomics, and the network was analyzed in stomach adenocarcinoma (TCGA, Nature 2014, tumor samples with sequencing and CNA data, 287 samples / 30 genes). B: The CSC model network is shown. Molecules were mapped with the localization information based on The Human Protein Atlas (http://www.proteinatlas.org/) and UniProt (http://www.uniprot.org/).

**Table 4.** Genes in CSC network.

| Gene symbol | Full name | Ratio in diffuse-type GC to MSC (GAPDH normalized) | Localization (The human protein atlas, supported) | Localization (UniProt) |
|---|---|---|---|---|
| BMI1 /// COMMD3-BMI1 | BMI1 proto-oncogene, polycomb ring finger /// COMMD3-BMI1 readthrough | 1.76 | Localized to the Nucleus (approved), Nuclear bodies (approved) | Nucleus Cytoplasm |
| CXCR4 | C-X-C motif chemokine receptor 4 | 1901.94 | Not available | Cell membrane; Multi-pass membrane protein; Cell junction; Early endosome; Late endosome; Lysosome |
| EFNA1 | ephrin A1 | 216.27 | Not available | Cell membrane; Lipid-anchor › GPI-anchor; Secreted |
| EFNB2 | ephrin B2 | 2.87 | Localized to the Nucleoplasm (supported) | Membrane |
| EPHA5 | EPH receptor A5 | Signal intensity is low | Not available | Cell membrane; Single-pass type I membrane protein; Cell projection › axon; Cell projection › dendrite |
| ERBB2 | erb-b2 receptor tyrosine kinase 2 | 3.04 | Localized to the Plasma membrane (supported) In addition localized to the Cytosol (supported) | Isoform 1: Cell membrane; Single-pass type I membrane protein; Cytoplasm › perinuclear region; Nucleus; Isoform 2 & 3: Cytoplasm; Nucleus |
| ITGB1 | integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) | 0.31 | Localized to the Plasma membrane (supported), Focal adhesion sites (supported) | Cell membrane; Single-pass type I membrane protein; Cell projection › invadopodium membrane; Single-pass type I membrane protein; Cell projection › ruffle membrane; Single-pass type I membrane protein; Recycling endosome; Melanosome; Cleavage furrow; Cell projection › lamellipodium; Cell projection › ruffle; Cell junction › focal adhesion; Cell surface |

*Continued on next page*

| Gene symbol | Full name | Ratio in diffuse-type GC to MSC (GAPDH normalized) | Localization (The human protein atlas, supported) | Localization (UniProt) |
|---|---|---|---|---|
| NANOG | Nanog homeobox | Signal intensity is low | Not available | Nucleus |
| NOTCH1 | notch 1 | 6.94 | Localized to the Nucleoplasm (approved) | Cell membrane; Single-pass type I membrane protein |
| POU5F1 | POU class 5 homeobox 1 | Signal intensity is low | Localized to the Nucleoplasm (supported)<br>In addition localized to the Cytosol (supported) | Cytoplasm<br>Nucleus |
| RUNX2 | runt related transcription factor 2 | 0.79 | Localized to the Nucleoplasm (validated) | Nucleus |
| SFRP1 | Secreted frizzled-related protein 1 | 5.22 | Nucleoli (approved) | Secreted |
| SHH | sonic hedgehog | Signal intensity is low | Not available | Sonic hedgehog protein C-product: Secreted › extracellular space;<br>Sonic hedgehog protein N-product: Cell membrane; Lipid-anchor |
| SNAI1 | snail family transcriptional repressor 1 | Signal intensity is low | Localized to the Nucleus (supported), Cytosol (supported) | Nucleus<br>Cytoplasm |
| SNAI2 | snail family transcriptional repressor 2 | 0.30 | Localized to the Nucleus (approved) | Nucleus<br>Cytoplasm |
| SOX2 | SRY-box 2 | 47.16 | Localized to the Nucleoplasm (supported) | Nucleus |
| APC | APC, WNT signaling pathway regulator | 1.03 | Not available | Cell junction › adherens junction;<br>Cytoplasm › cytoskeleton;<br>Cell projection › lamellipodium;<br>Cell projection › ruffle membrane;<br>Cytoplasm; Cell membrane |

