



G0/G1 Switch 2 Induces Cell Survival and Metastasis through Integrin-Mediated Signal Transduction in Human Invasive Breast Cancer Cells

Eunah Cho, Yeo-Jung Kwon, Dong-Jin Ye, Hyung-Seok Baek, Tae-Uk Kwon, Hyung-Kyoon Choi and Young-Jin Chun*

College of Pharmacy and Center for Metareceptome Research, Chung-Ang University, Seoul 06974, Republic of Korea

Abstract

Human breast cancer cell line, MDA-MB-231, is highly invasive and aggressive, compared to less invasive cell line, MCF-7. To explore the genes that might influence the malignancy of MDA-MB-231, DNA microarray analysis was performed. The results showed that G0/G1 switch 2 (G0S2) was one of the most highly expressed genes among the genes upregulated in MDA-MB-231. Although G0S2 acts as a direct inhibitor of adipose triglyceride lipase, action of G0S2 in cancer progression is not yet understood. To investigate whether G0S2 affects invasiveness of MDA-MB-231 cells, G0S2 expression was inhibited using siRNA, which led to decreased cell proliferation, migration, and invasion of MDA-MB-231 cells. Consequently, G0S2 inhibition inactivated integrin-regulated FAK-Src signaling, which promoted Hippo signaling and inactivated ERK1/2 signaling. In addition, G0S2 downregulation decreased β -catenin expression, while E-cadherin expression was increased. It was demonstrated for the first time that G0S2 mediates the Hippo pathway and induces epithelial to mesenchymal transition (EMT). Taken together, our results suggest that G0S2 is a major factor contributing to cell survival and metastasis of MDA-MB-231 cells.

Key Words: G0S2, FAK-Src signaling, Hippo pathway, EMT

INTRODUCTION

Triple-negative breast cancer is a type of breast cancer that lacks estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (Her2/neu receptor) (Mukherjee and Zhao, 2013; Yadav *et al.*, 2015). Triple-negative breast cancer accounts for 10% to 20% of human breast cancers (Zhang *et al.*, 2013). It shows high cell proliferation and metastasis (Thike *et al.*, 2010; Yadav *et al.*, 2015). Furthermore, triple-negative breast cancer patients exhibit poor prognosis and higher risk of recurrence (Pogoda *et al.*, 2013). Since triple-negative breast cancer lacks hormone receptors, hormone therapies and drugs that target ER, PR, and Her2 may be ineffective against it. Thus, targeted therapy and chemotherapy are the main therapeutic methods against triple-negative breast cancers. Thus, it is essential to elucidate aggressive characteristics of triple-negative breast cancers for finding novel therapeutic targets and approaches.

The Hippo pathway initially was known as a regulator which

controls organ size and proliferation. Recent studies have discovered that the Hippo pathway is strongly involved in cell-cell contraction, cell polarity, apoptosis, and cell cycle. In cancer, the Hippo pathway is a tumor suppressor, and its deregulation is recognized as an important feature in many cancers. The transcription coactivator YAP1, which is a major target of the Hippo pathway, is implicated with induction of EMT as well as proliferation, invasion, metastasis. YAP1 induces expression of genes such as amphiregulin, cysteine-rich angiogenic inducer 61 (CYR61), and connective tissue growth factor (CTGF) through TEA domain (TEAD) family transcription factors and they are highly activated in metastatic breast cancer cells (Lamar *et al.*, 2012).

The G0/G1 switch gene 2 (G0S2) is a protein comprising 103 amino acids (Zandbergen *et al.*, 2005). Expression of G0S2 is abundant in metabolically active tissues, such as adipose tissue, liver, heart, and skeletal muscle (Zhang *et al.*, 2017). In cell organelles, G0S2 is mainly located in the endoplasmic reticulum (ER), mitochondria (Zandbergen *et al.*,

Open Access <https://doi.org/10.4062/biomolther.2019.063>

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received Apr 15, 2019 Revised May 24, 2019 Accepted Jun 4, 2019

Published Online Jul 5, 2019

*Corresponding Author

E-mail: yjchun@cau.ac.kr

Tel: +82-2-820-5616, Fax: +82-2-825-5616

2005; Nielsen *et al.*, 2011), and lipid droplets (LDs) (Yang *et al.*, 2010). The major function of G0S2 is directly binding to adipose triglyceride lipase, inhibiting triglyceride hydrolase activity by influencing lipid metabolism (Yang *et al.*, 2010; Zhang *et al.*, 2017). A recent study showed that G0S2 promotes larger LDs in brown adipocytes (Heckmann *et al.*, 2014).

Besides, there have been several studies related to association between G0S2 and cancers. G0S2 is dramatically hypomethylated in MDA-MB-231 cells compared with MCF-7 cells, which results in overexpression of G0S2 in MDA-MB-231 cells (Cheishvili *et al.*, 2015). Moreover, lower G0S2 expression in glioma is associated with a longer overall survival, and inhibition of G0S2 expression suppresses the invasion of glioma cells (Fukunaga *et al.*, 2018). A previous study showed that G0S2 induces apoptosis by interacting with Bcl-2 in non-small cell lung carcinoma cell line, H1299, and colon cancer cell line, HCT116 (Welch *et al.*, 2009). In addition, G0S2 expression is inhibited in ER⁺ breast cancer cell lines, such as BT474, MCF-7, and T47D, and the low level of G0S2 is associated with poor outcomes in ER⁺ breast cancer patients (Yim *et al.*, 2016).

Several previous studies have focused on the roles of G0S2 in lipid metabolism. However, there have barely been any studies elucidating the functions and mechanisms of G0S2 in triple-negative breast cancer.

In the current study, we determined a strong correlation between the expression of G0S2 and malignancy of breast cancer cells. Our results demonstrated that knockdown of G0S2 reduced typical tumor progression features, such as cell proliferation, migration, and invasion of MDA-MB-231 cells. Furthermore, these results may be due to activation of the Hippo pathway as well as inactivation of the FAK-Src and ERK1/2 signaling. Thus, we suggested that G0S2 plays critical roles in cell survival and metastasis of MDA-MB-231 cells.

MATERIALS AND METHODS

Chemicals and reagents

RPMI 1640 medium and fetal bovine serum (FBS) were purchased from Hyclone (Logan, VI, USA). The bicinchoninic acid (BCA) protein assay kit, and goat-anti mouse IgM Alexa Flour 488 were purchased from Thermo Scientific (Waltham, MA, USA). The D-Plus™ CCK cell viability assay kit was purchased from Dongin LS (Seoul, Korea). Mitomycin C was obtained from Sigma (St. Louis, MO, USA). M-MLV reverse transcriptase and RNase inhibitor were purchased from Promega (Madison, WI, USA). dNTP mixture was obtained from TaKaRa Bio (Shiga, Japan). QGreen™ 2X SybrGreen qPCR Master Mix was purchased from CellSafe (Gyeonggi, Korea). Rabbit poly-clonal antibodies for FAK, p-FAK (Tyr925), Src, p-Src (Tyr416), p-ERK1/2 (Thr202/Tyr204), integrin α 5, integrin β 1, LATS1, p-LATS1 (Ser909), and p-YAP1 (S127) were purchased from Cell Signaling Technology (Beverly, MA, USA). Rabbit polyclonal antibodies for E-cadherin, β -catenin, ERK1, and CYR61, mouse monoclonal antibodies for YAP1 and α -tubulin, and goat anti-rabbit IgG Texas-Red were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit polyclonal antibody for G0S2 was obtained from CUSABIO (College Park, MD, USA). All other chemicals and reagents were of highest quality commercially available.

Cell culture

Human breast cancer cell lines BT-20, HCC-70, T47D, MDA-MB-231, and MCF-7 (Korean Cell Line Bank, Seoul, Korea) were cultured in RPMI 1640 medium containing 10% (v/v) heat-inactivated FBS, 100 μ g/mL streptomycin, and 100 U/mL penicillin. MCF-10A human breast epithelial cells were obtained by Dr. Aree Moon (Duksung Woman's University, Seoul, Korea) and cultured in DMEM/F12 medium (Welgene, Seoul, Korea) containing 5% horse serum, 0.5 mg/mL hydrocortisone, 10 μ g/mL insulin, 100 ng/mL cholera toxin, 20 ng/mL EGF, 100 μ g/mL streptomycin, and 100 U/mL penicillin. All cell lines were incubated at 37°C in a humidified atmosphere containing 5% CO₂.

Transient transfection with siRNA

Cells were transfected with G0S2-specific siRNA (37.5 nM, target sequence: 5'-TGCACTAGGGAGGAAGGATAA-3', QIAGEN) with Neon® Transfection System (Invitrogen, Carlsbad, CA, USA) and cultured in RPMI 1640 medium supplemented with 10% FBS without antibiotics overnight. The medium was replaced with RPMI 1640 medium containing 10% FBS with antibiotics and cultured for 24 h.

Cell viability assay

G0S2 siRNA-transfected cells (7×10³ cells/well) were seeded in 96-well cell culture plates. After stabilization for 48 h, 10 μ L of D-Plus™ CCK solution was treated to each well, followed by incubation at 37°C for 2 h. The formed formazan dyes were quantified by measuring the absorbance at 450 nm with a Sunrise™ microplate reader (Tecan, Männedorf, Switzerland).

