ORIGINAL ARTICLE

Basic Study

Regulated genes in mesenchymal stem cells and gastric cancer

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Abstract

AIM: To investigate the genes regulated in mesenchymal stem cells (MSCs) and diffuse-type gastric cancer (GC), gene expression was analyzed.

METHODS: Gene expression of MSCs and diffuse-type GC cells were analyzed by microarray. Genes related to stem cells, cancer and the epithelial-mesenchymal transition (EMT) were extracted from human gene lists using Gene Ontology and reference information. Gene panels were generated, and messenger RNA gene expression in MSCs and diffuse-type GC cells was analyzed. Cluster analysis was performed using the NCSS software.

RESULTS: The gene expression of regulator of G-protein signaling 1 (RGS1) was up-regulated in diffuse-type GC cells compared with MSCs. A panel of stem-cell related genes and genes involved in cancer or the EMT were examined. Stem-cell related genes, such as growth arrest-specific 6, musashi RNA-binding protein 2 and hairy and enhancer of split 1 (Drosophila), NOTCH family genes and Notch ligands, such as delta-like 1 (Drosophila) and Jagged 2, were regulated.

CONCLUSION: Expression of RGS1 is up-regulated, and genes related to stem cells and NOTCH signaling are altered in diffuse-type GC compared with MSCs.

Key words: Mesenchymal stem cells; Gastric cancer; Stem cells; Gene; Epithelial-mesenchymal transition

Core tip: Recent studies have revealed that epithelial-mesenchymal transition (EMT) regulators play important roles in cellular phenotypes. This study has shown that EMT-related genes are regulated in diffuse-type gastric cancer and mesenchymal stem cells (MSCs). Regulator of G-protein signaling 1 (RGS1) was significantly up-regulated in diffuse-type GC compared with MSCs. These results are interesting and

provide insights about the mechanisms of the stem cell phenotype transition and gastric cancer progression. These insights will examine the novel role of EMT-related genes and RGS1.

INTRODUCTION

It is a mystery how the epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs) are correlated[1,2]. Although it is known that the EMT is related to cancer migration and shares similar molecular characteristics with CSCs, whether the EMT occurs in CSCs or if there is any threshold for this phenotype between stem and cancer cells is largely unknown. The EMT is the cellular process involving the cell phenotype transitioning from an epithelial phenotype to a mesenchymal phenotype. Cancer cells experience the EMT when adhesion is less important, and the cells can freely migrate throughout the body. This mechanism is thought to be important in cancer cell invasion and migration and CSC development. The stem cell phenotype transforms from a differentiated stage to an undifferentiated stage and vice versa during the development of tissues and organs and during different disease states. Investigating stem cell-regulated genes is a highly important issue to elucidate the mechanisms of CSCs.

It has been shown that mesenchymal stem cells (MSCs) can promote the expression of an EMT phenotype in cancer cells *via* CD44, lysyl oxidase and TWIST[3]. The relationship between MSCs and cancer cells, including the mechanism of stemness exhibited by cancer cells, remains unclear. Moreover, the self-renewal of colorectal cancer initiating cells is considered to be a new therapeutic approach[4]. It is important to uncover the gene signature of stem and cancer cells to further understand the stemness of these cells. Studies to reveal the stemness phenotype have already been performed. A marker of the putative stem cell region of human intestinal crypts has been identified using a Fourier transform infrared microspectroscopy approach[5-7]. Genes, such as necdin homolog (mouse) (*NDN*), EPH receptor A5 (*EPHA5*), nephroblastoma overexpressed gene (*NOV*) and runt-related transcription factor 2 (*RUNX2*), have been correlated with MSC passage numbers[8]. Molecular signatures have also been investigated, and a prediction model of bone marrow stromal cell senescence has been discovered[9]. Even gene expression at the single cell level can be determined using a recent technique[10]. These new approaches, including a physical theory, may be important for the advancement of stem cell therapeutics.

Gastric cancer is classified into diffuse and intestinal types[11,12]. Diffuse-type gastric carcinomas supposedly originate from nonmetaplastic gastric epithelium, and the intestinal type originates from the regenerating epithelium in chronic atrophic gastritis[12]. Diffuse-type gastric cancer (GC) are known to correlate with the EMT[13]. We have previously shown that diffuse-type GC can be distinguished with combination of cadherin 1, type 1, E-cadherin (epithelial) (*CDH1*) and cadherin 2, type 1, N-cadherin (neuronal) (*CDH2*) gene expression in MSC and diffuse-type GC associated with the EMT[14]. Considering that hepatoma cells are converted into human induced pluripotent stem cell-like cells using microRNAs, the difference between stem cells and cancer cells should be deeply investigated[15]. Breast CSCs show both mesenchymal-like and epithelial-like phenotypes and transition between EMT- and mesenchymal-epithelial transition-like states[16]. Diffuse-type GC has been reported to be involved in the remodeling of the pulmonary artery, which suggests cancer niche formation by diffuse-type GC[17]. Considering the finding that MSCs provide microenvironment for CSCs, the features of MSC and cancer cells should be investigated[18].

In this article, MSC gene expression has been investigated and compared with diffuse-type GC cells that exhibit a mesenchymal phenotype more than intestinal-type GC cells do, and panels of regulated genes in MSCs and diffuse-type GC have been generated to clarify the important molecular patterns that distinguish stem and cancer cells.

MATERIALS AND METHODS

Cell culture

MSCs from human bone marrow were cultured according to the manufacturer's protocol (Lonza Walkersville, Inc., Walkersville, MD, United States). Detailed methods have been previously described[8]. Briefly, MSCs were cultured *in vitro* in mesenchymal stem cell growth medium (Lonza Catalog #PT-3001; mesenchymal stem cell basal medium plus SingleQuots™ of growth Supplements) at 37 ℃ in a CO_2 (5%) incubator. Each number in the MSC sample name indicates the passage number.

Total RNA extraction

Diffuse-type GC samples were surgically resected and obtained from the National Cancer Center Research Institute. Detailed information on total RNA extraction has been previously described[14,19]. Briefly, total RNA was obtained by suspending the cells in ISOGEN lysis buffer from surgically resected samples, which were provided by the Central Hospital or East Hospital at the National Cancer Center[14,19]. Total RNA of MSCs were purified using RNeasy kit (QIAGEN, Düsseldorf, Germany) as previously described[8].

Microarray analysis

MSC gene expression data are available to the public *via* the NCBI Gene Expression Omnibus (GEO)[8]. Diffuse-

type GC gene expression data are also available *via* the NCBI GEO[14]. The accession numbers of data used for gene expression analysis are GSE7888 and GSE42252 for MSCs and diffuse-type GC, respectively. Briefly, total RNA from MSCs and diffuse-type GC was analyzed using a GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix, Inc., Santa Clara, CA, United States). Probe set and Gene Ontology information is based on annotation version na34. Cluster analysis was performed using NCSS 2007 (NCSS, LLC., Kaysville, UT, United States).

Statistical analysis

All data are presented as the mean ± SE. Statistical significance was calculated with GraphPad Prism® 6 or Microsoft® Excel® using the unpaired Student's t test or one-way ANOVA with Bonferroni's multiple comparisons test.

RESULTS

Regulated genes in diffuse-type GC and MSCs

Genes regulated in diffuse-type GC and MSCs were investigated and analyzed using cluster analysis (NCSS) (Figure 1). Probe sets that have a more than a 50-fold significant difference between MSCs and diffuse-type GC ($P < 10^{-9}$ in the equal variance hypothesis and $P < 0.01$ in the non-equal variance hypothesis) are shown in Figure 1. Each panel shows the gene expression in 30 probe sets. Panels A, B and C show the up-regulated probe sets in diffuse-type GC compared with MSCs, whereas panel D shows 29 up-regulated probe sets and one down-regulated gene, pentraxin 3, long (*PTX3*). In this analysis, regulator of G-protein signaling 1 (*RGS1*) was the most up-regulated gene in diffuse-type GC compared with MSCs (Figure 2A). *RGS4* gene expression was down-regulated in diffuse-type GC compared with MSCs (Figure 2B). The regulated RGS family genes are shown in Table 1. Additional up-regulated genes in diffuse-type GC compared to MSCs included spleen tyrosine kinase (*SYK*) and *CDH1*, which have been suggested to be mutated in cancer, based on an assessment of the public database Cancer Gene Census (http://cancer.sanger.ac.uk/cancergenome/projects/census/).

