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Effect of Jagged1 on the proliferation and migration of colon cancer cells

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Abstract. The aim of this study was to determine the effect of Jagged1 on the proliferation and migration of colon cancer cells. HT-29 colorectal carcinoma cells were transfected with adenovirus carrying either the Jagged1 gene (Ad-Jagged1) or Jagged1 small interfering RNA (siRNA) (Ad-si/hJagged1). The protein was detected using western blot analysis. The proliferation of cells was observed by assessing the incorporation of [^{3}H]-thymidine. The migration of cells was detected using a modified Boyden chamber assay. The level of Jagged1 protein increased by 2.8-fold with Ad-Jagged1 transfection and decreased by 70% with Ad-si/hJagged1 transfection. Proliferation, migration and p-Akt protein expression were enhanced in the Ad-Jagged1-transfected group compared to that in the non-transfected control group. In addition, the proliferation, migration and phosphorylated Akt protein expression were reduced in the Ad-si/hJagged1-transfected group compared to that in the non-transfected control group. The Jagged1 protein may promote proliferation and migration of colon cancer cells, which may be related to its effect on Akt phosphorylation.

Introduction

Colon cancer is the third most common type of cancer, with an increasing incidence each year (1). The proliferation and migration of colon cancer cells are two significant determinants of the prognosis of colon cancer. Jagged1 is a ligand in the Notch1 signaling pathway. A recent study has demonstrated that Jagged1 is highly expressed in colon cancer (2). However, the role of Jagged1 in the occurrence and development of colon cancer remains poorly understood. Another study revealed that the Jagged1/Notch signaling pathway was involved in the proliferation and migration of certain types of cancer (3). This study aimed to investigate the effect of Jagged1 on the *in vitro* proliferation and migration of colon cancer cells.

Materials and methods

Materials. Human colon cancer cells (HT-29) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA), and DMEM-F12 and fetal bovine serum (FBS) were purchased from Gibco (Carlsbad, CA, USA). The Jagged1-carrying adenovirus (Ad-Jagged1) and the blank adenovirus (Ad-CMV) were kindly provided by Professor Weinmaster from the University of California, Los Angeles, CA, USA. The adenovirus carrying small interfering RNA (siRNA) targeting the Jagged1 gene (Ad-si/hJagged1) and the control adenovirus without siRNA expression were kindly provided by Professor Gabrilovich from the University of South Florida, FL, USA. Rabbit anti-human antibodies against Jagged1, phosphorylated Akt (p-Akt), Akt and GAPDH were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The modified Boyden chamber was purchased from Zhongshan Co., Ltd. (China).

Cell culture and adenovirus transfection. The HT-29 cells were maintained in DMEM-L containing 10% FBS at 37˚C in a 5% CO_2 atmosphere in a 100-ml flask. When the cell confluence reached 60-70%, the 1x10^6 pfu/ml adenovirus solution (10 μl) was added to the flask and the cells were incubated for 48 h.

[^{3}H]-thymidine ([^{3}H]-TdR) incorporation. The cells were maintained in serum-free medium for 4 h, then 10 μl of [^{3}H]-TdR was added at a final concentration of 37 kBq/ml. Following 24-h incubation, the medium was removed and the cells were washed with PBS of an equal volume. Then, these cells were treated with 500 μl of 0.125% trypsin and a cell suspension was prepared which was then transferred onto grade 49 glass-fiber filter paper. Following washing in 0.9% NaCl three times, the paper containing the cells was rinsed with 2 ml of 5% trichloroacetic acid for fixation. Then, the cells were washed once in 1 ml of absolute ethanol and dehydrated. The scintillation solution was added to the dry paper, which was kept in a dark room for 3 h. Detection was performed and the data were expressed as counts per min per well (cpm/well).

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The amount of incorporated [^{3}H]-TdR positively correlated with the DNA synthesis.

