Open Journal of Pathology, 2022, 12, 80-87
https://www.scirp.org/journal/ojpathology
ISSN Online: 2164-6783
ISSN Print: 2164-6775

Caveolin-1 Inhibits Proliferation and Migration of Gastric Cancer Cell via Inactivating BMI-1

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How to cite this paper: Wang, J. and Liu, S.S. (2022) Caveolin-1 Inhibits Proliferation and Migration of Gastric Cancer Cell via Inactivating BMI-1. *Open Journal of Pathology*, **12**, 80-87.
https://doi.org/10.4236/ojpathology.2022.123010

Received: April 8, 2022
Accepted: May 9, 2022
Published: May 12, 2022

Abstract

Objective: To investigate the effect of Caveolin-1on the proliferation and migration ability in gastric carcinoma cells MGC-803 and its mechanism. **Methods:** Plasmid DNA pcDNA3.1-Cav1 was extracted by extracted kits, and transfected Cav-1 gene sequences were found. The expression levels of Cav-1 protein were detected by Western blot. And the proliferation was analyzed by CCK8 assay. The effect of Cav-1 on migration was detected by wound healing. The expression levels of BMI-1 protein were detected by western blot. **Results:** 1) Western Blot showed that the expression levels of Cav-1 were higher in MGC-803/Cav-1 than control group, $P < 0.05$. 2) CCK8 showed, that the proliferation of MGC-803/Cav-1 was inhibited, but $P > 0.05$. Wound healing showed, that the migration ability of MGC-803/Cav-1 fell off, $P < 0.05$. 3) Western Blot showed that the expression levels of BMI-1 were lower in MGC-803/Cav-1 than in the control group, $P < 0.05$. **Conclusion:** Caveolin-1 can inhibit the proliferation and migration ability of gastric carcinoma cells and its mechanism may relate to BMI-1.

Keywords

Caveolin-1, Proliferation, Migration, BMI-1, GC

1. Introduction

Gastric cancer (GC) is a fast-growing malignant tumor in the digestive system, which is the third most common cause of cancer-related deaths. Despite the improvements in treatment and early detection reduced the incidence of GC, >1 million new cases were estimated worldwide in 2018, while China is one of the countries with the highest incidence of GC [1] [2]. The prognosis of the GC is poor, as a result of the middle or late clinical stage of diagnosis, presenting a serious threat to Chinese and even global health. GC is a multifactorial tumor, including sporadic and familial cases. The common risk factors consist of environmental factors (*Helicobacter pylori* infection, EBV infection, tobacco smoking, and dietary factors) and genetic factors. Among environmental factors, *Helicobacter pylori* infection is regarded as the most important cause of the GC. In recent years, molecular mechanisms have been noted associated with the GC. Many genetic alterations were detected, deepened human beings' understanding of the occurrence and development of GC. According to the WHO classification of the tumors series' fifth edition, several subtypes of GC are defined not only by microscopic characteristics but also by molecular phenotype, reflecting the important advancement in our understanding of GC. As a structural-functional protein, Caveolin-1 plays a part in membrane transport, signal transduction, and lipid homeostasis. Many kinds of malignant tumors were found to be related to the expression levels of Caveolin-1. But the exact mechanism is not fully understood. Further studies are needed to understand the role and mechanism of Caveolin-1 in malignant tumors.

2. Materials and Methods

2.1. Materials

RPMI 1640 and 10% foetal bovine serum were purchased from GIBCO Company (USA), penicillin and streptomycin were purchased from north China Pharmaceutical Co., Shijiazhuang, Hebei, China. Human GC cell lines MGC-803 was purchased from the Biotechnology Development Co. Ltd (Shanghai, China), and was maintained in a 37˚C incubator with 5% CO_2. QIAGEN extracted kits was obtained from QIAGEN Co. Ltd (Germany). Antibodies against Caveolin-1 and BMI-1 were purchased from Santa Cruz Co. Ltd (USA).

2.2. Cell Culture

Human GC cell lines MGC-803 were cultured in RPMI-1640 supplemented with 10% foetal bovine serum, 100 IU/mL penicillin, and 100 IU/mL streptomycin, maintained in an incubator at 37˚C and 5% CO_2.

2.3. Transfection

As described previously, Human GC cell lines MGC-803 were prepared. The cell line was maintained in DMEM medium, and seeded in 35-mm culture plates. The cell line was divided into the control group (without treatment), and MGC-803/Cav-1 groups (transfected with pcDNA3.1-Cav-1). The cells were in the logarithmic growth phase the next day, were transfected in experiments, and washed once with cold PBS. The pcDNA3.1-Cav-1 plasmid was extracted by QIAGEN plasmid extraction kit. The prepared cells of MGC-803/Cav-1 groups were transfected according to the specific steps referred to in kit instructions. After 6 h, the culture medium was changed to DMEM containing 10% FBS (without penicillin and streptomycin). After 48 h, 1500 lg/mL of G418 (Amersco, Inc., Solon, OH, USA) was added, and resistance screening was performed. The culture medium was changed once every 2 days. CAV-1 overexpression was verified using western blot analysis.