*Continued on next page*

| Gene symbol | Full name | Ratio in diffuse-type GC to MSC (GAPDH normalized) | Localization (The human protein atlas, supported) | Localization (UniProt) |
|---|---|---|---|---|
| CDH1 | cadherin 1 | 228.47 | Localized to the Plasma membrane (supported), Cell Junctions (supported) In addition localized to the Golgi apparatus (supported) | Cell junction; Cell membrane; Single-pass type I membrane protein; Endosome; Golgi apparatus › trans-Golgi network |
| CDH10 | cadherin 10 | Signal intensity is low | Not available | Cell membrane; Single-pass type I membrane protein |
| CDH12 | cadherin 12 | Signal intensity is low | Localized to the Vesicles (approved) | Cell membrane; Single-pass type I membrane protein |
| CDH18 | cadherin 18 | Signal intensity is low | Not available | Cell membrane; Single-pass type I membrane protein |
| CDH23 | cadherin 23 | Signal intensity is low | Not available | Cell membrane; Single-pass type I membrane protein |
| CDH4 | cadherin 4 | Signal intensity is low | Localized to the Plasma membrane (validated) | Cell membrane; Single-pass type I membrane protein |
| CDH9 | cadherin 9 | Signal intensity is low | Not available | Cell membrane; Single-pass type I membrane protein |
| CDKN2A | cyclin dependent kinase inhibitor 2A | 0.23 | Localized to the Nucleoli (validated) | Cytoplasm Nucleus |
| CELSR1 | cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo homolog, Drosophila) | Signal intensity is low | Localized to the Plasma membrane (supported) | Cell membrane; Multi-pass membrane protein |
| CELSR3 | cadherin EGF LAG seven-pass G-type receptor 3 | Signal intensity is low | Not available | Cell membrane; Multi-pass membrane protein |
| CNTN6 | contactin 6 | Signal intensity is low | Not available | Cell membrane; Lipid-anchor › GPI-anchor |
| COL12A1 | collagen type XII alpha 1 chain | 0.14 | Localized to the Nucleus (approved) | Secreted › extracellular space › extracellular matrix |
| COL14A1 | collagen type XIV alpha 1 chain | 1.59 | Localized to the Vesicles (approved) | Secreted › extracellular space › extracellular matrix |

*Continued on next page*

| Gene symbol | Full name | Ratio in diffuse-type GC to MSC (GAPDH normalized) | Localization (The human protein atlas, supported) | Localization (UniProt) |
|---|---|---|---|---|
| COL20A1 | collagen type XX alpha 1 chain | Signal intensity is low | Not available | Secreted › extracellular space |
| CREBBP | CREB binding protein | 1.89 | Localized to the Nucleoplasm (validated)<br>In addition localized to the Nuclear bodies (validated) | Cytoplasm<br>Nucleus |
| CUX1 | cut-like homeobox 1 | 3.87 | Localized to the Nucleoplasm (supported), Golgi apparatus (supported) | Nucleus |
| DCC | deleted in colorectal carcinoma | Signal intensity is low | Localized to the Golgi apparatus (approved) | Membrane; Single-pass type I membrane protein |
| EGFR | epidermal growth factor receptor | 2.08 | Localized to the Plasma membrane (validated) | Cell membrane; Single-pass type I membrane protein<br>Endoplasmic reticulum membrane; Single-pass type I membrane protein<br>Golgi apparatus membrane; Single-pass type I membrane protein<br>Nucleus membrane; Single-pass type I membrane protein<br>Endosome; Endosome membrane<br>Nucleus |
| ERBB3 | erb-b2 receptor tyrosine kinase 3 | 167.50 | Localized to the Actin filaments (approved)<br>In addition localized to the Plasma membrane (supported) | Isoform 1: Cell membrane; Single-pass type I membrane protein<br>Isoform 2: Secreted |
| ERBB4 | erb-b2 receptor tyrosine kinase 4 | Signal intensity is low | Not available | Cell membrane; Single-pass type I membrane protein |
| FAT1 | FAT atypical cadherin 1 | 0.88 | Not available | Cell membrane; Single-pass type I membrane protein<br>Nucleus<br>Cytoplasm › perinuclear region |