Cell cycle analysis

Cells were transfected with G0S2 siRNA for 48 h, and then, the cells were harvested with 0.05% trypsin-EDTA and washed with PBS. The obtained cell pellets were fixed in 70% ethanol overnight at -20°C. After washed with PBS to remove ethanol, the cells were incubated in 200 μ L of MUSE® cell cycle reagent (Merck Millipore, Darmstadt, Germany) for 30 min at room temperature in the dark. Cell cycle was measured by a MUSE® Cell Analyzer (Merck Millipore).

Apoptosis assay

After culturing for 48 h with G0S2 siRNA, the cells were harvested with 0.05% trypsin-EDTA and washed with PBS. The cell pellets were treated with caspase-3/7 working solution containing a DNA-binding dye at 37°C for 30 min, and then, treated with 7-AAD working solution for 5 min at room temperature. Apoptotic cells were measured by fluorescence-activated cell sorting using a MUSE® Cell Analyzer (Merck Millipore).

Colony forming assay

Cells were transfected with G0S2 siRNAs, and then, 200 cells were seeded in a well of a 6-well culture plate and cultured for 10 days. The culture medium was changed every two days. Subsequently, 4% formaldehyde solution in PBS was treated for 10 min at room temperature to fix the cells. After that, the fixed cells were washed with PBS several times and stained with 0.2% crystal violet for 10 min. After drying enough, the number of colonies containing over 50 cells was measured using a microscope (OLYMPUS, Tokyo, Japan).

In vitro scratch assay

Transfected cells (7×10⁵ cells/well) were seeded in 6-well culture plates. After stabilization for 48 h, cells with over 90% confluence were washed with PBS and incubated in mitomycin C solution (10 µg/mL) at 37°C for 2 h. After washing several times, a scratch into cell monolayer was created using a 200 µL pipette tip. Scratch images were captured using a microscope at the indicated time points.

Transwell invasion assay

Measurement of cell invasion was performed using the QC-M™ 24 well Invasion Assay Kit (Merck Millipore) in accordance with the manufacturer’s instructions. In brief, cells transfected with G0S2 siRNA were seeded in cell culture inserts with 8-µm pore size polycarbonate membrane coated with reconstituted basement membrane extract. Invasive cells through the membrane were stained with 4',6-diamidino-2-phenylindole (DAPI). Five fields per membrane in a cell culture insert were photographed using the ZEISS LSM700 Confocal Laser Scanning Microscope (Carl Zeiss, Jena, Germany) and the number of invasive cells was counted.

Quantitative PCR

Total RNA was extracted using Ribospin™ kit (GeneALL, Seoul, Korea). 0.5 µg of total RNA was reverse-transcribed as follows: initially at 37°C for 1 h, followed by at 95°C for 5 min in 20 µL total volume containing 5× RT buffer, 10 mM dNTPs, 100 pmol oligo-dT primer, 200 units of Moloney murine leukemia virus reverse transcriptase (M-MLV), and 40 units of RNase inhibitor. qPCR was conducted using the QGreen™ 2X SybrGreen qPCR Master Mix. Each reaction contained 10 µL of qPCR Master Mix, 2 µL of cDNA, 1 µM oligonucleotide primers in a final volume of 20 µL. Amplification was carried out as follows: one cycle of pre-denaturation 95°C for 5 min, 40 cycles of denaturation at 95°C for 15 s, followed by a combined annealing and extension step at each primer’s melting temperature (T_m) for 30 s. Data were analyzed using QIAGEN Rotor-Gene Q Series software (QIAGEN, Venlo, Netherlands).

Western blot

Whole cells were harvested and solubilized with ice-cold lysis buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% nonidet P-40, 0.1% SDS, 0.5% sodium deoxycholate, 2 mM EDTA, 50 mM NaF, and 1 mM sodium orthovanadate. The total amount of protein in the lysates was measured by the BCA method in accordance with the supplier’s recom-

mendations. Proteins (30 µg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 8 to 15% polyacrylamide gels and transferred onto 0.45 µm PVDF membranes. After that, membranes were blocked with 5% (w/v) bovine serum albumin or 5% skim milk in Tris-buffered saline with 0.1% Tween 20 (TBS-T) at 4°C for 2 h, and then, incubated overnight with primary antibodies at 4°C. After washed in TBS-T 3 times 10 min at room temperature, membranes were incubated with secondary antibody for 2 h at 4°C. All these steps related to membranes were conducted on a shaker. Each target protein band was visualized by the enhanced chemiluminescence (ECL) (Bionote, Gyeonggi, Korea), and the band intensities were analyzed using a Chemi-Doc XRS densitometer and quantified using Quantity One software (Bio-Rad, CA, USA).

Confocal microscopy

Cells were transfected with G0S2 siRNA and seeded on poly D-lysine-coated coverslips on a 6-well cell culture plate. After stabilization for 48 h, the cells were fixed with 4% formaldehyde solution in PBS at room temperature for 10 min. After washed with cold PBS, the fixed cells were incubated in 0.5% Triton X-100 at room temperature for 15 min to permeabilize the cells, followed by washing with cold PBS three times. The cells were blocked with 3% bovine serum albumin in PBS at room temperature for 1 h. Incubation steps of antibodies were conducted as follows: primary antibody (1:250) incubation at 4°C overnight, washing with cold PBS three times for 3 min, subsequently, incubation with goat anti-rabbit IgG-Texas Red (1:250) or goat anti-mouse IgM-Alexa Fluor 488 (1:250) at room temperature for 4 h. After washed with cold PBS several times for 3 min, the coverslips were mounted on glass slides using Ultra Cruz™ mounting medium containing DAPI (Santa Cruz Biotechnology). Fluorescence signals were measured and captured using a ZEISS LSM 800 confocal laser scanning microscope (Carl Zeiss).

Statistical analysis

All data are shown as mean ± standard errors of the means (SEM). Comparison of two groups with one characteristic was conducted using unpaired Student’s *t*-test and comparison of more than two groups with one characteristic was conducted using one-way ANOVA followed by Dunnett’s Multiple Comparison *t*-test on Graph-Pad Prism 7 software (GraphPad Software Inc., CA, USA). *p*-values of <0.05 were considered statistically significant.

Table 1. Microarray data analysis for invasive MDA-MB-231 cells and less invasive MCF-7 breast cancer cells (Cut-off ≥30-fold)

Gene name	Definition	MDA-MB-231 / MCF-7 ratio	Gene symbol
<i>G0S2</i>	G0/G1 switch 2	634.7	NM_015714
<i>ANKRD1</i>	Cardiac ankyrin repeat protein	574.2	NM_014391
<i>VIM</i>	Vimentin	411.9	NM_003380
<i>FOXA2</i>	Forkhead box A2	352.4	NM_021784
<i>ANXA1</i>	Annexin A1	348.6	NM_000700
<i>GPX1</i>	Glutathione peroxidase 1	319.6	NM_201397
<i>MMP1</i>	Matrix metalloproteinase 1	302.1	NM_002421
<i>LDHB</i>	Lactate dehydrogenase B	290.3	NM_002300
<i>FOXQ1</i>	Forkhead box Q1	258.6	NM_033260
<i>XAGE1B</i>	X antigen family, member 1B	254.7	NM_001097594

RESULTS

G0S2 is overexpressed in MDA-MB-231 cells

To explore which genes might affect the invasiveness of MDA-MB-231 cells, differential gene expressions were evaluated and compared between highly invasive MDA-MB-231 cells and less invasive MCF-7 cells by DNA microarray analysis. Table 1 shows the ten highly expressed genes in MDA-MB-231 cells compared with MCF-7 cells. The difference in *G0S2* expression was the greatest between MDA-MB-231 and MCF-7 cells. mRNA expression level was also measured by qPCR to confirm the results of DNA microarray analysis (Fig. 1). Consistent with DNA microarray analysis, the mRNA expression level of *G0S2* showed the greatest difference between the cell lines. These results indicated that *G0S2* is highly expressed in MDA-MB-231 cells.

G0S2 overexpression is associated with invasiveness of triple-negative breast cancer cell lines

To investigate whether *G0S2* overexpression is a characteristic of triple-negative breast cancer lines, mRNA levels of *G0S2* were measured and compared among triple-negative breast cancer lines, BT-20, HCC-70, and MDA-MB-231, hormone-dependent breast cancer cell lines, MCF-7 and T47D, and breast epithelial cell line, MCF-10A. We found that *G0S2* expression is considerably higher in triple-negative breast cancer cell lines compared with less invasive hormone-dependent breast cancer cells, such as MCF-7 and T47D cells (Fig. 2A). Moreover, consistent with the mRNA levels, we confirmed that *G0S2* protein levels were markedly higher in MDA-MB-231 cells (Fig. 2B).

To determine the association between *G0S2* expression and relapse-free survival (RFS) of triple-negative breast cancer patients, Kaplan-Meier analysis from the KM plotter database (<http://kmplot.com>) was used. As shown in Fig. 2C, higher *G0S2* expression leads to lower RFS in patient groups who have triple-negative breast cancers. This result indicated that *G0S2* overexpression is associated with local, regional,

or distant metastasis (Gelmon *et al.*, 2012). Taken together, *G0S2* expression is highly upregulated especially in triple-negative breast cancer, which indicates that higher *G0S2* expression affects invasive phenotypes of breast cancer and poor prognosis.