2.4. Western Blot

The cell line was maintained in DMEM medium, and seeded in 35-mm culture plates. After 48 h, the DMEM was removed, and the cells were washed once with cold PBS and cultured in DMEM without FBS for 4 h. RIPA lysis buffer was used for lysis cells and total protein of the cells was extracted. BCA assay was used to detect the concentrations of the total protein. Protein samples were separated by 10% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% nonfat dry milk for 2 h at room temperature, incubated with primary antibodies, rabbit anti-Caveolin-1 (1:2000), and GAPDH (1:1000), overnight at 4 C. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (1:4000) for 1.5 h at room temperature. The membranes were washed with TBST three times and then ECL (Beyotime, Haimen, Jiangsu, China) color and exposed in the darkroom. After being scanned, the stripes' width and grey value were analyzed by the Image J image analysis system (national Institutes of Health, Bethesda, MD, USA). Each experiment was repeated three times.

2.5. Cell Counting Kit-8 (CCK-8) Assay

MGC-803/Cav-1 and control group cells were seeded at a concentration of 5000 per well into a 96-well plate and routinely incubated for 36 h at 37˚C. The white well was used for zero adjustments. 10 μl CCK-8 reagent was added into every well at 0, 24, 48, 72 hours, the plates were maintained at 37˚C 2 h later, then the absorbance of cells was measured at 450 nm using a microplate reader. Each group of cells was set with 5 auxiliary holes and repeated three times. Cell proliferation rates were estimated according to the following formula:

$$\text{proliferation rate (\%)} = \text{optical density (OD) value in experimental group/OD value in normal control group} \times 100\%.$$

2.6. Wound Healing Assay

MGC-803/Cav-1 and control group cells were seeded at a density of 10,000 per well into a 24-well plate and routinely incubated for 36 h at 37˚C. A 10-μl pipette tip was used to scratch the cell monolayer once cells had reached 90% confluence. The floating cells were washed by PBS for two times. Subsequently, the cells were cultured in DMEM without FBS. Finally, the cells were imaged using an inverted microscope (Olympus, Japan) at 0, 24 and 48 h after wounding. The width of the scratch distance between the two phases was measured to estimate the migration of cells and can reflect the difference in the cell migration ability in different groups. The recovered wound area (%) at the indicated time point (24/48 h) was calculated according to the following formula:

$$[(\text{wound width at 0 h}) - (\text{wound width at 24/48 h})]/2/\text{wound width at 0 h} \times 100\%.$$

2.7. Statistical Analysis

SPSS19.0 statistical software was used for data analysis. Data are presented as the mean ± SEM. Data were compared between two groups by Student's t-test. And a value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. The Expression of Caveolin-1 Was Higher in MGC-803/Cav-1 than That in Control Group Detected via Western Blot

Expression of Caveolin-1 was detected in Human GC cell lines MGC-803, MGC-803/Cav-1 group, and the control group. Western blot results showed that the expression of Caveolin-1 was high in the MGC-803/Cav-1 group (7.31 ± 0.38) than that in control group (1.99 ± 0.06) (**Figure 1**), which is a significant difference and suggests that successful transfection ($P < 0.01$).

3.2. Caveolin-1 Inhibits the Proliferation of Human GC Cell Lines MGC-803 Detected by CCK8 Assay

To evaluate the effects of Caveolin-1 over-expression on the abilities of proliferation in MGC-803 cell lines, cell proliferation rates were estimated by CCK-8 assay. As shown in **Figure 2**, Caveolin-1 inhibited the cell viability compared

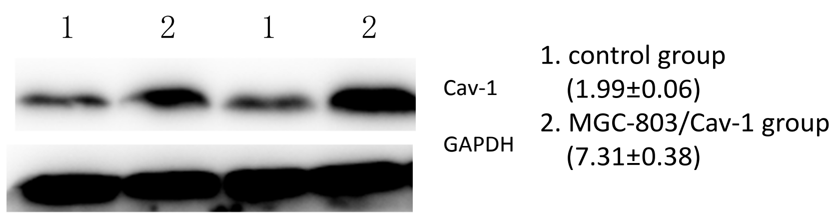

Figure 1. The differential expression of Caveolin-1 in MGC-803/Cav-1 group and control group after stably transfected with pcDNA3.1-Cav-1 plasmid detected by western-blot.

