Adhesion G protein-coupled receptor G2 accelerates the proliferation of cancer cells by promoting the formation of CDK4/CCND1

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Abstract

Gastric cancer is a common malignant tumor in humans. Analysis of clinical data of gastric cancer revealed that adhesion G protein-coupled receptor G2 (ADGRG2), endoplasmic reticulum oxidoreductase 1β, lactate dehydrogenase B and chromosome 1 open reading frame 115 were abnormally highly expressed in gastric cancer. ADGRG2 was not only highly expressed in gastric cancer tissues, but was also associated with poor prognosis in patients with gastric cancer. Numerous oncogenes and tumor suppressor genes are directly involved in the regulation of the cell cycle. ADGRG2 was shown to promote cell proliferation by promoting the G\textsubscript{1}/S transition. ADGRG2 did not affect the expression of CDK4 or cyclin D1 (CCND1), but was found to affect the cell cycle by promoting the formation of the cell cycle-dependent complex CDK4/CCND1, thereby promoting cell proliferation, and affecting the formation and development of gastric cancer.

Introduction

Gastric cancer is a common malignant tumor in humans, originating from gastric mucosal epithelial cells (1). Its morbidity and mortality continue to be high, which has a great impact on human health (2). Therefore, elucidating the mechanism underlying gastric cancer development and identifying novel therapeutic targets can provide a theoretical basis for the treatment of gastric cancer.

Adhesion G protein-coupled receptor G2 (ADGRG2) is a protein encoded by the ADGRG2 gene on the X chromosome (3). ADGRG2 is mainly expressed in human and mouse epididymis, human prostate and parathyroid glands, and in the central nervous system (4). In addition, ADRG2 was found to be highly expressed in fibroblast-like synoviocytes obtained from patients with osteoarthritis (5). ADGRG2 is significantly overexpressed in the Wnt signal-dependent subgroup of medulloblastoma tumors, as well as in Ewing sarcoma, and carcinoma derived from prostate, kidney or lung (6). It was previously reported that ADGRG2 promotes tumor invasion and metastasis through placental growth factor and MMP1 (7). However, to the best of our knowledge, the expression and mechanism of ADGRG2 in gastric cancer have not been reported to date (8).

Cancer may be considered a type of cell cycle-related disease (9). The activation of cancer genes and the inactivation of cancer suppressor genes exert functional effects on the mechanism of the cell cycle (10). Multiple oncogenes and tumor suppressor genes are directly involved in the regulation of the cell cycle (11). Their abnormal activation leads to alterations in the cell cycle and uncontrolled proliferation of cancer cells. CDKs are the main molecules involved in the cell cycle regulation mechanism (12). The activation of CDK depends on the cyclins that are expressed in the different phases of the cell cycle (13). CDK and cyclins combine to form a cell cycle-dependent complex that regulates the progression of the cell cycle (14), which may eventually affect the formation and development of tumors (15).
The main aim of the present study was to explore the influence of ADGRG2 on the formation and development of gastric cancer and provide a theoretical basis for its clinical treatment.

**Materials and methods**

*Flow cytometry analysis of the cell cycle*. The MGC803 cell line, which was authenticated by short tandem repeat analysis, was used in the present study.

Cells were collected by centrifugation, washed three times with ice-cold PBS and centrifuged again (Eppendorf Mastercycler Nexus SX1; Eppendorf). The supernatant was discarded and the cells were resuspended in 0.5 ml PBS. Next, 3.5 ml 70% pre-cooled ethanol was added, and the solution was stored at 4°C overnight up to 1 month until use. The ethanol-fixed cells were then centrifuged in a Mastercycler Nexus SX1 (Eppendorf). Next, the supernatant was discarded and the cells were washed three times with PBS to remove the residual ethanol. Next, the cells were resuspended in 1 ml PI/Triton X-100 staining solution containing 0.2 mg RNase A and stained at 37°C for 15 min. Subsequently, the cell cycle was determined using flow cytometry.