Cell migration. Following digestion, cells were collected and re-suspended in 500 μl of medium for counting. Then, 100 μl of medium was added to the lower chamber, and the cell suspension containing 1x10^5 cells (100 μl) was added to the upper chamber followed by incubation at 37˚C in a 5% CO_2 atmosphere for 6 h. The filter was collected and the cells that did not migrate were removed. The remaining cells were fixed in absolute ethanol and then underwent haemotoxylin staining. The filter was kept at 37˚C overnight and made transparent with xylene. Three fields were randomly selected at a magnification of x400 and the migrated cells were counted.

Western blot analysis. Cells were washed in PBS three times and then lysed in a lysis buffer containing phenylmethanesulfonylfluoride (PMSF) for 30 min. The lysate was transferred into an EP tube followed by centrifugation at 4˚C for 20 min at 12000 x g. The supernatant was collected and stored at -70˚C. Protein of equal content was subjected to SDS-PAGE at a constant 80 V, and then transferred onto a polyvinylidene fluoride (PVDF) membrane, which was blocked in 5% non-fat milk in phosphate-buffered saline Tween-20 (PBST) at room temperature for 1 h. Subsequently, the membrane was treated with rabbit anti-human Jagged1, p-Akt, Akt or GAPDH (1:200) independently at 4˚C overnight, and then with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG at 37˚C for 1 h. Visualization was conducted according to the manufacturer's instructions (ECL kit). Representative images were collected using a gel imaging system (Bio-Rad, Hercules, CA, USA). The expression of the target proteins were normalized by that of GAPDH.

Statistical analysis. Statistical analysis was performed using SPSS version 11.5 and quantitative data were expressed as the means ± standard deviation. Comparisons between the two groups were conducted using an independent samples t-test, and between three groups using one-way analysis of variance. A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Jagged1 intervention and detection. Ad-Jagged1 adenovirus and blank adenovirus (Ad-CMV) were used to transfect colon cancer cells. The results demonstrated that when compared with cells without transfection [mock (control) group], the Jagged1 expression in the Ad-Jagged1 group was significantly increased (2.17±0.45 vs. 0.78±0.06, P<0.01), and there was no significant difference between the Ad-CMV group and the mock group in the Jagged1 expression (0.81±0.05vs. 0.78±0.06, P>0.05) (Fig. 1A). Following transfection with adenovirus carrying siRNA targeting Jagged1 (Ad-si/hJagged1 group) and adenovirus without Jagged1 siRNA (Ad-NSC group), the results revealed that the Jagged1 expression in the Ad-si/hJagged1 group was markedly reduced when compared with the mock group (0.24±0.01 vs. 0.79±0.05, P<0.01), but no significant difference was observed between the Ad-NS group and the mock group (0.78±0.04 vs. 0.79±0.05, P>0.05) (Fig. 1B).

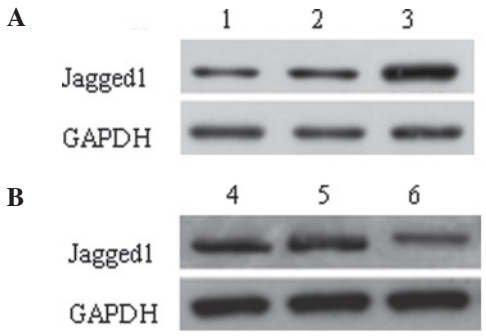

Figure 1. (A and B) Detection of Jagged1 expression in the colon cancer cells revealed by western blot analysis. Lanes 1 and 4, cells without transfection; lane 2, cells transfected with Ad-CMV; lane 3, cells transfected with Ad-Jagged1; lane 5, cells transfected with Ad-NSC; lane 6, cells transfected with Ad-si/hJagged1. Ad-CMV, blank adenovirus; Ad-Jagged1, Jagged1-carrying adenovirus; Ad-NSC, adenovirus without Jagged1 siRNA; Ad-si/hJagged1, adenovirus carrying siRNA targeting Jagged1.