Knockdown of G0S2 expression decreases tumorigenesis and enhances apoptosis

To explore the role of *G0S2* in tumorigenesis in MDA-MB-231 cells, cell viability and colony formation were deter-

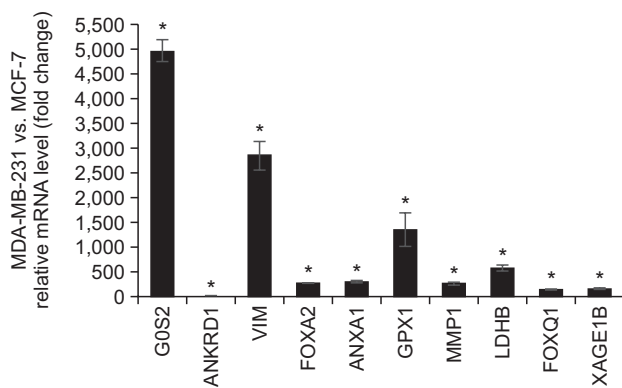


Fig. 1. Gene expression differences between MDA-MB-231 cells and MCF-7 cells. The ten highly expressed genes in MDA-MB-231 cells compared with MCF-7 cells in the microarray were verified using qPCR. The results show the fold change in MDA-MB-231 cells based on each target gene expression in MCF-7. All results were normalized to 18S ribosomal RNA (18S rRNA). The data is shown as mean \pm SEM (n=3). Statistically significant differences from control when * p <0.05.

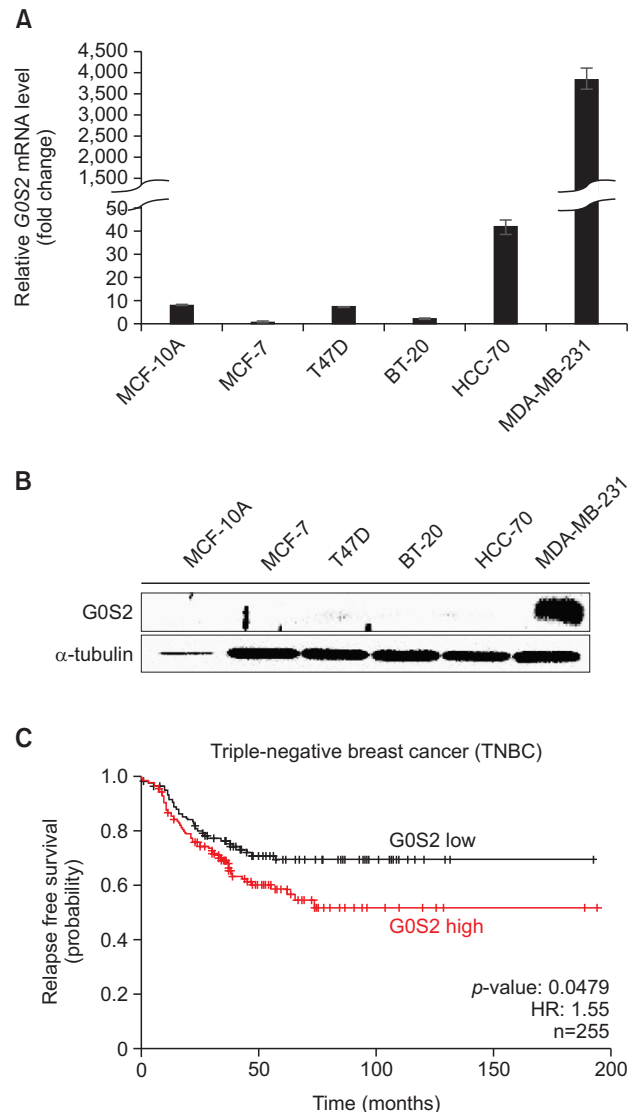


Fig. 2. Comparison of *G0S2* expression levels in human breast cancer cell lines. (A) qPCR was performed to identify *G0S2* expression in indicated cell lines. All results were normalized to 18S rRNA. The data is shown as mean \pm SEM (n=3). (B) Indicated cell lines were harvested and total cellular proteins were extracted for western blot assay. The proteins were separated by SDS-PAGE (15%) and western blot assay was performed with anti-*G0S2* antibody. (C) Kaplan-Meier analysis of relapse free survival for the *G0S2*-high and *G0S2*-low patient groups in triple-negative breast cancers. The data was obtained from Kaplan-Meier Plotter (<http://kmplot.com>).

mined. When cells were treated with G0S2 siRNA, approximately 20% of cell viability was decreased (Fig. 3A, 3B). In addition, G0S2 knockdown remarkably reduced the number of colonies (Fig. 3C). These results demonstrated that G0S2 induced cell proliferation and colony formation related to tumorigenesis in MDA-MB-231 cells.

To identify whether G0S2 affected apoptosis, cells were transfected with G0S2 siRNA, and then, cell cycle was analyzed by flow cytometry. As shown in Fig. 3D, the proportion of G0S2 knockdown cells in the G1 phase was higher in comparison to the control, whereas the proportion of cells in the S and G2/M phases was decreased relative to control. In addition, apoptosis was analyzed using caspase-3/7 staining and flow cytometry. Increase in the percentage of apoptotic cells (~7%) was observed in cells treated with G0S2 siRNA compared to the control (Fig. 3E). These results were consistent with the increased percentage of sub-G1 in the cell cycle assay, which indicated increase in apoptosis (Fig. 3D). Together, these results showed that G0S2 knockdown prevented MDA-MB-231 cells from entering the S phase and induced apoptosis.

Inhibition of G0S2 expression reduces migration and invasion

To further determine whether G0S2 is involved in the metastatic progression of MDA-MB-231 cells, wound healing and transwell invasion assays were performed. In wound healing assay, the results showed that the wound closure of the cells after treatment with G0S2 siRNA was inhibited by about 50% compared to the control at 24 h after scratching, while the wound of control cells was closed completely at 24 h after scratching (Fig. 4A). In the transwell invasion assay, the number of invaded cells across pores was significantly decreased in the group treated with G0S2 siRNA compared to the control (Fig. 4B). These results demonstrated that G0S2 promoted metastasis by inducing migration and invasion of MDA-MB-231 cells.

Knockdown of G0S2 inhibits activation of FAK-Src and ERK1/2 signaling

Integrins are well-known ECM receptors and crucial for cell migration and invasion (Hood and Cheresch, 2002). Of them,