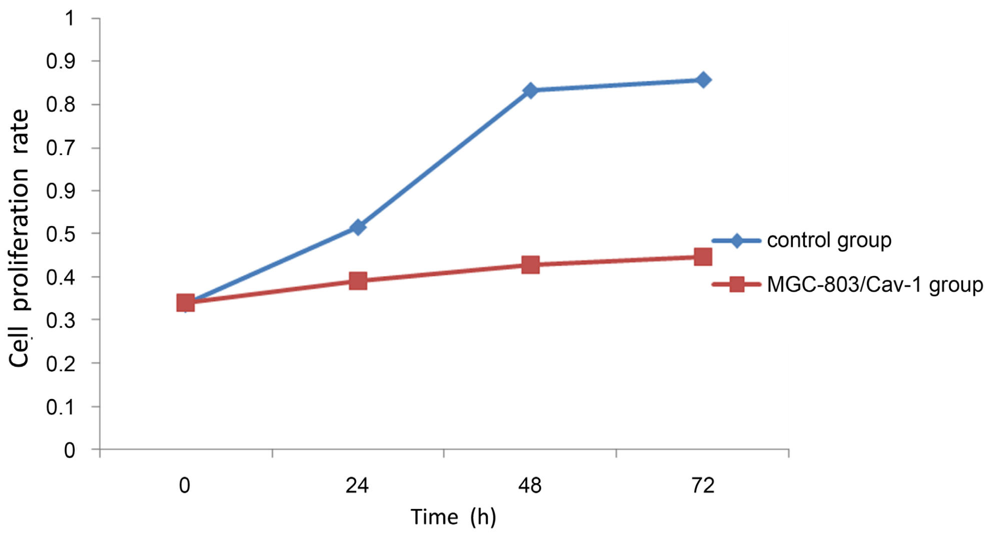

Figure 2. Caveolin-1 inhibits the proliferation of Human GC cell lines MGC-803 detected by CCK8 assay.

with the control group at 24, 48, and 72 h, particularly obvious at 72 h. There were no significant differences at any time point.

We deduced the Caveolin-1 gene may inhibit cell proliferation.

3.3. Caveolin-1 Inhibits the Invasion of Human GC Cell Lines MGC-803 Detected by Wound Healing Assay

The results of the wound healing assay also provided consistent conclusions same as with the CCK8 assay. In MGC-803/Cav-1 group, the recovered wound area at 0, 24, 48 h were (3.09 ± 0.41, 8.86 ± 1.83, 19.40 ± 3.09) separately, lower than that in the control group (4.15 ± 0.67, 13.47 ± 3.51, 35.63 ± 7.22), as showed in **Figure 3**. The difference was more obvious at 48h ($P < 0.05$). The results of the experiments indicated that the over-expression of Caveolin-1 may inhibit the invasion of Human GC cell lines MGC-803.

3.4. Caveolin-1 Inhibits the Expression of BMI-1 in Human GC Cell Lines MGC-803 Detected by Western Blot

Western blot is used to detect the expression of BMI-1 in MGC-803/Cav-1 group and control group. The expression of BMI-1 was found significantly decreased in the MGC-803/Cav-1 group (2.95 ± 0.16) compared with the control group (7.35 ± 0.15) (**Figure 4**). This shows that Caveolin-1 may decrease the expression level of BMI-1. Caveolin-1 can regulate proliferation and invasion and a series of biological behaviors of Human GC cell lines by affecting BMI-1.