*RNA extraction*. RNA was extracted using the RNA extraction kit RNeasy Mini Kit (Qiagen, Inc.) following the manufacturer’s instructions. The reagents used in the experiment were RNase-free. Briefly, cells were resuspended in 350 µl buffer RLT containing 1% β-mercaptoethanol. Next, 350 µl 70% absolute ethanol was added, mixed by pipetting, transferred to an adsorption column and centrifuged in a Mastercycler Nexus SX1 (Eppendorf) at 10,000 x g for 15 sec. Upon discarding the effluent, 350 µl buffer RW1 was added, followed by centrifugation in a Mastercycler Nexus SX1 (Eppendorf) at 10,000 x g for 15 sec. Upon discarding the supernatant, 10 µl DNase I was added to 70 µl buffer RDD. The sample was mixed evenly, added to the membrane of the adsorption column and incubated at room temperature for 15 min. Next, 350 µl buffer RW1 was added, followed by centrifugation in a Mastercycler Nexus SX1 (Eppendorf) at 10,000 x g for 20 sec. Next, the collection tube was replaced, and 500 µl buffer RPE was added, followed by centrifugation in a Mastercycler Nexus SX1 (Eppendorf) at 10,000 x g for 20 sec. Upon discarding the supernatant, 500 µl buffer RPE was added, followed by centrifugation in a Mastercycler Nexus SX1 (Eppendorf) at 10,000 x g for 2 min. Upon discarding the flow-through, the collection tube was replaced, and the sample was centrifuged in a Mastercycler Nexus SX1 (Eppendorf) at maximum speed for 2 min. Next, the adsorption column was placed in a 1.5-ml centrifuge tube (Eppendorf) tube, and ~30 µl RNase-free water was added, followed by incubation for 1 min and centrifugation in a Mastercycler Nexus SX1 (Eppendorf) at 8,000 rpm for 1 min to collect the RNA. Nanodrop 2000 (NanoDrop Technologies; Thermo Fisher Scientific, Inc.) was then used to measure the concentration and purity of RNA.

*Reverse transcription of RNA into cDNA*. M-MLV Reverse Transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.) was used according to the manufacturer’s instructions. Briefly, the following components were mixed in a 200-µl PCR tube: 1 µl oligo (dT) (500 µg/ml), 1 µl dNTP (10 mM), 2 µg total RNA and
RNase-free water up to a total volume of 12 µl. Next, the mixture was placed in the PCR instrument, where it was heated and denatured at 65°C for 5 min, before being readily placed on ice to cool. Next, the following components were mixed in another PCR tube: 4 µl 5X first strand buffer, 2 µl DTT (0.1 M), 1 µl RNase inhibitor (20 U/µl) and 1 µl M-MLV. Subsequently, 8 µl was added to the mixture prepared in the other aforementioned PCR tube, and then placed in the PCR instrument, where the reaction was performed at 37°C for 60 min followed by incubation at 70°C for 15 min to inactivate M-MLV. The obtained cDNA was stored at -20°C for long-term storage.

**Fluorescence quantitative PCR (qPCR).** The fluorescence qPCR system consisted of 10 µl SYBR Green Mix (Roche Diagnostics), 1 µl upstream primer (4 µM), 1 µl downstream primer (4 µM), 1 µl cDNA and ddH2O to a final volume of 20 µl. The reaction conditions were as follows: Pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 sec and annealing at 58°C for 1 min for 40 cycles. Fluorescence detection was performed after annealing and extension. When the amplification reaction was completed, a melting curve reaction was performed to analyze the product specificity, which started at 55°C, increased by 0.5°C every 30 sec and ended at 95°C for a total of 81 cycles. The $2^{-\Delta\Delta Cq}$ method was used for analysis, and GAPDH was used as an internal reference. The relevant sequences are provided in Table SI.