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| Gene symbol | Full name | Ratio in diffuse-type GC to MSC (GAPDH normalized) | Localization (The human protein atlas, supported) | Localization (UniProt) |
|---|---|---|---|---|
| FAT2 | FAT atypical cadherin 2 | Signal intensity is low | Not available | Cell membrane; Single-pass type I membrane protein<br>Cell junction<br>Nucleus |
| FAT3 | FAT atypical cadherin 3 | Signal intensity is low | Not available | Membrane; Single-pass type I membrane protein |
| FBN1 | fibrillin 1 | 0.20 | Localized to the Cytosol (approved) | Secreted |
| FBXW7 | F-box and WD repeat domain containing 7, E3 ubiquitin protein ligase | 2.53 | Localized to the Nucleoplasm (supported)<br>In addition localized to the Vesicles (approved) | Isoform 1: Nucleus › nucleoplasm<br>Isoform 2: Cytoplasm<br>Isoform 3: Nucleus › nucleolus |
| FLNA | filamin A | 1.45 | Localized to the Plasma membrane (validated), Actin filaments (validated), Cytosol (validated) | Cytoplasm › cell cortex<br>Cytoplasm › cytoskeleton |
| GLI3 | GLI family zinc finger 3 | 1.34 | Not available | Nucleus; Cytoplasm;<br>Cell projection › cilium |
| GRB7 | growth factor receptor-bound protein 7 | Signal intensity is low | Localized to the Plasma membrane (supported) | Cytoplasm;<br>Cell junction › focal adhesion;<br>Cell membrane; Peripheral membrane protein; Cytoplasmic side;<br>Cytoplasmic granule<br>Cell projection |
| HSPG2 | heparan sulfate proteoglycan 2 | 1.29 | Localized to the Nucleoplasm (approved), Plasma membrane (approved), Cytosol (approved) | Secreted › extracellular space › extracellular matrix › basement membrane |
| KRAS | KRAS proto-oncogene, GTPase | 1.75 | Not available | Cell membrane; Lipid-anchor; Cytoplasmic side<br>Cytoplasm › cytosol |

*Continued on next page*

| Gene symbol | Full name | Ratio in diffuse-type GC to MSC (GAPDH normalized) | Localization (The human protein atlas, supported) | Localization (UniProt) |
|---|---|---|---|---|
| LRP2 | low density lipoprotein receptor-related protein 2 | Signal intensity is low | Localized to the Vesicles (approved), Mitochondria (approved) | Membrane; Single-pass type I membrane protein<br>Membrane › coated pit |
| MYC | v-myc myelocytomatosis viral oncogene homolog (avian) | 2.85 | Localized to the Nucleoplasm (validated) | Nucleus › nucleoplasm<br>Nucleus › nucleolus |
| NFATC2 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 | 27.85 | Localized to the Nucleoplasm (supported), Cytosol (supported) | Cytoplasm<br>Nucleus |
| PCDH10 | protocadherin 10 | 0.07 | Localized to the Golgi apparatus (approved)<br>In addition localized to the Nucleus (approved), Vesicles (approved) | Cell membrane; Single-pass type I membrane protein |
| PCDH15 | protocadherin-related 15 | Signal intensity is low | Not available | Cell membrane; Single-pass type I membrane protein<br>Isoform 3 :Secreted |
| PIK3CA | phosphoinositide-3-kinase, catalytic, alpha polypeptide | 1.33 | Localized to the Cytosol (approved) | GO - Cellular component<br>cytosol Source: Reactome<br>lamellipodium Source: Ensembl<br>phosphatidylinositol 3-kinase complex Source: BHF-UCL<br>phosphatidylinositol 3-kinase complex, class IA Source: UniProtKB<br>plasma membrane Source: GO_Central |
| PSME4 | proteasome (prosome, macropain) activator subunit 4 | 1.97 | Localized to the Nucleoplasm (supported) | Cytoplasm › cytosol<br>Nucleus<br>Nucleus speckle |

*Continued on next page*

| Gene symbol | Full name | Ratio in diffuse-type GC to MSC (GAPDH normalized) | Localization (The human protein atlas, supported) | Localization (UniProt) |
|---|---|---|---|---|
| PTEN | phosphatase and tensin homolog | 15.55 | Localized to the Nucleoplasm (supported)<br>In addition localized to the Cytosol (supported) | Cytoplasm<br>Nucleus<br>Nucleus › PML body<br>Isoform alpha:<br>Secreted |
| RELN | reelin | Signal intensity is low | Localized to the Focal adhesion sites (approved)<br>In addition localized to the Plasma membrane (approved) | Secreted › extracellular space › extracellular matrix |
| SALL4 | sal-like 4 (Drosophila) | Signal intensity is low | Localized to the Nucleoplasm (validated) | Cytoplasm<br>Nucleus |
| SMAD4 | SMAD family member 4 | 2.32 | Localized to the Nucleoplasm (supported), Cytosol (supported)<br>In addition localized to the Centrosome (approved) | Cytoplasm<br>Nucleus |
| SPTA1 | spectrin, alpha, erythrocytic 1 (elliptocytosis 2) | Signal intensity is low | Not available | Cytoplasm › cytoskeleton<br>Cytoplasm › cell cortex |
| TP53 | tumor protein p53 | Signal intensity is low | Localized to the Nucleoplasm (validated) | Cytoplasm<br>Nucleus<br>Nucleus › PML body<br>Endoplasmic reticulum<br>Mitochondrion matrix |
| TRIO | triple functional domain (PTPRF interacting) | 0.92 | Localized to the Cytosol (approved)<br>In addition localized to the Vesicles (approved) | Cytoplasm |