Stem cell gene panel

The expression of genes related to stem cells was investigated (Table 2). Considering that gene combination is important to understand stem cell phenotype, several genes involving to stem cells have been analyzed[20]. *NANOG* and *SUZ12*, which have been found by gene expression analysis to be involved in MSC invasion was up-regulated in diffuse-type GC compared to MSCs[21]. Gene expression of tumor necrosis factor receptor superfamily, member 19 (*TNFRSF19*), tended to decrease in diffuse-type GC compared with MSCs, but it was not significantly altered in MSCs and diffuse-type GC (Figure 3). The gene expression of *TNFRSF8*, also known as

CD30, was slightly up-regulated in diffuse-type GC compared with MSCs. The gene expression of growth arrest-specific 6 (*GAS6*), musashi RNA-binding protein 2 (*MSI2*) and hairy and enhancer of split 1, (Drosophila) (*HES1*), which have stem cell terms in the Gene Ontology biological process, were regulated in diffuse-type GC and MSCs (Figure 4).

Investigation of EMT markers

To examine the cellular phenotype of cancer and stem cells, the expression levels of EMT-related genes were investigated (Table 3). The gene expression of the *TWIST*, *ZEB* and *SNAI* families were examined because these genes have been reported to be associated with the EMT and cancer regulation[22]. The gene expression of twist family bHLH transcription factor 1 (*TWIST1*) and paired related homeobox 1 (*PRRX1*) were down-regulated in diffuse-type GC compared with MSCs. The gene expression of zinc finger E-box binding homeobox 2 (*ZEB2*) was up-regulated in diffuse-type GC compared with MSCs. Previously, high mobility group AT-hook 2 (*HMGA2*) has been shown to be involved in the EMT, *WNT*/β-catenin signaling and malignant cancer[23,24]. *HMGA2* gene expression was down-regulated in diffuse-type GC compared with MSCs. The gene expression of one of the probe sets for catenin (cadherin-associated protein), beta 1, 88 kDa (*CTNNB1*) was up-regulated in diffuse-type GC compared with MSCs. The gene expression of the other two probe sets for *CTNNB1* was stable. Sp1 transcription factor (*SP1*) and lymphoid enhancer-binding factor 1 (*LEF1*) were up-regulated in diffuse-type GC compared with MSCs. *SP1* is known to be an important factor for cell cycle regulation and is up-regulated in various cancers[25]. The *AKT* gene family is known to be important in cancer and has been suggested to regulate the EMT and stem cells in cancer cells[26]. Three *AKT* isoforms were examined, and the gene expression of v-akt murine thymoma viral oncogene homolog 2 (*AKT2*) was up-regulated. However, some of the probe sets recognizing *AKT3* showed a down-regulation of *AKT3* gene expression. The gene expression of stratifin (*SFN*); F11 receptor (*F11R*); gap junction protein, beta 2, 26 kDa (*GJB2*); occluding (*OCLN*); coxsackie virus and adenovirus receptor (*CXADR*); and keratin 8 (*KRT8*) were up-regulated in diffuse-type GC compared with MSCs.

Messenger RNA expression of NOTCH signaling-related genes

NOTCH signaling-related genes are involved in cancer, and *NOTCH* signaling is one of the target signaling pathways of CSCs[27-30]. Next, *NOTCH* messenger RNA (mRNA) expression was examined (Table 4). *NOTCH1, 3* and *4* gene expression was up-regulated, whereas *NOTCH2* gene expression was down-regulated in diffuse-type GC compared with MSCs (Figure 5A). The gene expression of delta-like 1 (Drosophila) (*DLL1*) and Jagged 2 (*JAG2*) were up-regulated in diffuse-type GC compared with MSCs
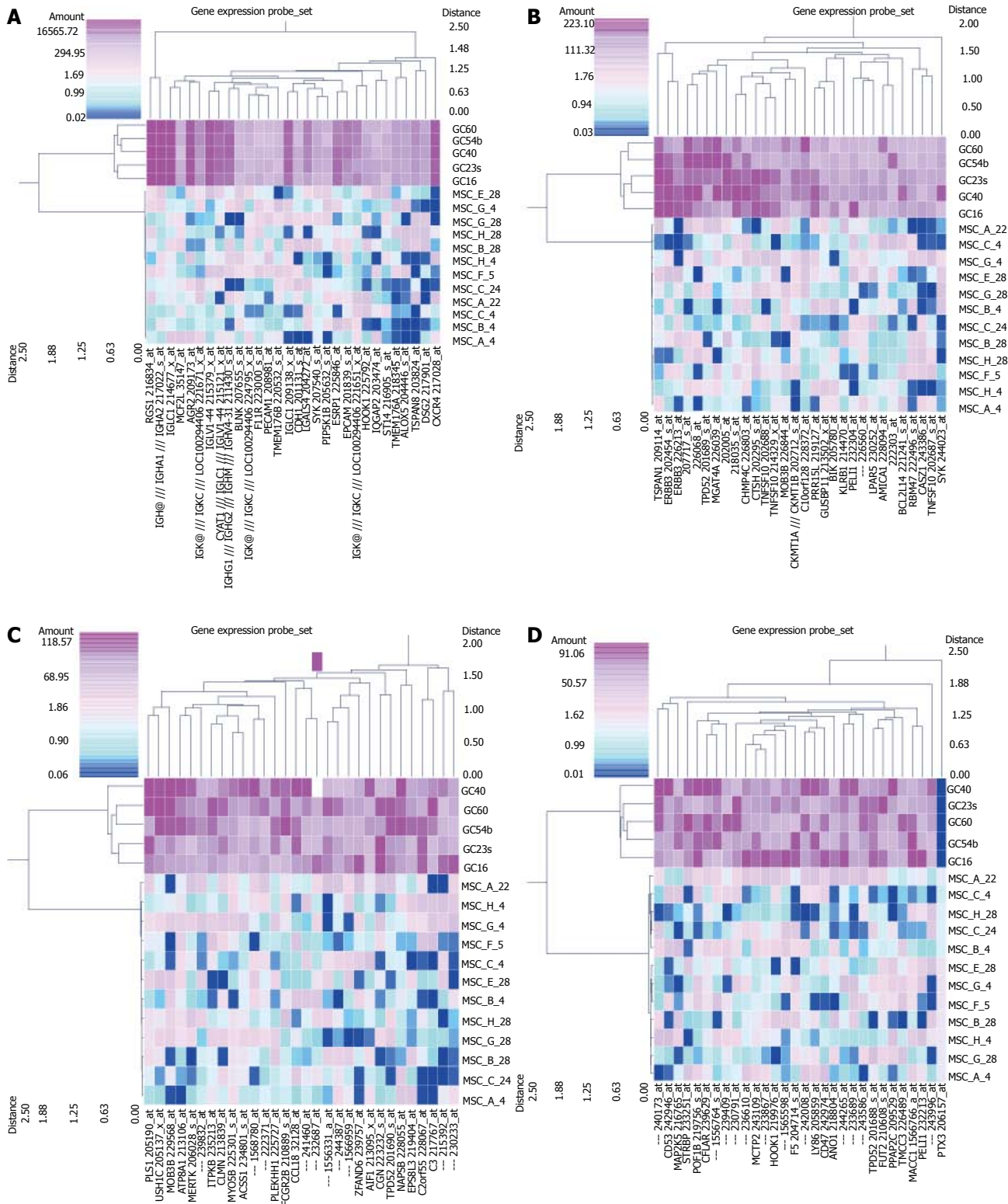
Figure 1 Genes regulated in mesenchymal stem cells and diffuse-type gastric cancer. A total of 120 probe sets that had more than a 50-fold change in mesenchymal stem cells (MSCs) and diffuse-type gastric cancer (GC) are shown. Each panel (A to D) shows 30 probe sets up-regulated or down-regulated in diffuse-type GC compared with MSCs. (Fold change > 50, $P < 0.01$ and $P < 10^{-9}$ using Student's *t* test in the non-equal variance hypothesis and equal variance hypothesis, respectively).

(Figure 5B and 5C). The scheme for *NOTCH* signaling and cross-talk between cells is presented in Figure 6A. The Gene Ontology biological process of the *NOTCH* family genes are shown in Table 5.