**Western blotting.** Cells were collected, washed with PBS, mixed with an appropriate quantity of lysis buffer and lysed for 30 min on ice. After ultrasonication for 1 min, the sample was centrifuged in a Mastercycler Nexus SX1 (Eppendorf) at 12,000 rpm for 10 min at 4°C. The supernatant was collected and the protein concentration was determined by using the BCA method. Next, an appropriate quantity of 5X SDS was added to the protein sample, which was then denatured by boiling in a metal bath at 95°C for 5 min and immediately subjected to SDS-PAGE or stored at -80°C until further use. After preparing the stacking and resolving gels, the protein sample was diluted to 5 µg/5 µl with 1X SDS for loading. Next, the sample was subjected to electrophoresis: A voltage of 80 V was used for concentration for 30 min, which was changed to 100 V for ~1 h when the sample entered the separation gel. Next, the samples were transferred to a membrane by applying 100 V in an ice water bath for 2 h. The membrane was then blocked by incubating with 5% skimmed milk powder in TBS-Tween 20 (TBST) at room temperature for 2 h. Next, the membrane was incubated with the primary antibody (diluted 1:1,000 in blocking solution) at room temperature for 2 h or at 4°C overnight. Next, the membrane was washed three times with TBST for 15 min each at room temperature on a shaker. Next, the membrane was incubated with the secondary antibody (diluted 1:5,000 in blocking solution) for 1 h at room temperature, followed by three washes with TBST at room temperature for 15 min each on a shaker. To develop the protein bands, solutions A and B of a chemiluminescence kit were mixed at 1:1 ratio, and ~500 µl mixture was added to each X-ray film, which were then wrapped in plastic film in a dark room and placed in an X-ray film holder. After pressing for 10 sec-3 min, the X-ray films were developed, fixed, dried and stored. The relevant antibody information is shown in supplementary 1.

**Immunofluorescence.** In the culture plate, the slides with climbed cells were washed three times with PBS for 3 min each and then fixed with 4% paraformaldehyde for 15 min. Next, the slides were washed three
times with PBS for 3 min each. Next, 0.5% Triton X-100 (diluted in PBS) was used at room temperature for 20 min to permeabilize the cells. Upon washing the slides with PBS three times for 3 min each, the PBS was removed with absorbent paper, and normal goat serum was then added to the slides for blocking at room temperature for 30 min. Next, absorbent paper was used to absorb the blocking solution without washing. Subsequently, diluted primary antibody was added to each slide, placed in a humid box and incubated overnight at 4°C. Next, a fluorescently labeled secondary antibody was added as follows: PBS-Tween 20 (PBST) was used to wash the slides three times (3 min each time). The excess liquid on the slide was removed with absorbent paper, and the aforementioned diluted fluorescent secondary antibody was added in a dropwise manner, followed by incubation in a humid box at 20–37°C for 1 h. Next, the section was immersed in PBST three times (3 min each time), and the nucleus was subjected to counterstaining as follows: DAPI was added in a dropwise manner and incubated for 5 min in the dark. Next, the excess of DAPI was removed by washing with PBST four times (5 min each time), and the liquid on the slide was removed with absorbent paper. Subsequently, the slide was mounted with a mounting solution containing a fluorescence quencher, and images were observed and collected under a fluorescence microscope.

**MTT assay to detect cell proliferation.** To determine the proliferation ability of cells, MTT assay was performed as previously described (24). Cellular suspensions were prepared and transferred into a 96-well culture plate, and 10 µl MTT reagent (Beyotime Institute of Biotechnology) was added to each well. The samples were then incubated for 4 h, and colorimetric changes were determined at an absorbance of 570 nm by using a microplate reader. The absorbance of the samples was normalized to that of a blank control.

**EdU staining to detect cell proliferation.** EdU is a thymidine analogue, which can insert thymine into replicated DNA during cell proliferation. EdU can react with Apollo fluorescent dye to detect the replicated DNA, thereby detecting the proliferative activity of cells (12). In the present study, the cell proliferation activity was detected via the specific reaction of EdU with Apollo488, and the resulting green fluorescence was indicative of proliferation in EdU-positive cells.