## 4. Discussion

The EFN/EPH signaling pathway is important for cancer. In the case of medulloblastoma, ephrinB1 is differentially expressed and mainly expressed in islands within the tumor comprised of dense neoplastic cells with a high mitotic proliferative index [26]. It has been reported that EphA2 is regulated by E-cadherin (CDH1 or cadherin 1) [27]. E-cadherin loss in breast cancer leads to a decrease in phosphorylated EphA2 and altered neoplastic cell growth and adhesion [27]. Single nucleotide polymorphisms (SNPs) in the *EFNA1* gene have been found to play an important role in GC susceptibility [28]. The results of an Identify Candidate Causal SNPs and Pathways (ICSNPathway) analysis using a GC genome-wide association study (GWAS) dataset indicated that SNPs rs4745 and rs12904 lead to an EFNA1 and ephrin receptor binding pathway in GC [28,29]. These SNPs, rs4745 and rs12904, are suggested to affect the regulatory roles in the ephrin receptor binding of EFNA1 [30]. Considering that the overexpression of EFNA1 is observed in 57% of GC tissue samples, the EFNA1 and EPH pathway may play a crucial role in GC [11].

The present study revealed that the EFNA1 was up-regulated in diffuse-type GC compared to MSCs. It has been described that the EFNA1 is up-regulated in GC in the previous studies [11,31], which shows the consistency of the present study with the previous findings. The present study demonstrated the novel EFNA1 network model showing the gene expression patterns in diffuse-type GC and MSCs. This EFNA1 molecular network contains EMT-related molecules such as CDH1 (E-cadherin) and CTNNB1 (β-catenin). The EFNA1 network also contains AKT signaling molecules and PTEN which are up-regulated in diffuse-type GC compared to MSCs. The generated EFNA1 network contains the molecules such as KRAS, SLIT2, APC and RAC1, although their gene expression were not altered in diffuse-type GC and MSCs. The up-regulated genes may include the important molecules for CSCs.

EFN/EPH signaling pathway networks and the Wnt/β-catenin signaling pathway are suggested to interact with each other [32]. *EFNB1* mRNA is expressed in human embryonic stem (ES) cells and diffuse-type GC. *EFNB3* has been identified as a potential transcriptional target of the Wnt/β-catenin signaling pathway [32]. The Wnt/β-catenin signaling pathway is implicated in GC and the self-renewal of stem cells, which suggests that the Wnt/β-catenin signaling pathway may be an interesting target for the investigation of CSCs in GC. EPH receptor tyrosine kinases are implicated in CSC regulation [33]. β-catenin accumulates in the nucleus of cells at the bottom of the small intestine crypts of $EphB2^{-/-}EphB3^{-/-}$ mice, which suggests cross-talk between β-catenin signaling and EFN/EPH signaling [34].

The gene expression of *EPHA2* and *EPHA7* had tendency to be up-regulated in diffuse-type GC compared to MSCs. It has been reported that the gene expression pattern of *EPHA2*, *EFNA1* and *EGFR* is significantly associated with poor response to treatment with cetuximab, an anti-EGFR antibody, in stage IV colorectal cancer, which suggests that the EGFR signaling pathway and EFN/EPH signaling pathway cross-talk in cancer cells [35]. A study that is involved in gene expression profiling of muscle stem cells has demonstrated that *EphA1*, *EphA2*, *EfnA1*, and *EphB1* were induced, whereas *EphA3*, *EphA4*, *EphA7*, *EfnA2*, *EfnA3*, *EfnA4*, *EfnA5*, *EphB2*, *EphB3*, *EphB4*, *EfnB1*, *EfnB2* and *EfnB3* were inhibited during postnatal myogenesis in mice [36]. In glioblastoma, EphA2 was overexpressed in stem cells, and the Akt signaling pathway experienced cross-talk with EphA2 to regulate stem cell properties [37]. Hepatic progenitor cell markers include EFNA1, EpCAM, CK7 (KRT7), CK19 (KRT19), alpha-fetoprotein (AFP) and CD90 (THY1) [38]. These

results demonstrate that the EFN/EPH signaling pathway is regulated in stem cell differentiation.

## 5. Conclusion

In conclusion, EFNA1 was up-regulated in diffuse-type GC compared to MSCs, and the network pathway analysis demonstrated that EFNA1 model network contains EMT-related molecules. The CSC model network was also generated, in which CSC-related genes such as EGFR, ERBB2, and NOTCH1 are included. To reveal the EMT and CSC mechanism, the expression analysis of network molecules in several types of cancer would be for the future investigation.

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## Conflict of interest

The author declares that no conflicts of interest exist.

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