RGS1 expression in normal tissues

To confirm that the up-regulation of *RGS1* was not stomach specific, *RGS1* gene expression in normal tissues was investigated using various data in the database, such

Figure 2 Regulator of G-protein signaling 1 and regulator of G-protein signaling 4 gene expression was up-regulated and down-regulated, respectively, in diffuse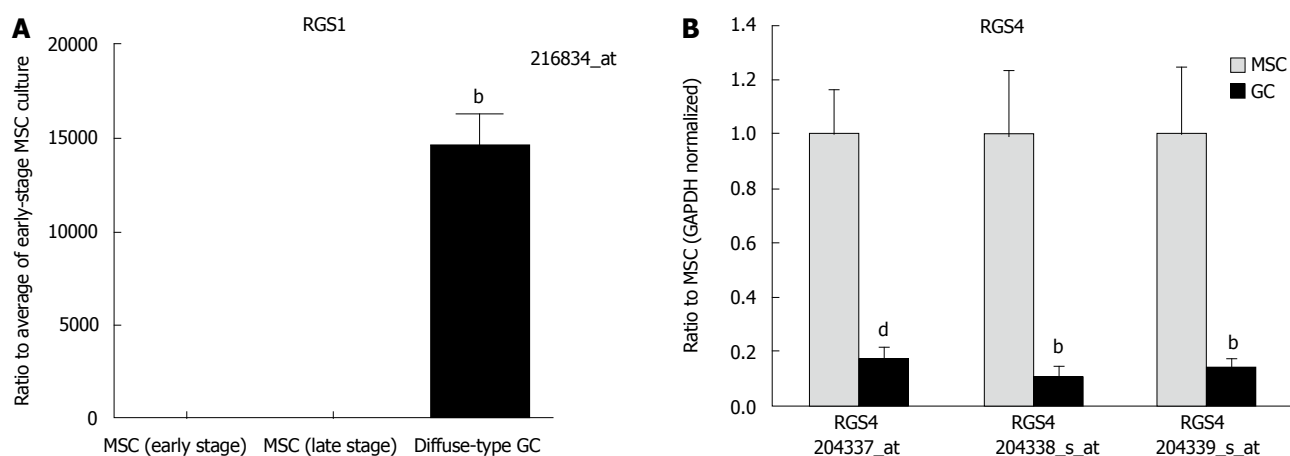-type gastric cancer. A: regulator of G-protein signaling 1 (*RGS1*) gene expression was up-regulated in diffuse-type gastric cancer (GC) compared with early-stage mesenchymal stem cell (MSC) cultures [*n* = 5 in early-stage MSC cultures (B#4, C#4, F#5, G#4, and H#4), *n* = 5 in late-stage MSC cultures (B#28, C#24, E#28, G#28, and H#28), and *n* = 5 in diffuse-type GC (GC16, GC40, GC54b, GC60, and GC23s)]. Results represent the mean ± SE. [b]*P* < 0.01 using a one-way analysis of variance with Bonferroni's multiple comparisons test; B: RGS4 gene expression was down-regulated in diffuse-type GC compared with MSCs. Results represent the mean ± SE (*n* = 12 in MSC cultures and *n* = 5 in diffuse-type GC). [b]*P* < 0.01 and [d]*P* < 0.001 using Student's t test with the non-equal variance hypothesis.

Table 1 Gene expression alterations of regulator of G-protein signaling family genes in mesenchymal stem cells and diffuse-type gastric cancer

Gene symbol	Detailed description	*P* value	Ratio of diffuse-type GC to MSCs	Probe set ID
RGS1	Immune response / signal transduction / adenylate cyclase-inhibiting G-protein coupled receptor signaling pathway / negative regulation of signal transduction / termination of G-protein coupled receptor signaling pathway / positive regulation of GTPase activity	0.013 0.001	1004 13808	202988_s_at 216834_at
RGS2	Regulation of translation / cell cycle / spermatogenesis / regulation of G-protein coupled receptor protein signaling pathway / negative regulation of signal transduction / negative regulation of phospholipase activity / negative regulation of cardiac muscle hypertrophy / termination of G-protein coupled receptor signaling pathway / negative regulation of mitogen-activated protein (MAP) kinase activity / positive regulation of GTPase activity / negative regulation of G-protein coupled receptor protein signaling pathway / brown fat cell differentiation / relaxation of cardiac muscle / relaxation of vascular smooth muscle / positive regulation of cardiac muscle contraction / regulation of adrenergic receptor signaling pathway	0.007	14.51	202388_at
RGS3	Inactivation of mitogen-activated protein kinase (MAPK) activity / signal transduction / regulation of G-protein coupled receptor protein signaling pathway / negative regulation of signal transduction / termination of G-protein coupled receptor signaling pathway / positive regulation of GTPase activity	1.00E-05	0.39	203823_at
RGS4	Inactivation of MAPK activity / regulation of G-protein coupled receptor protein signaling pathway / negative regulation of signal transduction / termination of G-protein coupled receptor signaling pathway / positive regulation of GTPase activity	3.00E-04 0.003 0.004	0.17 0.11 0.14	204337_at 204338_s_at 204339_s_at
RGS5	Signal transduction / regulation of G-protein coupled receptor protein signaling pathway / negative regulation of signal transduction / termination of G-protein coupled receptor signaling pathway / positive regulation of GTPase activity	0.01 0.008 0.009	47.79 81.82 95.52	209070_s_at 209071_s_at 218353_at
RGS10	Negative regulation of signal transduction / termination of G-protein coupled receptor signaling pathway / positive regulation of GTPase activity	0.003	3.92	204319_s_at
RGS19	Autophagy / G-protein coupled receptor signaling pathway / small GTPase mediated signal transduction / negative regulation of signal transduction / termination of G-protein coupled receptor signaling pathway / positive regulation of GTPase activity	9.00E-04	2.45	204336_s_at

RGS family genes that have significant alterations in MSCs and diffuse-type GC are shown. *RGS1, RGS2, RGS5, RGS10* and *RGS19* were up-regulated in diffuse-type GC compared with MSCs. *RGS3* and *RGS4* were down-regulated in diffuse-type GC compared with MSCs. RGS: Regulator of G-protein signaling; MSCs: Mesenchymal stem cells; GC: Gastric cancer.

as the GEO database. From data analysis of various types of normal human tissues (GSE2361 and GSE2193)[31,32], *RGS1* has been shown to be expressed in normal stomach, bladder, colon, corpus, liver, lung, placenta, prostate, skin, spinal cord, spleen, thymus, thyroid, and trachea, whereas *RGS1* gene expression is relatively high in the thymus and

Gene symbol	Gene name	*P* value	Ratio of diffuse-type GC to MSCs	Probe set ID
NANOG	Nanog homeobox	0.0037	14.9	220184_at
SUZ12	SUZ12 polycomb repressive complex 2 subunit	0.0022	35.4	1566191_at
TNFRSF8	Tumor necrosis factor receptor superfamily, member 8	0.0126	1.98	206729_at
CD9	CD9 molecule	0.0012	3.44	201005_at
ICAM3	Intercellular adhesion molecule-3	0.0143	2.69	204949_at
CD200	CD200 molecule	0.0013	4.79	209583_s_at
		0.0002	4.35	209582_s_at
THY1	Thy-1 cell surface antigen	0.0081	0.61	208850_s_at
		0.0301	0.79	208851_s_at
		0.0024	0.67	213869_x_at

Table 2 Gene expression alterations of stem cell-related genes in mesenchymal stem cells and diffuse-type gastric cancer

Genes related to stem cells in the literature are shown with their gene expression alterations in MSCs and diffuse-type GC. *NANOG* and *SUZ12*, which have been found by gene expression analysis to be involved in MSC invasion was up-regulated in diffuse-type GC compared to MSCs. RGS: Regulator of G-protein signaling; MSCs: Mesenchymal stem cells; GC: Gastric cancer.
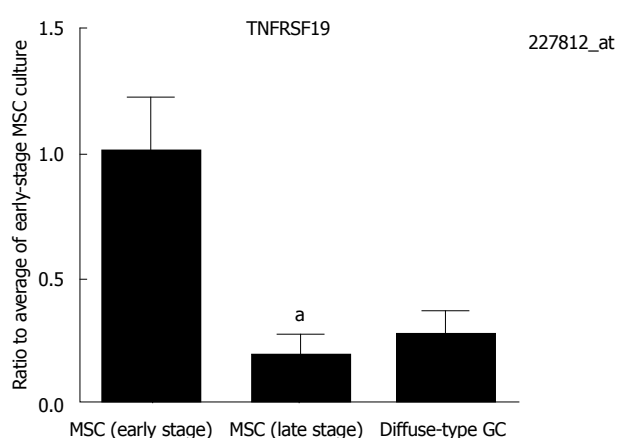

Figure 3 Tumor necrosis factor receptor superfamily, member 19 gene expression. Tumor necrosis factor receptor superfamily, member 19 (*TNFRSF19*) gene expression tended to decrease in diffuse-type gastric cancer (GC) but was not significantly altered compared with early-stage mesenchymal stem cell (MSC) cultures. *TNFRSF19* gene expression in late-stage MSC cultures was significantly down-regulated compared with early-stage MSC cultures. Results represent the mean ± SE [*n* = 5 in early-stage MSC cultures (B#4, C#4, F#5, G#4, and H#4), *n* = 5 in late-stage MSC cultures (B#28, C#24, E#28, G#28, and H#28), and *n* = 5 in diffuse-type GC (GC16, GC40, GC54b, GC60, and GC23s)]. a*P* < 0.05 using a one-way ANOVA with Bonferroni's multiple comparisons test.

spinal cord but not in the stomach.