**Results**

**Specific highly expressed marker genes in gastric cancer.** Gastric cancer is a major threat to human health; thus, it is important to explore the mechanism underlying its occurrence and development. Genevestigator (https://genevestigator.com/) was used to analyze the gene expression of cancer and normal tissues in 120 patients (Fig. S3). The results showed that the expression of ADGRG2, endoplasmic reticulum oxidoreductase 1β (ERO1B), lactate dehydrogenase B (LDHB) and chromosome 1 open reading frame 115 (C1orf115) was low in normal stomach tissues and abnormally high in gastric cancer (Fig. 1A). These four genes were considered as a set. The tumor and adjacent tissues of three patients were randomly selected to analyze the expression levels of these four genes. The results showed that the expression levels of these four genes in the adjacent tissues were significantly lower compared with those in cancer tissues (Fig. 1B). According to the clinical stage of the tumor, the patients were
divided into groups I-II and II-III, and the changes in gene expression were analyzed. The results showed that, as the condition of the patients with cancer deteriorated, the expression levels of the aforementioned four genes increased, which showed that the expression of these genes is closely associated with the development of gastric cancer (Fig. 1C). In addition, the changes in signaling pathways in samples with high expression of these four genes were analyzed (Fig. 1D), and the results revealed that the changes in these genes may affect the expression of genes involved in signaling pathways such as the cell cycle.

**ADGRG2 is highly expressed in gastric cancer and is associated with a poor prognosis.** The expression of ADGRG2, ER01B, LDHB and C1orf115 in 24 tumor tissues and their corresponding normal tissues was analyzed. The results showed that ADGRG2 was abnormally highly expressed in gastric cancer compared with its expression in normal tissues (Fig. 2A-D). The data were obtained from the Kaplan-Meier plotter (http://kmplot.com/) website. The median gene expression of all patients was calculated, and values above the median were defined as high expression, while values below the median were defined as low expression. In addition, the influence of these genes on the prognosis of patients with lung, gastric, breast and ovarian cancer was analyzed. The analysis showed that patients with gastric cancer and high expression of ADGRG2 had a poor prognosis (Fig. 2A).

**ADGRG2 affects the expression of cell cycle-related genes.** Signaling pathway changes were analyzed in gastric cancer samples with high expression of ADGRG2, ER01B, LDHB and C1orf115. The results showed that, among the gastric cancer samples that had high expression levels of these four genes, signaling pathways associated with the cell cycle were included in the top 20 signaling pathways that were upregulated or downregulated (Fig. 3A and B). The positive correlation of genes with ADGRG2, ER01B, LDHB and C1orf115 in these gastric cancer samples was analyzed. A total of 236 related genes and cell cycle-related genes were analyzed. There are i) 14 cell cycle-related genes among the genes associated with ADGRG2 (Fig. 3C and G); ii) 6 cell cycle-related genes among the genes associated with ER01B (Fig. 3D and H); iii) 3 cell cycle-related genes among the genes associated with LDHB (Fig. 3E and I); and iv) 3 cell cycle-related genes among the genes associated with C1orf115 (Fig. 3F and J). Therefore, it was hypothesized that ADGRG2 may affect the cell cycle.

**ADGRG2 expression affects cell cycle-related genes.** In two gastric cancer cell lines (AGS and MGC803), the expression of ADGRG2 was overexpressed and knocked down (Fig. S4). After overexpression of ADGRG2, changes in the expression of cell cycle-related genes were detected. The results showed that ADGRG2 expression was increased, leading to the upregulation of cell cycle inhibitory factors, including P19, P21 and P27 (Fig. 4A and C). Cell cycle inhibitors are divided into two major families: i) Ink4 (inhibitor of CDK4), such as P16, P15, P18 and P19, which specifically inhibit CDK4-cyclin D1 and CDK6-cyclin D1 complexes; and ii) kinase inhibition proteins, including P21, P27 and P57 (16), which can inhibit the activity of the majority of CDKs. Among them, P21l can also bind with the cofactor proliferating cell nuclear antigen of DNA polymerase α to directly inhibit DNA synthesis (17).