Effect of Jagged1 on the proliferation of colon cancer cells. The proliferation of the colon cancer cells was determined by [^{3}H]-TdR incorporation. The results demonstrated that cell proliferation in the Ad-Jagged1 group was higher than that in the mock group (22048±1235 vs. 14750±867 cpm/well, P<0.01). However, no significant difference was observed between the Ad-CMV group and the mock group (14946±722 vs. 14750±867 cpm/well±0.06, P>0.05). In addition, when compared with the mock group, the cell proliferation in the Ad-si/hJagged1 group was significantly reduced (10084±922 vs. 15027±1008 cpm/well, P<0.01). However, the cell proliferation in the mock group was comparable with that in the Ad-NSC group (14895±964 vs. 15027±1008 cpm/well, P>0.05).

Effect of Jagged1 on the migration of colon cancer cells. A modified Boyden chamber assay was conducted to determine the migration of the colon cancer cells. When compared with the mock group, the migration in the Ad-Jagged1 group was significantly enhanced (42.24±2.37 cells vs. 21.22±1.95 cells, P<0.01), but there was no marked difference between the Ad-CMV group and the mock group. Moreover, the migration in the Ad-si/hJagged1 group was markedly increased when compared with the mock group (14.89±1.45 cells vs. 20.89±1.56 cells, P<0.01), but a significant difference was not observed between the Ad-NSC group and the mock group (21.32±2.04 cells vs. 20.89±1.56 cells, P>0.05) (Fig. 2B).

Effect of Jagged1 on Akt expression in colon cancer cells. A western blot assay was performed to detect the expression of Akt and p-Akt. The results demonstrated that the p-Akt expression in the Ad-Jagged1 group was markedly higher than that in the Ad-CMV group and the mock group (1.06±0.13 vs. 0.82±0.09 vs. 0.81±0.07, P<0.01). In addition, p-Akt expression in the Ad-si/hJagged1 group was markedly lower than that in the Ad-NSC group and the mock group (0.61±0.05 vs. 0.80±0.03 vs. 0.79±0.06, P<0.01). However, no significant difference was observed in the Akt expression between the different groups (Fig. 3).

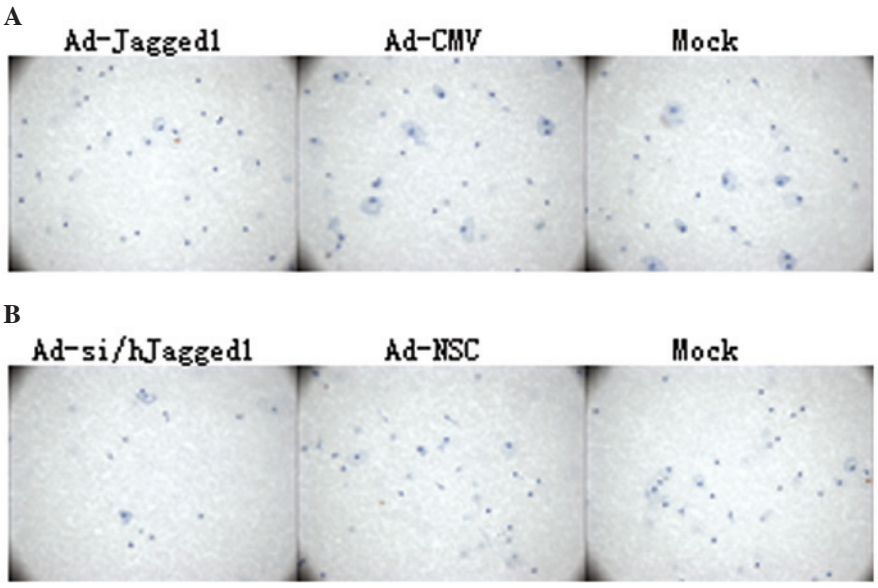

Figure 2. (A and B) Detection of migration of colon cancer cells by modified Boyden chamber assay. Ad-CMV, blank adenovirus; Ad-Jagged1, adenovirus carrying Jagged1; Ad-NSC, adenovirus without Jagged1 siRNA; Ad-si/hJagged1, adenovirus carrying siRNA targeting Jagged1.