DISCUSSION

Gastric cancer progression is associated with *Helicobacter pylori* and gastric stem cells are suggested to involve in the progression from chronic atrophic gastritis to gastric cancer[33]. It may be assumed that *RGS1* plays a role in regulating G protein signaling and mitogen-activated protein (MAP) kinase signaling during gastric cancer progression, which may provide some hypotheses in which *RGS1* promotes stem cell transition into CSCs, CSCs induce *RGS1* expression in gastric cancer, or *RGS1* orchestrates with other molecules in EMT. In gastric cancer cells, *CDH1* isoform is found to increase gastric cancer cell invasion and angiogenesis, and some inflammatory mechanism is suggested to be involved in *CDH1* regulation[34]. Analysis of *Rgs1*(-/-) has shown that B cell responses to chemokines, plasma cell localization, and immune tissue architecture are disturbed when *Rgs1* is deleted[35]. In our studies, *RGS1* was up-regulated in diffuse-type GC compared to MSC, which may suggest that *RGS1* plays a role to response cancer cells and indicate the possibility to involve in cancer immunity. It may be possible that *RGS1* protects the cells from cancer progression by enhancing anti-cancer immunity, or promote cell phenotype transition to cancer. The possibility that diffuse-type GC sample contains a variety of cells in cancer microenvironment should be considered. It may also be possible that *RGS1* is up-regulated in some cancer microenvironment cells. Because the dedifferentiation of epithelial cells into stem cells may play a role in regenerative medicine or diseases, including cancer, investigating the genes regulated in stem and cancer cells is important[36]. Changes in the gene expression profiles of early- and late-stage MSC cultures have provided evidence regarding the importance of studying gene combinations to understand cellular phenotypes[8]. Cell surface antigens, such as SSEA4 or TRA-1-81, should also be included for future quality control in stem cells[37-39]. Furthermore, cell type-specific transcriptional profiles have been suggested for neural lineage cells, hepatic lineage cells, embryonic stem cells and induced pluripotent stem cells (iPSCs)[40]. Interestingly, insulin-like growth factor-1 receptor signaling and EMT crosstalk is sufficient to down-regulate erlotinib function in cells, while exon 19 of the erlotinib-sensitive epidermal growth factor receptor is mutated in non-small cell lung carcinomas[41]. Many cell-based iPSC models that are derived from patient cells have been investigated[42], and successful genetic or epigenetic reprogramming of cellular identity is crucial for the development of these stem-cell based disease models[42]. The investigation of the molecular combinations associated with different cell types is important for determining cellular phenotypes.

RGS1 has been shown to be related to cancer in some studies[43-45]. In late-stage non-small cell lung cancer, single nucleotide polymorphisms in *RGS1* have been shown to

Table 3 Gene expression alterations of epithelial-mesenchymal transition-related genes in mesenchymal stem cells and diffuse-type gastric cancer

Gene symbol	Gene name	*P* value	Ratio of diffuse-type GC to MSCs	Probe set ID
TWIST1	Twist family bHLH transcription factor 1	0.0004	0.2	213943_at
PRRX1	Paired related homeobox 1	1.00E-05	0.3	226695_at
ZEB2	Zinc finger E-box binding homeobox 2	0.027	2.1	203603_s_at
		0.03	1.7	228333_at
		6.00E-05	3.9	235593_at
TJP1	Tight junction protein 1	0.184	1.2	202011_at
		0.795	1	214168_s_at
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa	0.001	9.6	223679_at
VIM	Vimentin	0.031	4.7	1555938_x_at
		0.016	0.7	201426_s_at
FN1	Fibronectin 1	0.204	0.6	1558199_at
		0.015	0.7	210495_x_at
		0.004	0.7	211719_x_at
		0.001	0.6	212464_s_at
		4.00E-08	0.04	214701_s_at
		1.00E-10	0.02	214702_at
		0.008	0.7	216442_x_at
AKT1	v-akt murine thymoma viral oncogene homolog 1	0.187	1.2	207163_s_at
AKT2	v-akt murine thymoma viral oncogene homolog 2	8.00E-09	3.1	225471_s_at
		0.002	4.4	226156_at
		0.0006	5.6	236664_at
		0.0006	7.6	1560689_s_at
AKT3	v-akt murine thymoma viral oncogene homolog 3	0.0006	6.2	242876_at
		0.008	0.6	212607_at
		0.549	1.2	212609_s_at
		0.385	0.8	219393_s_at
		0.772	1.1	222880_at
		0.0003	0.5	242879_x_at
SFN	Stratifin	0.003	66	33322_i_at
		0.008	111	33323_r_at
EPCAM	Epithelial cell adhesion molecule	0.0009	725	201839_s_at
F11R	F11 receptor	0.0004	35	221664_s_at
		0.002	21	222354_at
		0.0009	201	223000_s_at
		0.012	25	224097_s_at
GJB2	Gap junction protein, beta 2, 26 kDa	0.023	69	223278_at
LEF1	Lymphoid enhancer-binding factor 1	0.002	26	221558_s_at
JUN	Jun proto-oncogene	0.042	4.8	201464_x_at
		0.022	5.1	201466_s_at
		0.079	3.8	201465_s_at
		0.019	4.1	213281_at
TGFB1	Transforming growth factor, beta 1	0.002	2	203085_s_at
TGFB2	Transforming growth factor, beta 2	0.07	0.4	209909_s_at
		0.055	0.6	220407_s_at
		0.588	0.8	228121_at
TGFB3	Transforming growth factor, beta 3	0.015	6.8	209747_at
SP1	Sp1 transcription factor	0.0002	3.4	224754_at
		2.00E-05	2.7	224760_at
HMGA2	High mobility group AT-hook 2	0.003	0.3	208025_s_at
ITGA5	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	0.007	0.5	201389_at
OCLN	Occludin	0.0008	8	231022_at
		0.012	9.4	235937_at
		0.027	11	209925_at
		0.0045	11	227492_at
CXADR	Coxsackie virus and adenovirus receptor	0.013	265	203917_at
		0.014	297	226374_at
		0.021	75	239155_at
KRT8	Keratin 8	0.012	128	209008_x_at

RGS: Regulator of G-protein signaling; MSCs: Mesenchymal stem cells; GC: Gastric cancer.

be associated with overall survival in patients who received chemotherapy[43]. Silencing of *RGS1* and *RGS13* mRNAs resulted in enhanced responsiveness to chemoattractants in a human B lymphoma line[44]. *RGS1* has been suggested to be involved in regulatory T cell trafficking[45]. Furthermore, *RGS1* is up-regulated in T cells from the

Table 4 Gene expression alterations of NOTCH signaling-related genes in mesenchymal stem cells and diffuse-type gastric cancer

Gene symbol	Gene name	*P* value	Ratio of diffuse-type GC to MSCs	Probe set ID
NOTCH1	Notch 1	0.004	6.9	218902_at
NOTCH2	Notch 2	0.001	0.7	202445_s_at
		1.00E-05	0.5	202443_x_at
		1.00E-05	0.5	212377_s_at
DLL1	Delta-like 1 (Drosophila)	0.007	31	224215_s_at
JAG2	Jagged 2	0.003	11	32137_at
JAG1	Jagged 1	0.003	1.5	209099_x_at
		0.022	1.4	216268_s_at
		0.49	1.1	209098_s_at
WNT9A	Wingless-type MMTV integration site family, member 9A	0.027	5.4	230643_at
WNT2B	Wingless-type MMTV integration site family, member 2B	0.026	4.4	206458_s_at
WNT5A	Wingless-type MMTV integration site family, member 5A	8.00E-05	0.3	213425_at
		3.00E-05	0.2	205990_s_at
WNT5B	Wingless-type MMTV integration site family, member 5B	0.0002	0.2	221029_s_at