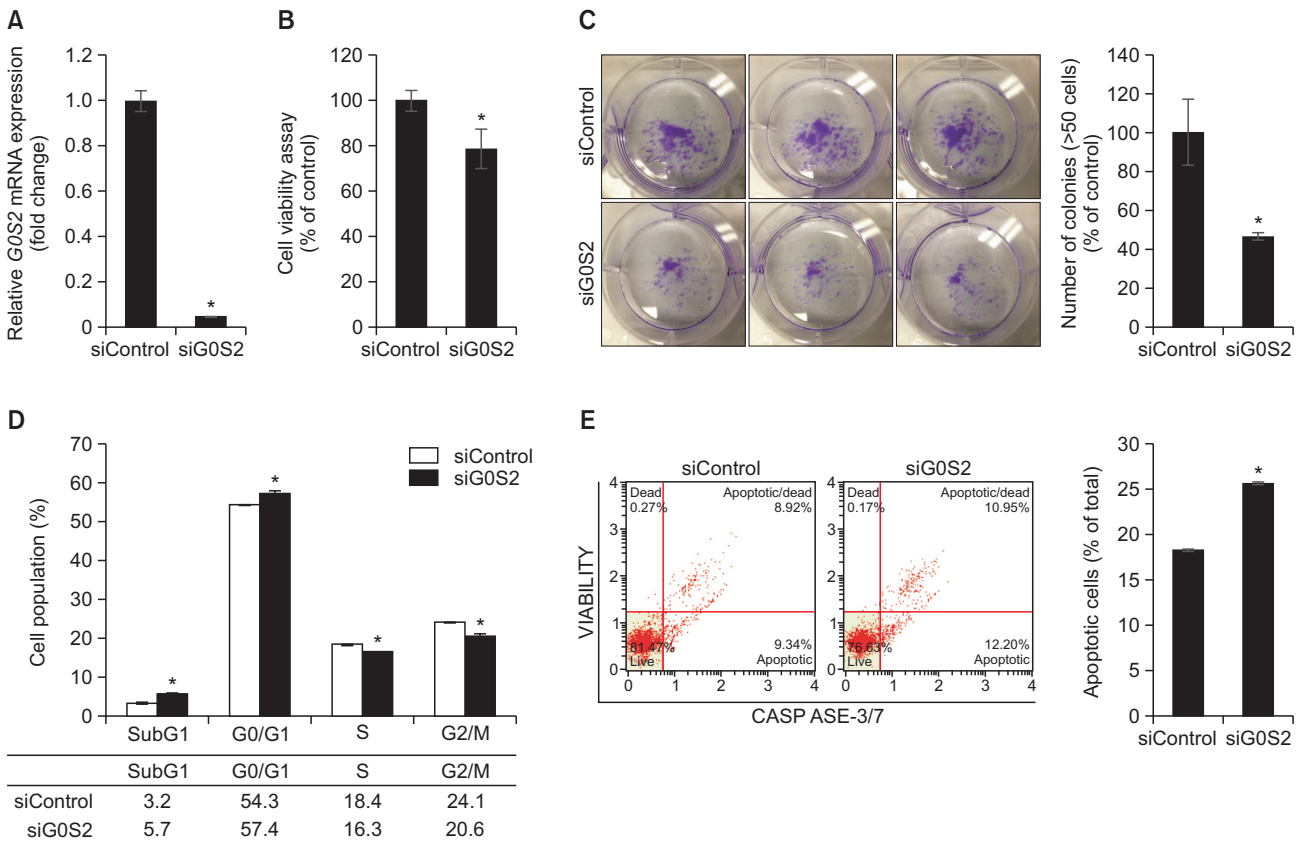


Fig. 3. Knockdown of G0S2 decreases cell proliferation and promotes apoptosis in MDA-MB231 cells. MDA-MB-231 cells were treated with G0S2 siRNA for 48 h. (A) Total RNA was isolated, and qPCR was performed to confirm knockdown of G0S2 by G0S2 siRNA. All results were normalized to 18S rRNA. The data is shown as mean ± SEM (n=3). (B) Cell viability assay. The percentage of cells surviving after G0S2 siRNA treatment was calculated. (C) Colony formation assay. Cells treated with G0S2 siRNA were seeded in 6-well plates at 200 cells/well and incubated. After 48 h, the medium was replaced with fresh growth medium every 2 days. After 10 days, the cells were fixed, stained, and photographed. (D) Cell cycle analysis. Cells were treated with G0S2 siRNA for 48 h, and then, cell cycle was examined using flow cytometry. (E) Apoptosis analysis. After G0S2 siRNA treatment for 48 h, apoptosis was evaluated using flow cytometry. The plots from FACS show percentage of live, early and late apoptotic, and dead cells. The data shows percentage of total apoptotic cells which means sum of percentage of early and late apoptotic cells. All data are shown as mean ± SEM (n=3). Statistically significant differences from control when *p<0.05.

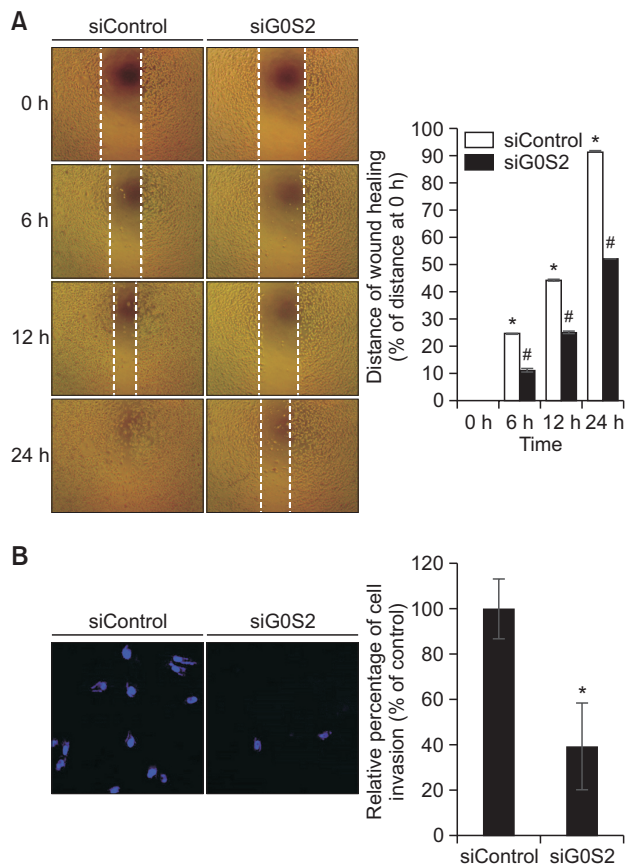


Fig. 4. G0S2 inhibition suppresses migration and invasion of MDA-MB-231 cells. MDA-MB-231 cells were treated with G0S2 siRNA for 48 h. (A) Cell migration was assessed by the wound healing assay. The wound area was observed at the indicated time points (6, 12, and 24 h) by microscopy and photographed. The data is shown as mean \pm SEM (n=3). Statistically significant differences from control when $^{*}p < 0.05$. (B) Invaded cells were stained with DAPI and photographed by confocal microscopy. The number of cells was counted in five randomly chosen microscope fields and the percentage of invaded cells was calculated. The quantitative data are represented as the mean \pm SEM (n=3). Statistically significant differences from control when $^{*}p < 0.05$.

integrin $\alpha 5\beta 1$ is highly expressed in invasive breast cancer cell lines. Focal adhesion kinase (FAK) is a key mediator of intracellular signaling through integrin $\alpha 5\beta 1$, and FAK-Src signaling in cancer promotes cell migration, invasion, and metastasis through cytoskeleton remodeling and MMP-mediated extracellular matrix proteolysis (Guan, 2010; Mierke *et al.*, 2011). ERK1/2, which is associated with tumor invasion and metastasis, is also regulated by integrin $\alpha 5\beta 1$ (Danen and Yamada, 2001).

To further investigate the mechanism of action of G0S2 on proliferation, migration, and invasion, the expression of proteins involved in integrin $\alpha 5\beta 1$ -mediated FAK-Src and ERK1/2 signaling was evaluated in MDA-MB-231 cells. First, decreased levels of G0S2 protein were confirmed using G0S2 siRNA (Fig. 5A). Interestingly, we found that the knockdown of G0S2 decreased expression of integrin $\alpha 5\beta 1$ protein in MDA-MB-231 cells (Fig. 5B). Furthermore, phosphorylation of FAK at Tyr915, induced by activation of integrin $\alpha 5\beta 1$, and phos-

phorylation of Src at Tyr416, induced by FAK activation, was also reduced. In addition, phosphorylation of ERK1/2 was decreased by G0S2 inhibition (Fig. 5C). These results indicated that G0S2 activated FAK-Src and ERK1/2 signaling by inducing integrin $\alpha 5\beta 1$ expression to promote proliferation, migration, and invasion in MDA-MB-231 cells.

Knockdown of G0S2 activates Hippo signaling pathway

Recent studies have revealed that Hippo signaling pathway may contribute to tumorigenesis and cancer development (Zygulska *et al.*, 2017). Integrin-induced FAK-Src is an upstream regulator of the Hippo signaling pathway, where Src, phosphorylated at Tyr 416, induces the active form of yes-associated protein 1 (YAP1) and its translocation to the nucleus by inactivating large tumor suppressor kinase 1 (LATS1) (Si *et al.*, 2017). Upregulation of YAP1 in the nucleus is one of the pre-metastatic events of breast cancer (Lamar *et al.*, 2012), and it promotes transcription of YAP1 target genes, including CYR61 (Si *et al.*, 2017). To determine the effect of G0S2 knockdown on the Hippo pathway in MDA-MB-231 cells, the mRNA levels of *LATS1*, *YAP1*, and *CYR61* were measured by qPCR. Our results showed that treatment with G0S2 siRNA increased the mRNA level of *LATS1*, whereas the mRNA level of *YAP1* was strongly suppressed. Interestingly, we also found that the expression of *CYR61*, which is a YAP1 target gene, was significantly suppressed by G0S2 inhibition (Fig. 6A).

To further elucidate whether the Hippo signaling pathway is activated by G0S2 knockdown, the degrees of phosphorylation of LATS1 and YAP1 were evaluated. As a result, phosphorylation of LATS1 at Ser909 and YAP1 at Ser127 were significantly increased, and YAP1 and CYR61 protein levels were decreased after G0S2 inhibition (Fig. 6B). Furthermore, confocal microscopy analysis revealed that knockdown of G0S2 expression might inhibit YAP nuclear localization by increasing the accumulation of phosphorylated YAP1 protein in the cytoplasm or inducing degradation of phosphorylated YAP1, because 14-3-3 proteins binds to the phosphorylation site, Ser127, of YAP1, or phosphorylation of YAP1 induces proteasome-mediated degradation (Fig. 6C) (Freeman and Morrison, 2011; Lamar *et al.*, 2012). Thus, these findings suggested that G0S2 inhibition activated the Hippo pathway by activating phosphorylation of LATS1 and YAP1 and preventing YAP1 nuclear localization.

Knockdown of G0S2 expression induces E-cadherin expression and represses β -catenin expression

EMT is a complicated process that epithelial cells gain the properties of migratory and invasive mesenchymal cells. EMT affects cancer progression and metastasis through lose cell-cell adhesion and polarity, and reorganizes their cytoskeletons (Son and Moon, 2010). E-cadherin which is one of the typical epithelial cell markers in EMT, and its downregulated expression is associated with highly invasive and metastatic cancers (Elisha *et al.*, 2018). β -catenin is critical in cell-cell adhesions by linking E-cadherin to the actin cytoskeleton (Hatsell *et al.*, 2003), and the aberrant expression of β -catenin is significantly associated with breast cancer cells (Lopez-Knowles *et al.*, 2010). We observed that the expression of E-cadherin was increased while the expression of β -catenin was decreased by G0S2 suppression (Fig. 7A). Consistently, the data from western blot analysis also showed that G0S2 inhibition significantly increased E-cadherin protein levels, whereas it re-