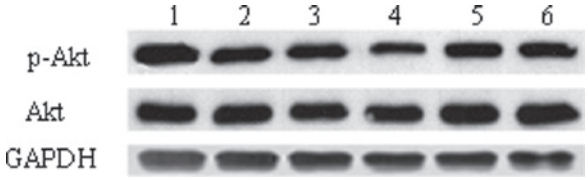

Figure 3. Detection of p-Akt and Akt in the colon cancer cells revealed by western blot assay. Lane 1, cells transfected with Ad-Jagged1; lane 2, cells transfected with Ad-CMV; lanes 3 and 6, cells without transfection; lane 4, cells transfected with Ad-si/hJagged1; lane 6, cells transfected with Ad-NSC. p-Akt, phosphorylated Akt; Ad-CMV, blank adenovirus; Ad-Jagged1, Jagged1-carrying adenovirus; Ad-NSC, adenovirus without Jagged1 siRNA; Ad-si/hJagged1, adenovirus carrying siRNA targeting Jagged1.

Discussion

Colon cancer is a common type of gastrointestinal malignancy. In recent years, the incidence of colon cancer has increased due to the changes in diet structure, and therefore it has become a significant malignancy threatening human health. A previous study has demonstrated that over-proliferation and compromised apoptosis are crucial for the occurrence and development of cancer (4). In clinical practice, chemotherapeutics, including 52Fu, cisplatin and mitomycin have the biological activity to reduce the proliferation of cancer cells, exerting an anti-tumor effect. However, the evident side-effects of these drugs, and the resistance of cancer cells to these drugs significantly limits the application of these chemotherapeutics (5). To identify the endogenous pro- and antitumor molecules from the molecular biological point of view may be a solution to this dilemma. Invasion and metastasis are significant biological characteristics of cancers. During invasion, cancer cells migrate from the primary site to the surrounding or distant tissues. The degree of migration of the cancer cells determines the degree of metastasis of the cancer (6). In certain types of cancer, studies have demonstrated that Jagged1 is crucial in the regulation of proliferation and migration of cancer cells (3). Moreover, in colon cancer, Jagged1 expression has been reported to be markedly increased when compared with that in adjacent normal tissues (2). However, the specific role of the increased expression of Jagged1 in colon cancer remains unknown.

Our results reveal that Jagged1 overexpression can promote the proliferation and migration of colon cancer cells, while Jagged1 silencing using siRNA significantly reduces proliferation and migration. These findings indicate that Jagged1 is a key molecule involved in the *in vitro* proliferation and migration of colon cancer cells. The Notch signaling pathway is widely conserved in a variety of animals, and is involved in the determination of cell fate. Studies have revealed that the Notch signaling pathway is closely involved with the occurrence and development of certain types of cancer. Jagged1 is a significant ligand in the Notch signaling pathway, where it activates Notch1, 2 and 4. The binding of Jagged1 to Notch may activate the transcription factors, including Hes and Hey, and initiate the proliferation and migration-related signaling pathways, including MAPK and PI3K/Akt (7), exerting the biological effects of Jagged1. In the present study, our results demonstrate that Jagged1 can increase p-Akt expression, but has no effect on Akt expression. The PI3K/Akt signaling pathway is a classic pathway involved in the proliferation and differentiation of cells. Studies have confirmed that the PI3K/Akt signaling pathway plays a crucial role in the occurrence and development of breast cancer (8). Akt is a downstream target protein and also a key molecule in the PI3K/Akt signaling pathway. The activation of PI3K may lead to the phosphorylation of Akt which can regulate the proliferation, apoptosis and migration of several types of cancer cells. There is evidence demonstrating that the phosphorylation of Akt is increased in a number of malignancies, including lung, gastric, breast, cervical and prostate cancer (9,10), which was consistent with our findings.

In conclusion, the overexpression of Jagged1, a key component in the Notch signaling pathway, is capable of promoting

the proliferation and migration of colon cancer cells, which may be related to the phosphorylation of Akt by Jagged1. Our findings suggest that Jagged1 plays a pivotal role in the occurrence and development of colon cancer, and thus may be a promising target in the prevention and treatment of this disease. However, the detailed mechanisms of the antitumor effect of Jagged1 require further study.

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