RGS: Regulator of G-protein signaling; MSCs: Mesenchymal stem cells; GC: Gastric cancer.
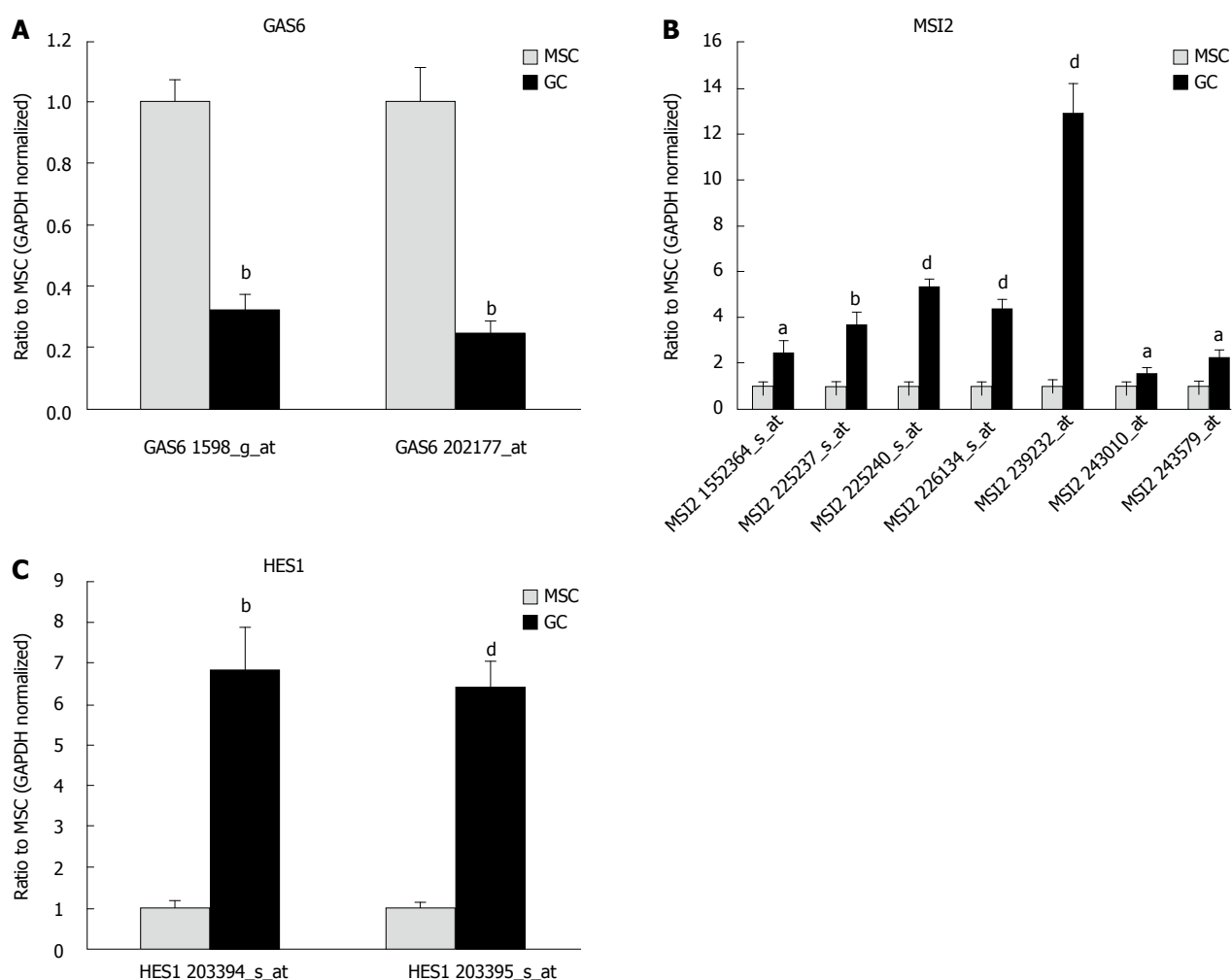

Figure 4 Gene expression of stem cell-related genes. A: The gene expression of growth arrest-specific 6 (*GAS6*) was down-regulated in diffuse-type gastric cancer (GC) compared with mesenchymal stem cell (MSCs). Results represent the mean ± SE (*n* = 12 in MSC and *n* = 5 in diffuse-type GC). [b]$P < 0.001$ using Student's *t* test; B: The gene expression of musashi RNA-binding protein 2 (MSI2) was up-regulated in diffuse-type GC compared with MSCs. Results represent the mean ± SE (*n* = 12 in MSC and *n* = 5 in diffuse-type GC). [a]$P < 0.05$, [b]$P < 0.01$, and [d]$P < 0.001$ using Student's *t* test; C: The gene expression of hairy and enhancer of split 1 (Drosophila) (HES1) was up-regulated in diffuse-type GC compared with MSCs. Results represent the mean ± SE (*n* = 12 in MSC and *n* = 5 in diffuse-type GC). [b]$P < 0.01$ and [d]$P < 0.001$ using Student's *t* test. All three genes have stem cell terms in the Gene Ontology biological process.

human gut compared with T cells from peripheral blood, and *RGS1* regulates gut T cell trafficking[46]. Cancer cells

Table 5 Gene ontology of NOTCH family genes

Gene symbol	Gene ontology biological process
NOTCH1	0000122 // negative regulation of transcription from RNA polymerase II promoter // inferred from sequence or structural similarity /// 0001525 // angiogenesis // inferred from electronic annotation /// 0001701 // in utero embryonic development // inferred from electronic annotation /// 0001708 // cell fate specification // inferred from electronic annotation /// 0001837 // epithelial to mesenchymal transition // inferred from sequence or structural similarity /// 0001889 // liver development // inferred from electronic annotation /// 0001947 // heart looping // inferred from sequence or structural similarity /// 0002040 // sprouting angiogenesis // inferred from electronic annotation /// 0002052 // positive regulation of neuroblast proliferation // inferred from electronic annotation /// 0002437 // inflammatory response to antigenic stimulus // inferred from electronic annotation /// 0003157 // endocardium development // inferred from sequence or structural similarity /// 0003160 // endocardium morphogenesis // inferred from sequence or structural similarity /// 0003162 // atrioventricular node development // inferred from electronic annotation /// 0003169 // coronary vein morphogenesis // inferred from sequence or structural similarity /// 0003180 // aortic valve morphogenesis // inferred from mutant phenotype /// 0003181 // atrioventricular valve morphogenesis // inferred from sequence or structural similarity /// 0003184 // pulmonary valve morphogenesis // inferred from mutant phenotype /// 0003192 // mitral valve formation // inferred from mutant phenotype /// 0003198 // epithelial to mesenchymal transition involved in endocardial cushion formation // inferred from sequence or structural similarity /// 0003203 // endocardial cushion morphogenesis // inferred from sequence or structural similarity /// 0003207 // cardiac chamber formation // inferred from sequence or structural similarity /// 0003208 // cardiac ventricle morphogenesis // inferred from sequence or structural similarity /// 0003209 // cardiac atrium morphogenesis // inferred from sequence or structural similarity /// 0003213 // cardiac right atrium morphogenesis // inferred from sequence or structural similarity /// 0003214 // cardiac left ventricle morphogenesis // inferred from sequence or structural similarity /// 0003219 // cardiac right ventricle formation // inferred from electronic annotation /// 0003222 // ventricular trabecula myocardium morphogenesis // inferred from sequence or structural similarity /// 0003241 // growth involved in heart morphogenesis // inferred from sequence or structural similarity /// 0003256 // regulation of transcription from RNA polymerase II promoter involved in myocardial precursor cell differentiation // inferred from sequence or structural similarity /// 0003264 // regulation of cardioblast proliferation // inferred from electronic annotation /// 0003270 // Notch signaling pathway involved in regulation of secondary heart field cardioblast proliferation // inferred from electronic annotation /// 0003273 // cell migration involved in endocardial cushion formation // inferred from sequence or structural similarity /// 0003344 // pericardium morphogenesis // inferred from sequence or structural similarity /// 0006351 // transcription, DNA-dependent // inferred from electronic annotation /// 0006355 // regulation of transcription, DNA-dependent // traceable author statement /// 0006357 // regulation of transcription from RNA polymerase II promoter // inferred from electronic annotation /// 0006367 // transcription initiation from RNA polymerase II promoter // traceable author statement /// 0006955 // immune response // non-traceable author statement /// 0006959 // humoral immune response // inferred from electronic annotation /// 0007219 // Notch signaling pathway // inferred from mutant phenotype /// 0007219 // Notch signaling pathway // traceable author statement /// 0007220 // Notch receptor processing // traceable author statement /// 0007221 // positive regulation of transcription of Notch receptor target // inferred from sequence or structural similarity /// 0007275 // multicellular organismal development // inferred from electronic annotation /// 0007283 // spermatogenesis // inferred from electronic annotation /// 0007368 // determination of left/right symmetry // inferred from sequence or structural similarity /// 0007386 // compartment pattern specification // inferred from electronic annotation /// 0007409 // axonogenesis // inferred from electronic annotation /// 0007440 // foregut morphogenesis // inferred from electronic annotation /// 0007492 // endoderm development // inferred from electronic annotation /// 0007507 // heart development // inferred from mutant phenotype /// 0008284 // positive regulation of cell proliferation // inferred from direct assay /// 0008284 // positive regulation of cell proliferation // inferred from mutant phenotype /// 0008285 // negative regulation of cell proliferation // inferred from direct assay /// 0008544 // epidermis development // inferred from electronic annotation /// 0009790 // embryo development // inferred from electronic annotation /// 0009912 // auditory receptor cell fate commitment // inferred from electronic annotation /// 0010001 // glial cell differentiation // inferred from electronic annotation /// 0010467 // gene expression // traceable author statement /// 0010468 // regulation of gene expression // inferred from electronic annotation /// 0010718 // positive regulation of epithelial to mesenchymal transition // inferred from mutant phenotype /// 0010812 // negative regulation of cell-substrate adhesion // inferred from direct assay /// 0014031 // mesenchymal cell development // inferred from sequence or structural similarity /// 0014807 // regulation of somitogenesis // inferred from electronic annotation /// 0021515 // cell differentiation in spinal cord // inferred from electronic annotation /// 0021915 // neural tube development // inferred from electronic annotation /// 0030154 // cell differentiation // inferred from electronic annotation /// 0030182 // neuron differentiation // inferred from electronic annotation /// 0030216 // keratinocyte differentiation // inferred from electronic annotation /// 0030279 // negative regulation of ossification // inferred from sequence or structural similarity /// 0030324 // lung development // inferred from electronic annotation /// 0030326 // embryonic limb morphogenesis // inferred from electronic annotation /// 0030334 // regulation of cell migration // inferred from sequence or structural similarity /// 0030335 // positive regulation of cell migration // inferred from electronic annotation /// 0030513 // positive regulation of BMP signaling pathway // inferred from sequence or structural similarity /// 0030514 // negative regulation of BMP signaling pathway // inferred from sequence or structural similarity /// 0030900 // forebrain development // inferred from electronic annotation /// 0031069 // hair follicle morphogenesis // inferred from electronic annotation /// 0031100 // organ regeneration // inferred from electronic annotation /// 0031960 // response to corticosteroid stimulus // inferred from electronic annotation /// 0032495 // response to muramyl dipeptide // inferred from electronic annotation /// 0032496 // response to lipopolysaccharide // inferred from electronic annotation /// 0035116 // embryonic hindlimb morphogenesis // inferred from electronic annotation /// 0035148 // tube formation // inferred from mutant phenotype /// 0035914 // skeletal muscle cell differentiation // inferred from electronic annotation /// 0035924 // cellular response to vascular endothelial growth factor stimulus // inferred from direct assay /// 0042127 // regulation of cell proliferation // inferred from electronic annotation /// 0042246 // tissue regeneration // inferred from electronic annotation /// 0042640 // anagen // inferred from electronic annotation /// 0043065 // positive regulation of apoptotic process // inferred from electronic annotation /// 0043066 // negative regulation of apoptotic process // inferred from electronic annotation /// 0045596 // negative regulation of cell differentiation // inferred from electronic annotation /// 0045603 // positive regulation of endothelial cell differentiation // inferred from electronic annotation /// 0045618 // positive regulation of keratinocyte differentiation // inferred from electronic annotation /// 0045662 // negative regulation of myoblast differentiation // inferred from mutant phenotype /// 0045665 // negative regulation of neuron differentiation // inferred from electronic annotation /// 0045668 // negative regulation of osteoblast differentiation // inferred from sequence or structural similarity /// 0045687 // positive regulation of glial cell differentiation // inferred from electronic annotation /// 0045892 // negative regulation of transcription, DNA-dependent // inferred from sequence or structural similarity /// 0045893 // positive regulation of