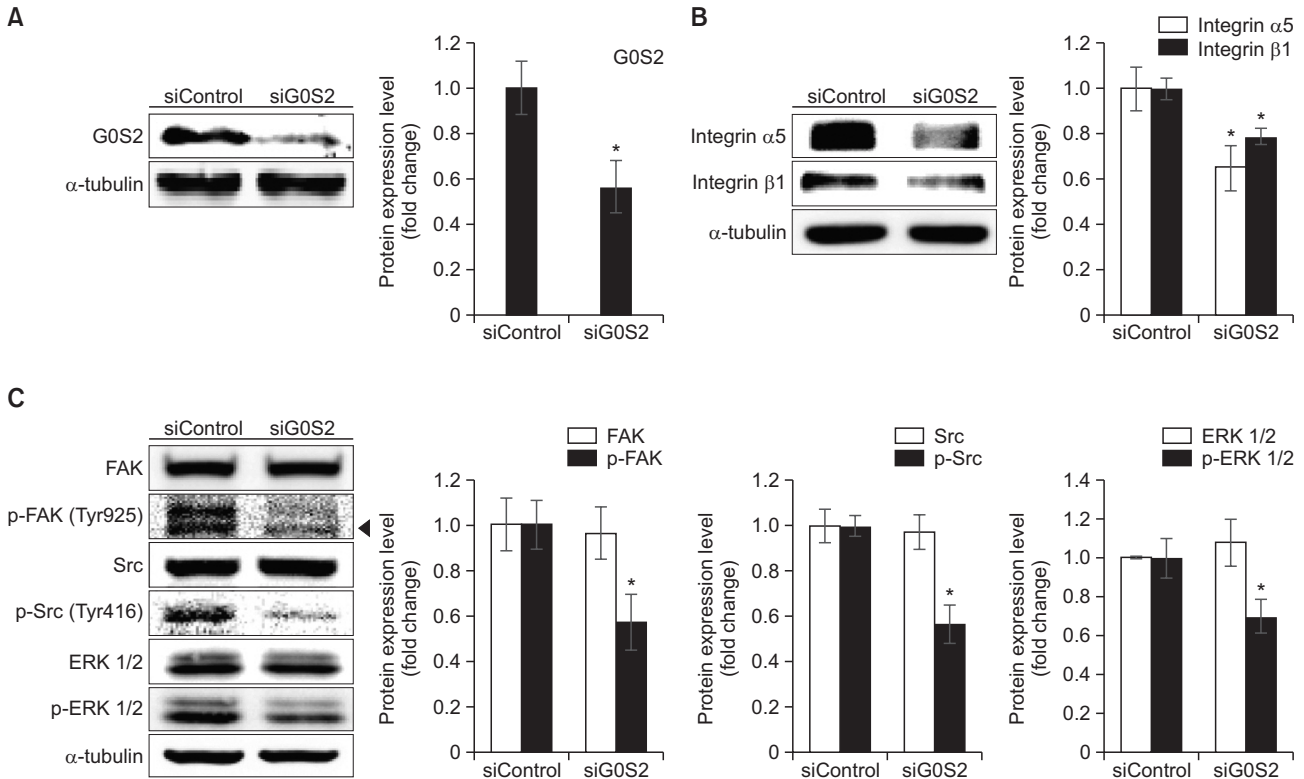


Fig. 5. G0S2 causes invasive phenotype of MDA-MB-231 cells via integrin-mediated FAK-Src and ERK1/2 signaling. MDA-MB-231 cells were treated with G0S2 siRNA for 48 h. (A-C) Transfected cells were harvested and total cellular proteins were extracted for western blot assay. The proteins were separated by SDS-PAGE (8-15%) and western blot assay was performed using indicated antibodies. Band intensities were measured with Quantify One software. Protein levels were normalized against α -tubulin levels. The data is shown as mean \pm SEM (n=3). Statistically significant differences from control when * p <0.05.

duced β -catenin protein levels in MDA-MB-231 cells (Fig. 7B). Taken together, these data demonstrated that reduction in G0S2 expression enhanced cell-cell adhesion by increasing E-cadherin and decreasing β -catenin in MDA-MB-231 cells, which leads to suppression of EMT process.

G0S2 inhibition affects expressions of MMPs and inflammatory cytokines associated with migration and invasion

MMPs are well-known markers of cell invasion. To determine whether the inhibition of invasion is associated with MMPs when cells were treated with G0S2 siRNA, the levels of different MMPs (1, 2, 3, 7, 9, 12, 13, and 14) were measured by qPCR. Our results showed that the knockdown of G0S2 expression reduced mRNA levels of *MMP1*, *MMP2*, *MMP3*, *MMP7*, *MMP9*, and *MMP14*. Interestingly, *MMP12* mRNA level was strongly increased (Fig. 8A). This result coincides with the function of MMP12 in protecting against tumor growth and invasion of breast cancer cells (Margheri et al., 2009).

Inflammatory cytokines, such as *IL6*, *IL8*, *VEGFA*, and *TNFA*, have also been reported as tumor progression factors which promote cell proliferation, invasion, and angiogenesis (Landskron et al., 2014). To confirm the effect of G0S2 on inflammatory cytokine expressions, their mRNA levels were measured by qPCR after knockdown of G0S2. The results showed that mRNA expression level of those cytokines was decreased. Especially, the mRNA levels of *IL8* and *TNFA* were

markedly decreased by 52% and 49% in G0S2 knockdown cells (Fig. 8B). These results indicated that G0S2 might induce tumor progression and metastasis by modulating expression of MMPs and inflammatory cytokines, including *IL8* and *TNFA*.

DISCUSSION

The first step of metastasis is migration of cancer cells away from the primary tumor site, a process called tumor invasion (Glentis et al., 2014). Therefore, cell migration and invasion are considered hallmarks of cancer cells along with cell proliferation (Hanahan and Weinberg, 2011). Specially in triple-negative breast cancers, with fatal metastasis, suppression of migration and invasion is very important for treatment. Here, we hypothesized that G0S2 overexpression could be involved in invasive phenotypes of MDA-MB-231 cells.

The CpG site in the G0S2 gene has been demonstrated to be overwhelmingly hypomethylated in MDA-MB-231 cells compared with MCF-7 cells, and the mRNA expression of G0S2 was found to be higher in MDA-MB-231 (Cheishvili et al., 2015). Moreover, when G0S2 is reduced in MDA-MB-231 cells, tumor invasiveness was significantly decreased. In contrast, a few studies have shown that G0S2 induces apoptosis (Welch et al., 2009), and low expression of G0S2 results in high recurrence of hormone-receptor positive breast cancer cell lines (Yim et al., 2016). However, the role of G0S2 in