The cell cycle regulatory proteins form a cell cycle regulation system that provides a strict and orderly timetable for the occurrence of various events in the cell cycle. When the internal and external
environment of the cell changes, these proteins form a corresponding regulatory mechanism that enables the cell cycle to adapt to the changes in the internal and external environment of the cell and ensures the accurate delivery of genetic material. Among the cells that exhibited overexpression of ADGRG2, 16 cell cycle inhibitory factors showed altered expression levels (Fig. 4B). RT-qPCR detection of gene expression revealed the same results (Fig. 4D).

**ADGRG2 expression affects cell cycle-related genes in cancerous tissues.** In the cancer tissues of patients with gastric cancer, the expression of cell cycle inhibitory factors was significantly lower than that of adjacent tissues (Fig. 5A). This is similar to the effect of overexpressing ADGRG2, which results in a decrease in the expression of cell cycle inhibitors (Fig. 5A). This suggests that there is an association between cell cycle-related signaling pathways, and the occurrence and development of tumors. The tumors were divided into stages I-II and II-III, and the expression changes of genes involved in cell cycle-related signaling pathways were detected. The results showed that the abnormal expression of genes involved in cell cycle-related signaling pathways becomes more pronounced with the development of tumors (Fig. 5B). Additionally, it was observed that abnormal expression of ADGRG2 also caused abnormal expression of genes involved in cell cycle-related signaling pathways. This suggests that ADGRG2 may affect the occurrence and development of tumors by affecting the cell cycle (Fig. 5C and D).

**ADGRG2 promotes the $G_1$/$S$ phase transition in the cell cycle.** Cell cycle changes were detected after overexpression and knockdown of ADGRG2 in the gastric cancer cell line AGS (Fig. 6A). The results showed that, when ADGRG2 is overexpressed, gastric cancer cells go through the $G_1$ phase and enter the $S$ phase (Fig. 6C), while, when ADGRG2 is knocked down, gastric cancer cells undergo $G_1$/$S$ phase arrest (Fig. 6B). Thus, expression of ADGRG2 is responsible for the $G_1$/$S$ transition of the cell cycle (Fig. S5).

**ADGRG2 promotes the formation of the cell cycle-dependent complex CDK4/cyclin D1 (CCND1).** When the expression of ADGRG2 was modified in the gastric cancer cell line AGS, the expression of CDK4 and CCND1, a cell cycle $G_1$/$S$ phase-dependent gene, was detected. Overexpression or knockdown of ADGRG2 did not change the expression of CDK4 or CCND1 (Fig. 7A-C). Cell cycle transition depends on the formation of CDK4/CCND1, a complex dependent on the $G_1$/$S$ phase of the cell cycle. After overexpressing or knocking down ADGRG2, the formation of the CDK4/CCND1 complex was detected by immunoprecipitation (Fig. 7D). The results showed that the overexpression of ADGRG2 promotes the formation of the cell cycle-dependent complex CDK4/CCND1. Knockdown of ADGRG2 inhibits the formation of the cell cycle-dependent complex CDK4/CCND1. In addition, immunofluorescence was used to detect the colocalization of CDK4 and CCND1 after changing the expression of ADGRG2 (Fig. 7E). The results showed that, after overexpressing ADGRG2, the colocalization signal of CDK4 and CCND1 became stronger. Upon knocking down ADGRG2, the CDK4 and CCND1 co-localization signal became weak. To verify whether the effect of ADGRG2 on the formation of the cell cycle-dependent complex CDK4/CCND1 is conserved, the effect of ADGRG2 on the expression of CDK4 and CCND1 was assessed in another gastric cancer cell line, MGC803 (Fig. S2A and B).
Immunoprecipitation was used to detect the effect of ADGRG2 on the formation of the cell cycle-dependent complex CDK4/CCND1 (Fig. S2C). The results revealed that, in MGC803 cells, ADGRG2 did not affect the expression of CDK4 or CCND1, but affected the formation of the cell cycle-dependent complex CDK4/CCND1.