transcription, DNA-dependent // inferred from sequence or structural similarity /// 0045944 // positive regulation of transcription from RNA polymerase II promoter // inferred from direct assay /// 0045944 // positive regulation of transcription from RNA polymerase II promoter // inferred from sequence or structural similarity /// 0045955 // negative regulation of calcium ion-dependent exocytosis // inferred from electronic annotation /// 0046427 // positive regulation of JAK-STAT cascade // inferred from sequence or structural similarity /// 0046533 // negative regulation of photoreceptor cell differentiation // inferred from electronic annotation /// 0048103 // somatic stem cell division // inferred from electronic annotation /// 0048663 // neuron fate commitment // inferred from electronic annotation /// 0048708 // astrocyte differentiation // inferred from electronic annotation /// 0048709 // oligodendrocyte differentiation // inferred from electronic annotation /// 0048711 // positive regulation of astrocyte differentiation // inferred from sequence or structural similarity /// 0048715 // negative regulation of oligodendrocyte differentiation // inferred from sequence or structural similarity /// 0048754 // branching morphogenesis of an epithelial tube // inferred from electronic annotation /// 0050678 // regulation of epithelial cell proliferation // inferred from electronic annotation /// 0050679 // positive regulation of epithelial cell proliferation // inferred from sequence or structural similarity /// 0050767 // regulation of neurogenesis // inferred from electronic annotation /// 0050768 // negative regulation of neurogenesis // inferred from sequence or structural similarity /// 0050793 // regulation of developmental process // inferred from electronic annotation /// 0055008 // positive regulation of cardiac muscle tissue morphogenesis // inferred from electronic annotation /// 0060038 // cardiac muscle cell proliferation // inferred from electronic annotation /// 0060045 // positive regulation of cardiac muscle cell proliferation // inferred from sequence or structural similarity /// 0060253 // negative regulation of glial cell proliferation // inferred from sequence or structural similarity /// 0060317 // cardiac epithelial to mesenchymal transition // inferred from sequence or structural similarity /// 0060411 // cardiac septum morphogenesis // inferred from sequence or structural similarity /// 0060412 // ventricular septum morphogenesis // inferred from mutant phenotype /// 0060528 // secretory columnal luminal epithelial cell differentiation involved in prostate glandular acinus development // inferred from electronic annotation /// 0060548 // negative regulation of cell death // inferred from electronic annotation /// 0060740 // prostate gland epithelium morphogenesis // inferred from electronic annotation /// 0060768 // regulation of epithelial cell proliferation involved in prostate gland development // inferred from electronic annotation /// 0060842 // arterial endothelial cell differentiation // inferred from sequence or structural similarity /// 0060843 // venous endothelial cell differentiation // inferred from sequence or structural similarity /// 0060948 // cardiac vascular smooth muscle cell development // inferred from sequence or structural similarity /// 0060956 // endocardial cell differentiation // inferred from sequence or structural similarity /// 0060979 // vasculogenesis involved in coronary vascular morphogenesis // inferred from sequence or structural similarity /// 0060982 // coronary artery morphogenesis // inferred from sequence or structural similarity /// 0061314 // Notch signaling involved in heart development // inferred from mutant phenotype /// 0061384 // heart trabecula morphogenesis // inferred from sequence or structural similarity /// 0061419 // positive regulation of transcription from RNA polymerase II promoter in response to hypoxia // inferred from sequence or structural similarity /// 0070986 // left/right axis specification // inferred from electronic annotation /// 0071372 // cellular response to follicle-stimulating hormone stimulus // inferred from direct assay /// 0072017 // distal tubule development // inferred from electronic annotation /// 0072044 // collecting duct development // inferred from electronic annotation /// 0090051 // negative regulation of cell migration involved in sprouting angiogenesis // inferred from direct assay /// 0072144 // glomerular mesangial cell development // inferred from electronic annotation /// 0072602 // interleukin-4 secretion // inferred from electronic annotation /// 0090090 // negative regulation of canonical Wnt receptor signaling pathway // inferred from electronic annotation /// 2000737 // negative regulation of stem cell differentiation // inferred from mutant phenotype /// 2000811 // regulation of anoikis // inferred from mutant phenotype /// 2000974 // negative regulation of pro-B cell differentiation // inferred from sequence or structural similarity /// 0097150 // neuronal stem cell maintenance // inferred from expression pattern /// 1901201 // regulation of extracellular matrix assembly // inferred from mutant phenotype /// 2001027 // negative regulation of endothelial cell chemotaxis // inferred from direct assay

NOTCH2 0001701 // in utero embryonic development // inferred from electronic annotation /// 0001709 // cell fate determination // inferred from electronic annotation /// 0001890 // placenta development // inferred from electronic annotation /// 0002011 // morphogenesis of an epithelial sheet // inferred from electronic annotation /// 0002437 // inflammatory response to antigenic stimulus // inferred from electronic annotation /// 0003184 // pulmonary valve morphogenesis // inferred from mutant phenotype /// 0006351 // transcription, DNA-dependent // inferred from electronic annotation /// 0006355 // regulation of transcription, DNA-dependent // traceable author statement /// 0006367 // transcription initiation from RNA polymerase II promoter // traceable author statement /// 0006915 // apoptotic process // traceable author statement /// 0006917 // induction of apoptosis // traceable author statement /// 0006959 // humoral immune response // inferred from electronic annotation /// 0007050 // cell cycle arrest // inferred from direct assay /// 0007219 // Notch signaling pathway // traceable author statement /// 0007220 // Notch receptor processing // traceable author statement /// 0007275 // multicellular organismal development // non-traceable author statement /// 0007368 // determination of left/right symmetry // inferred from electronic annotation /// 0007399 // nervous system development // non-traceable author statement /// 0008285 // negative regulation of cell proliferation // inferred from direct assay /// 0009887 // organ morphogenesis // inferred from expression pattern /// 0010467 // gene expression // traceable author statement /// 0016049 // cell growth // inferred from direct assay /// 0019827 // stem cell maintenance // traceable author statement /// 0030097 // hemopoiesis // traceable author statement /// 0030154 // cell differentiation // inferred from electronic annotation /// 0030326 // embryonic limb morphogenesis // inferred from electronic annotation /// 0042246 // tissue regeneration // inferred from electronic annotation /// 0043065 // positive regulation of apoptotic process // inferred from direct assay /// 0043066 // negative regulation of apoptotic process // traceable author statement /// 0046579 // positive regulation of Ras protein signal transduction // inferred from direct assay /// 0046849 // bone remodeling // inferred from mutant phenotype /// 0050793 // regulation of developmental process // inferred from electronic annotation /// 0060413 // atrial septum morphogenesis // inferred from mutant phenotype /// 0060674 // placenta blood vessel development // inferred from electronic annotation /// 0061314 // Notch signaling involved in heart development // inferred by curator /// 0072602 // interleukin-4 secretion // inferred from electronic annotation