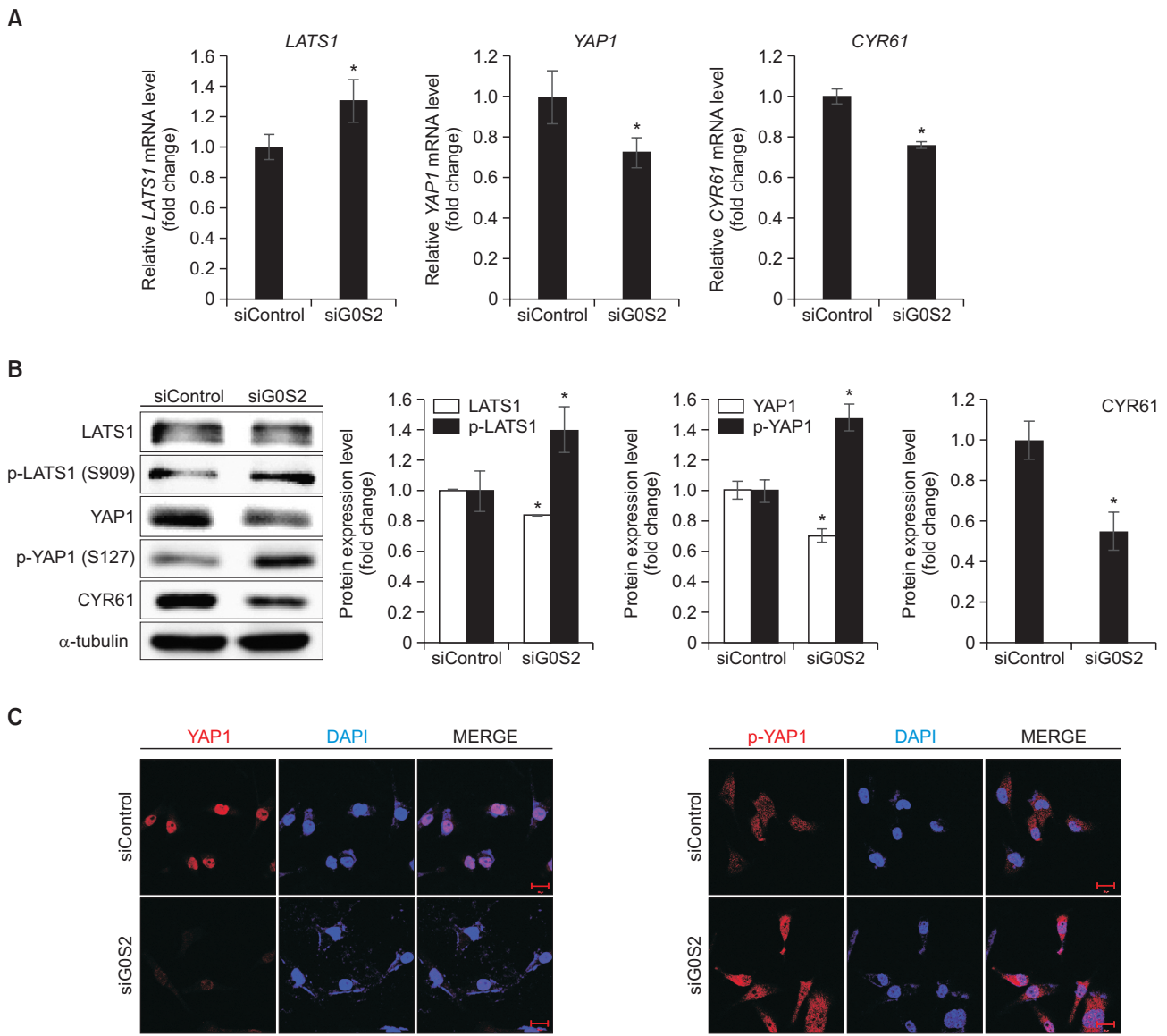


Fig. 6. Knockdown of G0S2 activates the Hippo pathway via integrin-mediated FAK-Src. MDA-MB-231 cells were treated with G0S2 siRNA for 48 h. (A) Alteration of *LATS1*, *YAP1*, and *CYR61* mRNA levels was identified using qPCR. All results were normalized to 18S rRNA. The data is shown as mean \pm SEM (n=3). Statistically significant differences from control when $*p < 0.05$. (B) Transfected cells were harvested, and total cellular proteins were extracted for western blot assay. The proteins were separated by SDS-PAGE (8%) and western blot assay was performed using indicated antibodies. The band intensities were measured with Quantity One software. Protein levels were normalized against α -tubulin levels. The data is shown as mean \pm SEM (n=3). Statistically significant differences from control when $*p < 0.05$. (C) Confocal microscopy was performed to observe alteration in expressions of YAP1 (left) and phosphorylated YAP1 (right) by G0S2 inhibition. Scale bar indicates 20 μ m.

breast cancer cells, especially in triple-negative breast cancer, remains controversial. Therefore, we investigated whether G0S2 plays a crucial role in invasive breast cancer cell survival and metastasis.

Our results demonstrated that G0S2 tended to be over-expressed in triple-negative breast cancer cell lines. Consistently, overexpression of G0S2 increases accumulation of LDs (Antalis *et al.*, 2011), and higher levels of LDs are related to higher aggressiveness in estrogen receptor-negative breast cancer cell lines (Nieva *et al.*, 2012). Taken together, the oncogenic characteristics induced by overexpressed G0S2 could

be correlated with high levels of LDs in MDA-MB-231 cells.

Our studies showed that G0S2 promoted cell proliferation, migration, and invasion in MDA-MB-231 cells by activating integrin $\alpha 5 \beta 1$ -mediated FAK-Src and ERK1/2 signaling through phosphorylation of Tyr925, Tyr416, or Thr202, and Tyr204. This is consistent with previous results that revealed that FAK-Src signaling activation promotes cell survival, adhesion, angiogenesis, migration, and metastasis by upregulating integrin $\alpha 5 \beta 1$ expressions (Mitra and Schlaepfer, 2006; Mierke *et al.*, 2011; Ye *et al.*, 2017). We found that G0S2 knockdown significantly repressed phosphorylation of Src at Tyr416. Tyr416

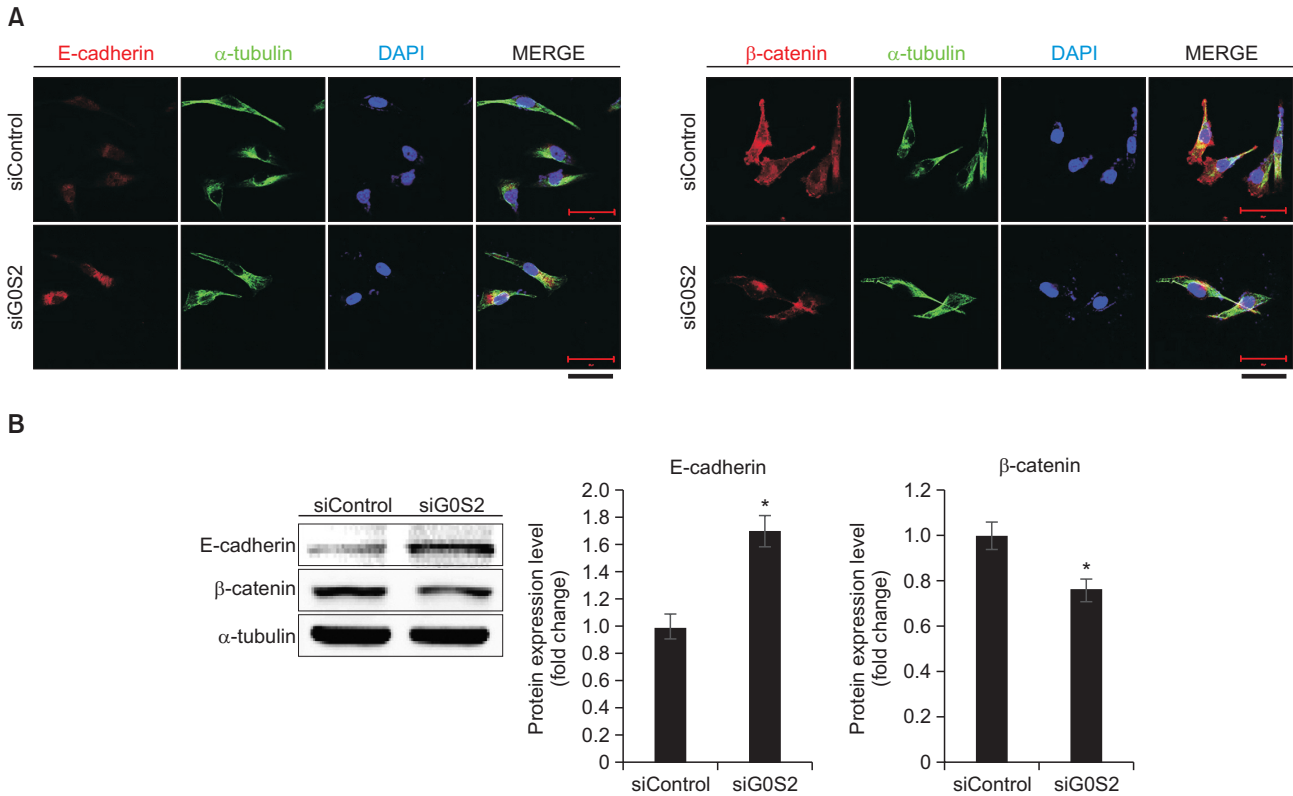


Fig. 7. Reduction of G0S2 expression downregulates β-catenin and upregulates E-cadherin. MDA-MB-231 cells were treated with G0S2 siRNA for 48 h. (A) The expressions of E-cadherin (left) and β-catenin (right) were observed by confocal microscopy. Scale bar indicates 20 μm. (B) Whole cell lysate was subjected to western blot analysis for E-cadherin (left) and β-catenin (right) expression. The band intensities were measured with Quantity One software. Protein levels were normalized against α-tubulin levels. The data is shown as mean ± SEM (n=3). Statistically significant differences from control when **p*<0.05.

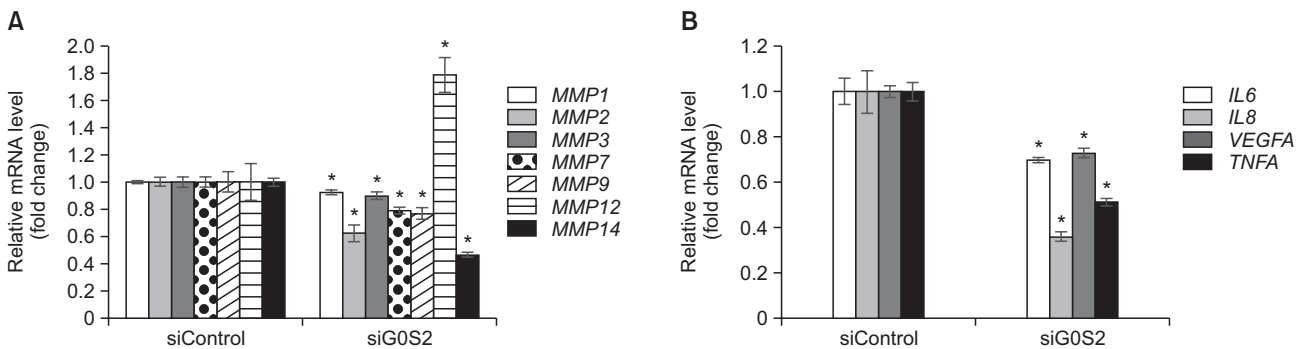


Fig. 8. The alteration in mRNA expressions of MMPs and inflammatory cytokines by G0S2 inhibition. MDA-MB-231 cells were treated with G0S2 siRNA for 48 h (A, B) Total RNA was isolated, and qPCR was performed to confirm the changes in mRNA levels of MMPs and inflammatory cytokines by G0S2 siRNA. All results were normalized to 18S rRNA. The data is shown as mean ± SEM (n=3). Statistically significant differences from control when **p*<0.05.

phosphorylation is strongly associated with Src activity, and the active Src can phosphorylate FAK at Tyr925, promoting recruitment of the Grb2-SH2 domain and activation of ERK pathway (Schlaepfer *et al.*, 1994; Canel *et al.*, 2010). Moreover, integrin β1 induces phosphorylation of Src at Tyr416 to promote Src tyrosine kinase activity in human colon cancer cells (Gupta and Vlahakis, 2009). Thus, knockdown of G0S2 may cause downregulation of Src and FAK activity through

suppression of integrin α5 and β1 expression. Furthermore, ERK1/2 is also activated by induction of integrin α5β1 expression (Danen and Yamada, 2001) and induce cell-matrix adhesion (Kim and Gumbiner, 2015; Lai *et al.*, 2018). Since p130 Crk-associated substrate (CAS) phosphorylation by integrin α5β1 enhances adhesion-mediated cell survival and migration by interaction with focal adhesion proteins, paxillin, and tensin (Huveneres and Danen, 2009), it may be possible that