**ADGRG2 promotes gastric cancer cell proliferation.** After altering the expression of ADGRG2, EdU staining was used to detect changes in the cell proliferation rate. The results showed that the EdU-positive rate with high expression of ADGRG2 in gastric cancer cells was higher compared with that of gastric cancer cells with low expression of ADGRG2 (Fig. 8A).

Flow cytometry and MTT staining were used to detect the effect of ADGRG2 on cell proliferation, and the results showed that the proliferation of gastric cancer cells with high expression of ADGRG2 increased (Fig. 8B).

Fig. 8C shows subcutaneous tumor formation in nude mice. After overexpressing ADGRG2 in AGS cells, nude mice were injected subcutaneously for tumor formation, and the difference in tumor volume was observed 1 month later. The results showed that the tumor volume after increased expression of ADGRG2 was larger than that of the control group. In addition, the present study analyzed the spatiotemporal expression of ADGRG2, ER01B and LDHB in mice (notably, there is no C1orf115 gene in mice) (Fig. S6). The results showed that ADGRG2 was highly expressed during the rapid growth period of mice, likely reflecting the promoting effect of ADGRG2 on cell proliferation.

**Discussion**

Gastric cancer is a major threat to human health. Previous studies have attempted to identify an effective treatment for gastric cancer, which was also the aim of the present study. First, via analysis of clinical data, it was found that ADGRG2 is abnormally highly expressed in gastric cancer tissues (18, 19). The expression of ADGRG2 in normal stomach tissues is higher compared with that in other normal organ tissues, which indicates that the ADGRG2 gene plays an important role in the normal function of the stomach. Abnormally high expression of ADGRG2 may induce tumorigenesis (20), while abnormally low expression of ADGRG2 may induce other diseases that should be explored in future research.

The driving and regulatory mechanisms of the cell cycle are closely associated with tumorigenesis. When the regulatory mechanism of the cell cycle is altered, it can lead to uncontrolled proliferation of normal cells, which may transform into tumor cells. Tumor cells are also derived from normal cells in the body. Cells are affected by internal and external factors at different stages of division and proliferation, which can also cause abnormal differentiation and proliferation, which can lead to the occurrence of tumors. Therefore, these two processes are closely associated.

It was found that abnormal expression of ADGRG2 in gastric cancer induced disorders in the cell cycle regulation (21). Low expression of ADGRG2 induces cell cycle G\textsubscript{1}/S arrest, which may be an important mechanism through ADGRG2 affects the occurrence and development of gastric cancer. ADGRG2 did not
alter the expression of CDK4 or CCND1, but affected the expression of the cell cycle-dependent complex CDK4/CCND1, which ultimately affected the proliferation of gastric cancer cells (Fig. 9). Analysis of clinical data revealed that ERO1B, LDHB and C1orf115 are abnormally highly expressed in gastric cancer, which suggests that it may have an effect on the occurrence and development of gastric cancer.

References


**Supplementary Table**

Supplementary Table S1 is not available with this version.