NOTCH3 0006351 // transcription, DNA-dependent // inferred from electronic annotation /// 0006355 // regulation of transcription, DNA-dependent // inferred from electronic annotation /// 0006367 // transcription initiation from RNA polymerase II promoter // traceable author statement /// 0007219 // Notch signaling pathway // traceable author statement /// 0007220 // Notch receptor processing // traceable author statement /// 0030154 // cell differentiation // inferred from electronic annotation /// 0007275 // multicellular organismal development // inferred from electronic annotation /// 0010467 // gene expression // traceable author statement /// 0030154 // cell differentiation // inferred from electronic annotation /// 0030900 // forebrain development // inferred from electronic annotation /// 0045596 // negative regulation of cell differentiation // inferred from electronic annotation /// 0045665 // negative regulation of neuron differentiation // inferred from electronic annotation /// 0048661 // positive regulation of smooth muscle cell proliferation // inferred from electronic annotation /// 0048663 // neuron fate commitment // inferred from electronic annotation /// 0050793 // regulation of developmental process // inferred from electronic annotation /// 0072104 // glomerular capillary formation // inferred from electronic annotation

NOTCH4	0001569 // patterning of blood vessels // inferred from sequence or structural similarity /// 0001709 // cell fate determination // traceable author statement /// 0001763 // morphogenesis of a branching structure // inferred from sequence or structural similarity /// 0001886 // endothelial cell morphogenesis // inferred from electronic annotation /// 0006351 // transcription, DNA-dependent // inferred from electronic annotation /// 0006355 // regulation of transcription, DNA-dependent // inferred from electronic annotation /// 0006367 // transcription initiation from RNA polymerase II promoter // traceable author statement /// 0007219 // Notch signaling pathway // traceable author statement /// 0007220 // Notch receptor processing // traceable author statement /// 0007221 // positive regulation of transcription of Notch receptor target // traceable author statement /// 0007275 // multicellular organismal development // inferred from electronic annotation /// 0009790 // embryo development // inferred from sequence or structural similarity /// 0010467 // gene expression // traceable author statement /// 0030097 // hemopoiesis // traceable author statement /// 0030154 // cell differentiation // non-traceable author statement /// 0030879 // mammary gland development // inferred from direct assay /// 0045446 // endothelial cell differentiation // inferred from electronic annotation /// 0045596 // negative regulation of cell differentiation // non-traceable author statement /// 0045602 // negative regulation of endothelial cell differentiation // inferred from sequence or structural similarity /// 0045893 // positive regulation of transcription, DNA-dependent // traceable author statement /// 0050793 // regulation of developmental process // inferred from electronic annotation

interacting with the immune system may be future targets for cancer treatment. Considering that *RGS1* is up-regulated in diffuse-type GC compared with MSCs, *RGS1* may be a potential target for cancer treatment. *RGS1* is involved in an adenylate cyclase-inhibiting G-protein coupled receptor signaling pathway, immune response, positive regulation of GTPase activity, and termination of a G-protein coupled receptor signaling pathway in the Gene Ontology biological process [Traceable author statement (TSA); Inferred from electronic annotation (IEA)] (NCBI). From the investigation of EMT-related genes and *RGS1*, this study may suggest the possibility of future gene applications, including *RGS1*, to evaluate cancer cells to their sensitivity to treatment. microRNAs have been reported to be involved in *RGS5* regulation in neuroblastoma[48]. Further assessments are needed to examine the possibility that *RGS1* could be a marker for responsiveness to cancer treatment.

NOTCH gene expression was altered in diffuse-type GC and MSCs. Gene expression of *NOTCH1, 3* and *4* was up-regulated in diffuse-type GC compared with MSCs, whereas *NOTCH2* was down-regulated in diffuse-type GC. Upon Notch ligand cellular stimulation, the intracellular domain of the Notch receptor (NICD) translocates into the nucleus and regulates gene transcription, which leads to cell communication in signaling[49,50]. The regulation of *NOTCH* family gene expression may play an important role in the transcriptional regulation of cell phenotype transitions. *NOTCH1* expression is increased in gastric cancer with non-cardia location and diffuse type[51]. The up-regulation of *NOTCH1* in diffuse-type GC compared to MSCs in our studies is consistent with the results in previous reports. *NOTCH2* has been reported to be a candidate marker for prognosis of neoadjuvant-treated gastric cancer[52]. Combination of expression of *GSK3B, CTNNB1, NOTCH1* was suggested to be correlated with patient survival. The previous study indicated that *NOTCH2* may be involved in chemotherapy resistance in GC[52]. Considering that CSCs may be involved in drug resistance, the role of *NOTCH2* should be investigated in CSCs or EMT. *NOTCH2* expression was increased in post-therapeutic tumors[52]. Since *NOTCH2* was down-regulated in diffuse-type GC compared to MSCs, *NOTCH2* may mark some cancer phenotype in different stages. *NOTCH3* was found to be increased in diffuse-type GC[51,53]. The previous reports are consistent with the results in this study indicating that *NOTCH3* is up-regulated in diffuse-type GC compared to MSCs. Interestingly, *NOTCH4* is suggested to be related to gastric carcinogenesis targeted by microRNA *miR-181c*[54]. Our data indicating *NOTCH4* up-regulation in diffuse-type GC compared to MSCs is consistent with the previous findings suggesting involvement of *NOTCH4* in gastric carcinogenesis. *NOTCH1* and *NOTCH2* exhibit opposite functions and play important roles in cancer as oncogenes or cancer suppressors depending on the cancer species. The relationship with *NOTCH* signaling and gastric cancer signaling should be investigated in the future (Figure 6A). In the EMT hypothesis, it is possible that epithelial-phenotype cells expressing *NOTCH1* transition to mesenchymal-phenotype cells that express *NOTCH2* (Figure 6B). Integrin beta 1 expression is important for the cancer cell population, and the insights into the mechanisms of cell phenotype conversion by gene regulation may provide fundamental understanding of cancer development[55]. Several pathways, including the claudin family genes and MAP kinase signaling pathways, have been investigated by gene set enrichment analysis, which may be the next step for the precise investigation of cancer drug response signaling[56].
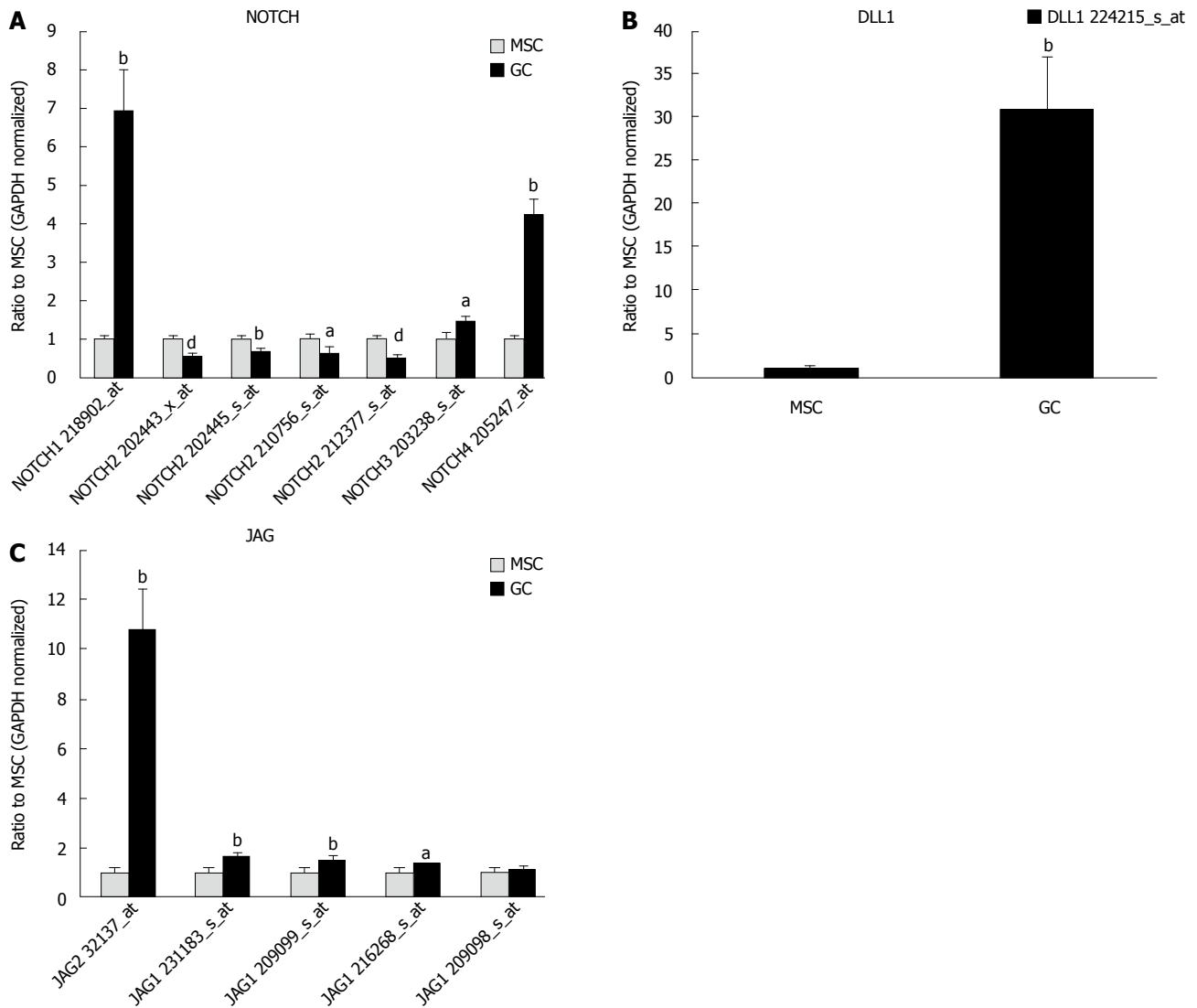
ACKNOWLEDGMENTS