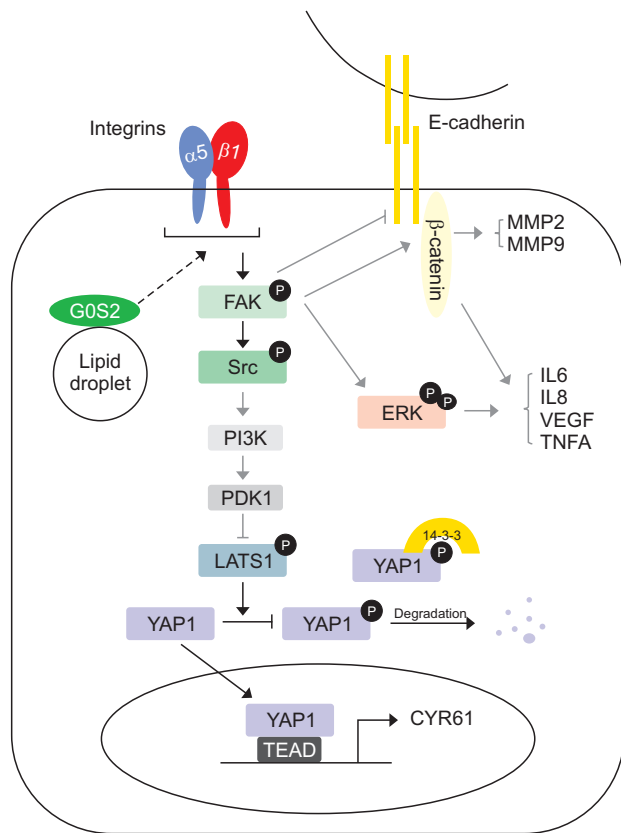


Fig. 9. Hypothetical schematic model for a novel oncogenic mechanism of G0S2. G0S2-induced invasive phenotypes seem to be related to integrin-mediated FAK-Src and ERK1/2 signaling and inactivation of the Hippo pathway, subsequently leading to alteration in expressions of E-cadherin, β -catenin, MMPs, and cytokines.

G0S2 activates CAS phosphorylation to promote cell motility and metastasis.

Previously, Hou *et al.* (2016) reported that malignant phenotypes, such as changes in cell shape and EMT markers or formation of invasive protrusion, in MDA-MB-231 cells are mainly mediated by integrin $\beta 1$. Our data also demonstrated that G0S2 induced EMT because G0S2 siRNA strongly upregulates expression of E-cadherin; therefore, integrin $\beta 1$ -mediated activation by G0S2 may play a crucial role in malignancy of MDA-MB-231 cells. However, further studies are needed to determine whether G0S2 interacts directly with integrins.

Previous studies have demonstrated that integrins directly bind to fibronectin and then stimulate FAK-Src signaling. After activation of FAK-Src signaling, YAP1 is activated in a PI3K-PDK1-dependent manner (Kim and Gumbiner, 2015). Subsequently, dephosphorylated LATS1/2 activates YAP1, resulting in YAP1 accumulation in the nucleus (Fan *et al.*, 2013; Kim and Gumbiner, 2015). In many human cancers, the nuclear YAP1 protein expression was found to be upregulated compared with that in normal tissues (Pan, 2010; Bao *et al.*, 2011), which results in enhancement of cell migration and invasion (Lamar *et al.*, 2012). The expression of CYR61, which is the target gene of YAP1, is promoted by transcriptional regulation of YAP1 and TEAD (Zhao *et al.*, 2008). CYR61 protein is linked to the cell surface and extracellular matrix (Perbal,

2004) and promotes tumorigenesis in breast cancer via integrin-mediated signaling (Tsai *et al.*, 2002). Based on these studies, we suggested that the knockdown of G0S2 expression suppressed CYR61 expression via the transcriptional inactivation of YAP1. However, further studies would be necessary to demonstrate how G0S2 controls upstream regulators of the Hippo pathway, including fibronectin.

In addition, our results showed that G0S2 knockdown suppressed β -catenin protein levels and mRNA levels of *MMP1*, *MMP2*, *MMP3*, *MMP7*, *MMP9*, and *MMP14* and *IL6*, *IL8*, *VEGF*, and *TNFA*. Inhibition of β -catenin not only decreased the secretion of inflammatory cytokines, such as IL6, IL8, VEGF, and TNFA (Liu *et al.*, 2016) but also the expression of MMP2 and MMP9 involved in EMT (Son and Moon, 2010). Based on these studies, we suggested that G0S2 indirectly alters the expressions of the abovementioned molecules through integrin-mediated activation of FAK-Src and ERK1/2 signaling, leading to promotion of cell proliferation, migration, and invasion of MDA-MB-231 cells.

G0S2 is highly expressed in MDA-MB-231 cells. However, Heckmann *et al.* (2016) showed that G0S2 is a short-lived protein due to proteasomal degradation by polyubiquitination at Lys25. Thus, further studies would be needed to elucidate why high G0S2 expression is maintained without its degradation in MDA-MB-231 cells.

In summary, inhibition of G0S2 suppressed proliferation, migration, and invasiveness of invasive breast cancer MDA-MB-231 cells. The scheme in Fig. 9 summarizes novel findings about the mechanisms induced by G0S2. Taken together, overexpression of G0S2 in MDA-MB-231 cells plays a key role in malignant tumor progression. Thus, G0S2 might be an effective therapeutic target for triple-negative breast cancers.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGMENTS

This research was supported by the National Research Foundation of Korea (NRF) funded by the Korean government (MSIP) (grant numbers. NRF-2015R1A5A1008958 and NRF-2017R1E1A1A01074032).

REFERENCES

- Antalis, C. J., Uchida, A., Buhman, K. K. and Siddiqui, R. A. (2011) Migration of MDA-MB-231 breast cancer cells depends on the availability of exogenous lipids and cholesterol esterification. *Clin. Exp. Metastasis* **28**, 733-741.
- Bao, Y., Hata, Y., Ikeda, M. and Withanage, K. (2011) Mammalian Hippo pathway: from development to cancer and beyond. *J. Biochem.* **149**, 361-379.
- Canel, M., Serrels, A., Miller, D., Timpson, P., Serrels, B., Frame, M. C. and Brunton, V. G. (2010) Quantitative *in vivo* imaging of the effects of inhibiting integrin signaling via Src and FAK on cancer cell movement: effects on E-cadherin dynamics. *Cancer Res.* **70**, 9413-9422.
- Cheishvili, D., Stefanska, B., Yi, C., Li, C. C., Yu, P., Arakelian, A., Tanvir, I., Khan, H. A., Rabbani, S. and Szyf, M. (2015) A common promoter hypomethylation signature in invasive breast, liver and