**Figures**
Figure 1

Analysis of gene expression changes in gastric cancer. (A) Genevestigator (https://genevestigator.com/) was used to analyze the gene expression of cancer and normal tissues in 120 patients. (B) Analysis of the differential expression of four genes (adhesion G protein-coupled receptor G2, endoplasmic reticulum oxidoreductase 1β, lactate dehydrogenase B and chromosome 1 open reading frame 115) in gastric cancer and adjacent tissues of three patients. (C) According to the clinical stage of patients with gastric cancer, the changes in gene expression were analyzed. (D) Gene Ontology analysis of the differentially expressed genes in adjacent and cancer tissues of patients with gastric cancer.
Expression of four genes in tumors, and effect of gene expression on the survival and prognosis of patients with gastric cancer. (A-D) The expression of adhesion G protein-coupled receptor G2, endoplasmic reticulum oxidoreductase 1β, lactate dehydrogenase B and chromosome 1 open reading frame 115 in 24 tumor and corresponding normal tissues was analyzed. In addition, the influence of these genes on the prognosis of patients with lung, gastric, breast and ovarian cancer was analyzed.
Figure 3

ADGRG2 affects the expression of cell cycle-related genes. (A and B) ADGRG2, ERO1B, LDHB and C1orf115 lead to increased expression of signaling pathways in gastric cancer tissues. Analysis of genes associated with (C) ADGRG2, (D) ERO1B, (E) LDHB and (F) C1orf115 expression. (G) ADGRG2-related genes and cell cycle-related genes were jointly analyzed. (H) ERO1B-related genes and cell cycle-related genes were jointly analyzed. (I) LDHB-related genes and cell cycle-related genes were jointly analyzed. (J)
C1orf115-related genes and cell cycle-related genes were jointly analyzed. ADGRG2, adhesion G protein-coupled receptor G2; ER01B, endoplasmic reticulum oxidoreductase 1β; LDHB, lactate dehydrogenase B; C1orf115, chromosome 1 open reading frame 115.

Figure 4

ADGRG2 expression affects cell cycle-related genes in AGS cells. (A) After overexpression of ADGRG2, the expression changes of cell cycle-related genes were detected. (B) After overexpression of ADGRG2, the number of cell cycle inhibitory factors was altered. (C) Transcriptome data analysis of the expression of cell cycle inhibitory factors after overexpression of ADGRG2. (D) Quantitative PCR was used to detect the expression of cell cycle inhibitory factors after overexpression of ADGRG2. ADGRG2, adhesion G protein-coupled receptor G2.
Figure 5

ADGRG2 promotes $G_1$/S phase transition in the cell cycle. (A) ADGRG2 was overexpressed and knocked down in the gastric cancer cell line AGS. (B) After altering the expression of ADGRG2 in the gastric cancer cell line AGS, changes in the cell cycle were detected. (C) After overexpressing ADGRG2 in the gastric cancer cell line AGS, changes in the cell cycle were detected. ADGRG2, adhesion G protein-coupled receptor G2.
Figure 7

ADGRG2 promotes the formation of the cell cycle-dependent complex CDK4/CCND1. After altering the expression of ADGRG2, expression changes in (A) CDK4 and (B) CCND1 were detected. (C) After altering the expression of ADGRG2, changes in CDK4 and CCND1 protein expression were detected. (D) After altering the expression of ADGRG2, co-immunoprecipitation was used to detect the formation of the CDK4/CCND1 complex. (E) After altering the expression of ADGRG2, the co-localization of CDK4 and CCND1 was detected by immunofluorescence. ADGRG2, adhesion G protein-coupled receptor G2; CCND1, cyclin D1.
Figure 8

ADGRG2 promotes gastric cancer cell proliferation. (A) After altering the expression of ADGRG2, EdU staining was used to detect changes in the cell proliferation rate. (B) After altering the expression of ADGRG2, flow cytometry and MTT staining were used to detect the effect of ADGRG2 on cell proliferation. (C) Subcutaneous tumor formation in nude mice. After overexpression of ADGRG2 in AGS cells, nude mice were injected subcutaneously with such cells for tumor formation, and the difference in tumor volume was observed 1 month later. The results revealed that the volume of the tumor in the ADGRG2-expressing group was larger than that of the control group. ADGRG2, adhesion G protein-coupled receptor G2.
Figure 9

Mechanism of adhesion G protein-coupled receptor G2 effect on tumor formation.

Supplementary Files

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