Figure 5 Gene expression of *NOTCH*, delta-like 1 (Drosophila) (DLL1) and JAG. A: The gene expression of *NOTCH1*, 3 and 4 was up-regulated in diffuse-type gastric cancer (GC) compared with mesenchymal stem cell (MSCs), whereas NOTCH2 was down-regulated. Results represent the mean ± SE (n = 12 in MSC cultures and n = 5 in diffuse-type GC). [a]$P < 0.05$, [b]$P < 0.01$, and [d]$P < 0.001$ using Student's t test; B: DLL1, a NOTCH ligand, was up-regulated in diffuse-type GC compared with MSCs. Results represent the mean ± SE (n = 12 in MSC cultures and n = 5 in diffuse-type GC). [b]$P < 0.01$ using Student's t test; C: Jagged 2 (JAG2), a NOTCH ligand, was up-regulated in diffuse-type GC compared with MSCs. Jagged 1 (JAG1) was up-regulated in diffuse-type GC compared with MSCs. Results represent the mean ± SE (n = 12 in MSC cultures and n = 5 in diffuse-type GC). [a]$P < 0.05$ and [b]$P < 0.01$ using Student's t test.

COMMENTS

Background

Mesenchymal stem cells (MSCs) exhibit mesenchymal features and are useful in regenerative medicine. Gastric cancer (GC) can be classified into two types, including diffuse-type GC that has mesenchymal features and intestinal-type GC that has epithelial features. The epithelial-mesenchymal transition (EMT) is an important mechanism in cancer development. The EMT is known to be involved in cancer cell migration and malignancy. Cancer stem cells (CSCs) are known to be resistant to anti-cancer drugs and involved in cancer recurrence.

Research frontiers

Gene regulation in MSCs and diffuse-type GC may provide new insights into CSCs. In this study, regulator of G-protein signaling (*RGS*) has been identified to be up-regulated in diffuse-type GC compared with MSCs. It is a novel hypothesis where *RGS* signaling may be involved in the EMT pathway.

Innovations and breakthroughs

Recent progress in genome-wide analysis in cancer has revealed that the gene signature is important for cancer diagnosis and exploring the targets of cancer therapy. CSCs are one of the targets for cancer treatment because these cells

tend to be resistant to anti-cancer drugs and are involved in cancer recurrence. This study is the first to demonstrate that *RGS1* may distinguish diffuse-type GC and MSCs.

Applications

By understanding how *RGS1* is involved in the EMT and CSC signaling, this study represents potential future strategies for GC including diffuse-type GC treatment and prediction.

Terminology

RGS1 is protein involved in G-protein signaling and the regulation of GDP dissociation from the G alpha subunit and GTP binding to form trimetric G proteins. The EMT is the mechanism by which epithelial cells change into mesenchymal cells with a migratory capacity. Several adhesion molecules are involved in the EMT, and *RGS1* may contribute to the changes in signaling.

Peer review

The authors described a global gene expression analysis in comparison between MSCs and diffuse-type GC cells. The authors found that *RGS1* gene was upregulated in diffuse-type GC cells compared with MSCs, and that genes related to stem cells and *NOTCH* signaling were altered in diffuse-type GC cells compared with MSCs. The results may provide some insights in correlation
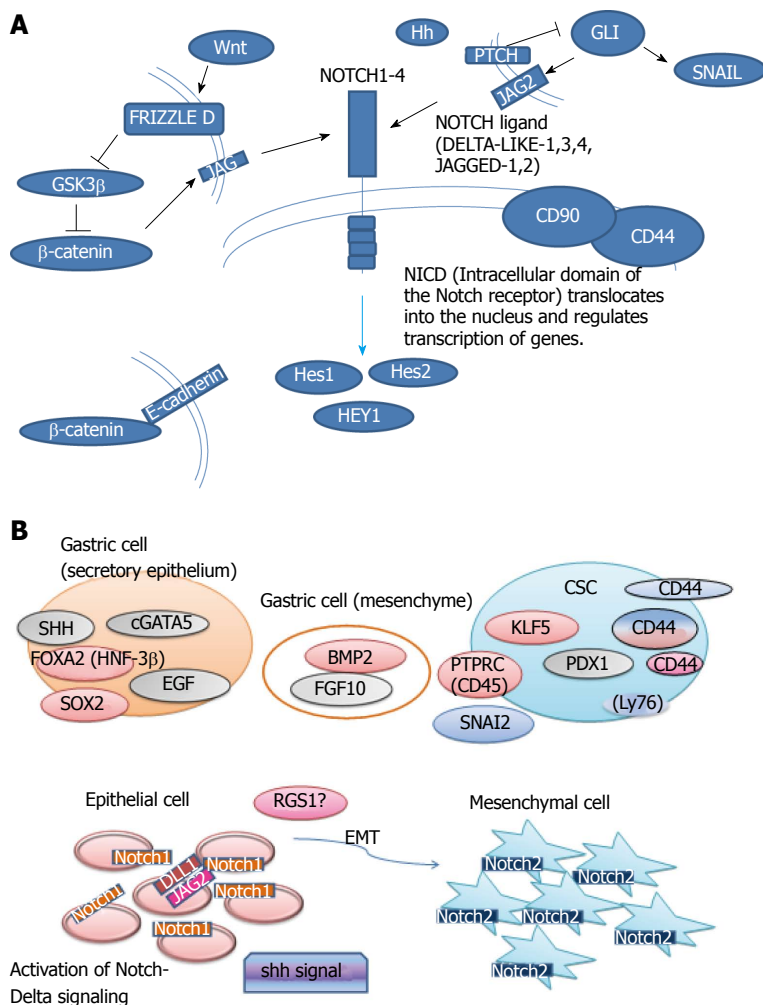
Figure 6 Notch signaling and the epithelial-mesenchymal transition hypothesis. A: Upon cellular stimulation by the Notch ligand, the intracellular domain of the Notch receptor (NICD) translocates into the nucleus and regulates gene transcription; B: In the EMT hypothesis, epithelial-phenotype cells expressing NOTCH1 transition to mesenchymal-phenotype cells expressing NOTCH2. RGS1: Regulator of G-protein signaling 1; EMT: Epithelial-mesenchymal transition; JAG2: Jagged 2; CSCs: Cancer stem cells.

between the EMT and CSCs. This paper summarizes important and actual data, it fulfils all criteria and demands put on articles published in World Journal of Stem Cells.

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