- prostate cancer cell lines reveals novel targets involved in cancer invasiveness. *Oncotarget* **6**, 33253-33268.
- Danen, E. H. and Yamada, K. M. (2001) Fibronectin, integrins, and growth control. *J. Cell. Physiol.* **189**, 1-13.
- Elisha, Y., Kalchenko, V., Kuznetsov, Y. and Geiger, B. (2018) Dual role of E-cadherin in the regulation of invasive collective migration of mammary carcinoma cells. *Sci. Rep.* **8**, 4986.
- Fan, R., Kim, N. G. and Gumbiner, B. M. (2013) Regulation of Hippo pathway by mitogenic growth factors via phosphoinositide 3-kinase and phosphoinositide-dependent kinase-1. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 2569-2574.
- Freeman, A. K. and Morrison, D. K. (2011) 14-3-3 Proteins: diverse functions in cell proliferation and cancer progression. *Semin. Cell Dev. Biol.* **22**, 681-687.
- Fukunaga, T., Fujita, Y., Kishima, H. and Yamashita, T. (2018) Methylation dependent down-regulation of G0S2 leads to suppression of invasion and improved prognosis of IDH1-mutant glioma. *PLoS ONE* **13**, e0206552.
- Gelmon, K., Dent, R., Mackey, J. R., Laing, K., McLeod, D. and Verma, S. (2012) Targeting triple-negative breast cancer: optimising therapeutic outcomes. *Ann. Oncol.* **23**, 2223-2234.
- Glentis, A., Gurchenkov, V. and Matic Vignjevic, D. (2014) Assembly, heterogeneity, and breaching of the basement membranes. *Cell Adhes. Migr.* **8**, 236-245.
- Guan, J. L. (2010) Integrin signaling through FAK in the regulation of mammary stem cells and breast cancer. *IUBMB Life* **62**, 268-276.
- Gupta, S. K. and Vlahakis, N. E. (2009) Integrin $\alpha 9 \beta 1$ mediates enhanced cell migration through nitric oxide synthase activity regulated by Src tyrosine kinase. *J. Cell Sci.* **122**, 2043-2054.
- Hanahan, D. and Weinberg, R. A. (2011) Hallmarks of cancer: the next generation. *Cell* **144**, 646-674.
- Hatsell, S., Rowlands, T., Hiremath, M. and Cowin, P. (2003) β -catenin and Tcfs in mammary development and cancer. *J. Mammary Gland Biol. Neoplasia* **8**, 145-158.
- Heckmann, B. L., Zhang, X., Saarinen, A. M. and Liu, J. (2016) Regulation of G0/G1 switch gene 2 (G0S2) protein ubiquitination and stability by triglyceride accumulation and ATGL interaction. *PLoS ONE* **11**, e0156742.
- Heckmann, B. L., Zhang, X., Xie, X., Saarinen, A., Lu, X., Yang, X. and Liu, J. (2014) Defective adipose lipolysis and altered global energy metabolism in mice with adipose overexpression of the lipolytic inhibitor G0/G1 switch gene 2 (G0S2). *J. Biol. Chem.* **289**, 1905-1916.
- Hood, J. D. and Cheresch, D. A. (2002) Role of integrins in cell invasion and migration. *Nat. Rev. Cancer* **2**, 91-100.
- Hou, S., Isaji, T., Hang, Q., Im, S., Fukuda, T. and Gu, J. (2016) Distinct effects of $\beta 1$ integrin on cell proliferation and cellular signaling in MDA-MB-231 breast cancer cells. *Sci. Rep.* **6**, 18430.
- Huvneers, S. and Danen, E. H. (2009) Adhesion signaling - crosstalk between integrins, Src and Rho. *J. Cell Sci.* **122**, 1059-1069.
- Kim, N. G. and Gumbiner, B. M. (2015) Adhesion to fibronectin regulates Hippo signaling via the FAK-Src-PI3K pathway. *J. Cell Biol.* **210**, 503-515.
- Lai, H., Zhao, X., Qin, Y., Ding, Y., Chen, R., Li, G., Labrie, M., Ding, Z., Zhou, J., Hu, J., Ma, D., Fang, Y. and Gao, Q. (2018) FAK-ERK activation in cell/matrix adhesion induced by the loss of apolipoprotein E stimulates the malignant progression of ovarian cancer. *J. Exp. Clin. Cancer Res.* **37**, 32.
- Lamar, J. M., Stern, P., Liu, H., Schindler, J. W., Jiang, Z. G. and Hynes, R. O. (2012) The Hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain. *Proc. Nat. Acad. Sci. U.S.A.* **109**, E2441-E2450.
- Landskron, G., De la Fuente, M., Thuwajit, P., Thuwajit, C. and Hermoso, M. A. (2014) Chronic inflammation and cytokines in the tumor microenvironment. *J. Immunol. Res.* **2014**, 149185.
- Liu, L., Zhi, Q., Shen, M., Gong, F. R., Zhou, B. P., Lian, L., Shen, B., Chen, K., Duan, W., Wu, M. Y., Tao, M. and Li, W. (2016) β -catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4262-4266.
- Lopez-Knowles, E., Zardawi, S. J., McNeil, C. M., Millar, E. K., Crea, P., Musgrove, E. A., Sutherland, R. L. and O'Toole, S. A. (2010) Cytoplasmic localization of β -catenin is a marker of poor outcome in breast cancer patients. *Cancer Epidemiol. Biomarkers Prev.* **19**, 301-309.
- Margheri, F., Serrati, S., Lapucci, A., Anastasia, C., Giusti, B., Pucci, M., Torre, E., Bianchini, F., Calorini, L., Albini, A., Ventura, A., Fibbi, G. and Del Rosso, M. (2009) Systemic sclerosis-endothelial cell antiangiogenic pentraxin 3 and matrix metalloprotease 12 control human breast cancer tumor vascularization and development in mice. *Neoplasia* **11**, 1106-1115.
- Mierke, C. T., Frey, B., Fellner, M., Herrmann, M. and Fabry, B. (2011) Integrin $\alpha 5 \beta 1$ facilitates cancer cell invasion through enhanced contractile forces. *J. Cell Sci.* **124**, 369-383.
- Mitra, S. K. and Schlaepfer, D. D. (2006) Integrin-regulated FAK-Src signaling in normal and cancer cells. *Cur. Opin. Cell Biol.* **18**, 516-523.
- Mukherjee, D. and Zhao, J. (2013) The Role of chemokine receptor CXCR4 in breast cancer metastasis. *Am. J. Cancer Res.* **3**, 46-57.
- Nielsen, T. S., Vendelbo, M. H., Jessen, N., Pedersen, S. B., Jorgensen, J. O., Lund, S. and Moller, N. (2011) Fasting, but not exercise, increases adipose triglyceride lipase (ATGL) protein and reduces G(0)/G(1) switch gene 2 (G0S2) protein and mRNA content in human adipose tissue. *J. Clin. Endocr. Metab.* **96**, E1293-E1297.
- Nieva, C., Marro, M., Santana-Codina, N., Rao, S., Petrov, D. and Sierra, A. (2012) The lipid phenotype of breast cancer cells characterized by Raman microspectroscopy: towards a stratification of malignancy. *PLoS ONE* **7**, e46456.
- Pan, D. (2010) The hippo signaling pathway in development and cancer. *Dev. Cell* **19**, 491-505.
- Perbal, B. (2004) CCN proteins: multifunctional signalling regulators. *Lancet* **363**, 62-64.
- Pogoda, K., Niwinska, A., Murawska, M. and Pienkowski, T. (2013) Analysis of pattern, time and risk factors influencing recurrence in triple-negative breast cancer patients. *Med. Oncol.* **30**, 388.
- Schlaepfer, D. D., Hanks, S. K., Hunter, T. and van der Geer, P. (1994) Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. *Nature* **372**, 786-791.
- Si, Y., Ji, X., Cao, X., Dai, X., Xu, L., Zhao, H., Guo, X., Yan, H., Zhang, H., Zhu, C., Zhou, Q., Tang, M., Xia, Z., Li, L., Cong, Y. S., Ye, S., Liang, T., Feng, X. H. and Zhao, B. (2017) Src inhibits the Hippo tumor suppressor pathway through tyrosine phosphorylation of Lats1. *Cancer Res.* **77**, 4868-4880.
- Son, H. and Moon, A. (2010) Epithelial-mesenchymal transition and cell invasion. *Toxicol. Res.* **26**, 245-252.
- Thike, A. A., Cheok, P. Y., Jara-Lazaro, A. R., Tan, B., Tan, P. and Tan, P. H. (2010) Triple-negative breast cancer: clinicopathological characteristics and relationship with basal-like breast cancer. *Modern Pathol.* **23**, 123-133.
- Tsai, M. S., Bogart, D. F., Castaneda, J. M., Li, P. and Lupu, R. (2002) Cyr61 promotes breast tumorigenesis and cancer progression. *Oncogene* **21**, 8178-8185.
- Welch, C., Santra, M. K., El-Assaad, W., Zhu, X., Huber, W. E., Keys, R. A., Teodoro, J. G. and Green, M. R. (2009) Identification of a protein, G0S2, that lacks Bcl-2 homology domains and interacts with and antagonizes Bcl-2. *Cancer Res.* **69**, 6782-6789.
- Yadav, B. S., Chanana, P. and Jhamb, S. (2015) Biomarkers in triple negative breast cancer: a review. *World J. Clin. Oncol.* **6**, 252-263.
- Yang, X., Lu, X., Lombes, M., Rha, G. B., Chi, Y. I., Guerin, T. M., Smart, E. J. and Liu, J. (2010) The G(0)/G(1) switch gene 2 regulates adipose lipolysis through association with adipose triglyceride lipase. *Cell Metab.* **11**, 194-205.
- Ye, D. J., Kwon, Y. J., Shin, S., Baek, H. S., Shin, D. W. and Chun, Y. J. (2017) Induction of integrin signaling by steroid sulfatase in human cervical cancer cells. *Biomol. Ther. (Seoul)* **25**, 321-328.
- Yim, C. Y., Sekula, D. J., Hever-Jardine, M. P., Liu, X., Warzecha, J. M., Tam, J., Freemantle, S. J., Dmitrovsky, E. and Spinella, M. J. (2016) G0S2 suppresses oncogenic transformation by repressing a MYC-regulated transcriptional program. *Cancer Res.* **76**, 1204-1213.
- Zandbergen, F., Mandard, S., Escher, P., Tan, N. S., Patsouris, D., Jatkoa, T., Rojas-Caro, S., Madore, S., Wahli, W., Tafuri, S., Muller, M. and Kersten, S. (2005) The G0/G1 switch gene 2 is a novel

- PPAR target gene. *Biochem. J.* **392**, 313-324.
- Zhang, J., Wang, Y., Yin, Q., Zhang, W., Zhang, T. and Niu, Y. (2013) An associated classification of triple negative breast cancer: the risk of relapse and the response to chemotherapy. *Int. J. Clin. Exp. Pathol.* **6**, 1380-1391.
- Zhang, X., Heckmann, B. L., Campbell, L. E. and Liu, J. (2017) G0S2: a small giant controller of lipolysis and adipose-liver fatty acid flux. *Biochem. Biophys. Acta* **1862**, 1146-1154.
- Zhao, B., Ye, X., Yu, J., Li, L., Li, W., Li, S., Lin, J. D., Wang, C. Y., Chinnaiyan, A. M., Lai, Z. C. and Guan, K. L. (2008) TEAD mediates YAP-dependent gene induction and growth control. *Genes Dev.* **22**, 1962-1971.
- Zygulska, A. L., Krzemieniecki, K. and Pierzchalski, P. (2017) Hippo pathway - brief overview of its relevance in cancer. *J. Physiol. Pharmacol.* **68**, 311-335.