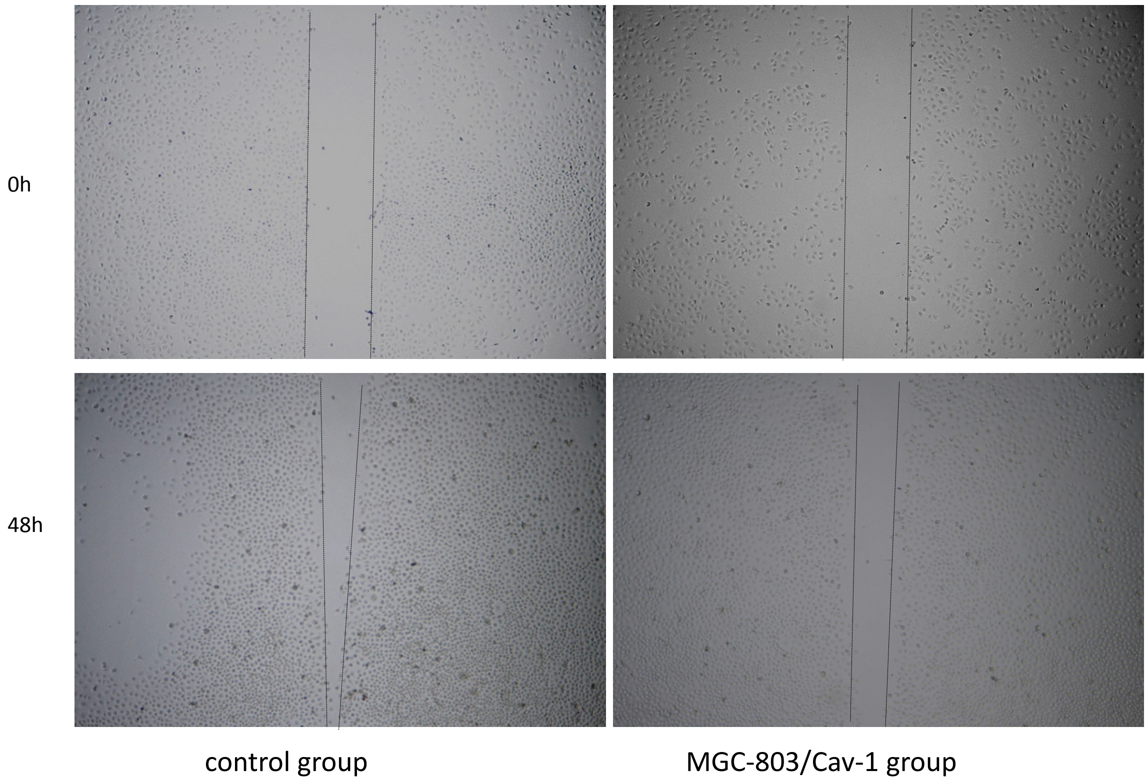

Figure 3. Caveolin-1 inhibits the invasion of Human GC cell lines MGC-803 detected by wound healing assay.

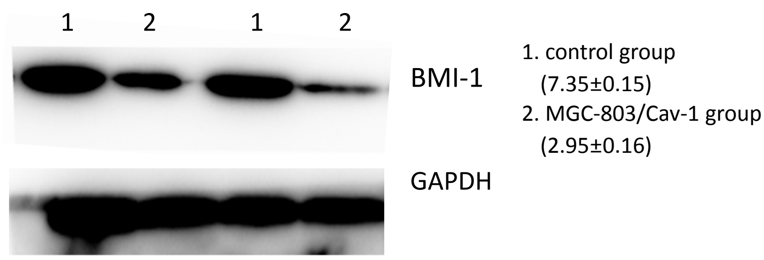

Figure 4. Caveolin-1 inhibits the expression of BMI-1 in Human GC cell lines MGC-803 detected by western blot.

4. Discussion

Gastric cancer (GC) is an often seen malignant tumor with a high incidence and mortality in China. Worldwide, GC is the third most common cause of cancer-related mortality, >1 million new cases were estimated worldwide in 2018 [2]. Because of the poor prognosis, GC presents a serious threat to global health. The main histological subtypes of GC include tubular, papillary, poorly cohesive, mucinous, and mixed adenocarcinomas. Due to the individual difference in epidemiological characteristics, clinicopathological features, and tumor biological characteristics, the treatment patterns and drug selection vary from person to person. Till now, HP infection is thought to be responsible for most GCs, in particular non-cardia GCs [3] [4]. In addition to this, genetic susceptibility and autoimmune gastritis were also involved. The molecular alterations of GCs refer to many genes, Caveolin-1 proteins, and Caveolin-1 genes were one of the hot fields in GC. The function and mechanism of Caveolin-1 are still indefinite, so deep study is needed.

As a component of membrane caveolae, identified in the 1950s [5] [6], Caveolin-1 is ubiquitous in many cells and tissues, contributing to numerous cell and tissue functions, such as endocytosis, signal transduction, cell adhesion, lipid homeostasis, membrane transport, and so on. Gradually, Caveolin-1 was found enriched in multiple signaling molecules, tyrosine kinases, and receptors, and its role in oncogenesis and progression have been the point of contention among biologists. The role of Caveolin-1 is controversial in different kinds of tumors, being a tumor suppressor gene in some tumors, for example, reduced expression was detected in lung, colon, ovarian [7] [8] [9], and several sarcomas (glioblastoma, osteosarcoma, and so on) [10]. On the opposite, in ameloblastoma and ameloblastic carcinoma, caveolin-1played a possible role in protumoral events, but may not necessarily participate in the malignant transformation process [11]. The study on prostate cancer showed that Caveolin-1 is closely related to the pathological grade and clinical stage, significantly higher in prostate cancer samples than in benign prostatic hyperplasia samples [12]. In view of such evidence, it is not surprised that Caveolin-1 played a dual role in tumors, depending on cancer type and stage. In the early stages of the tumor, Caveolin-1 is deduced to function as a tumor suppressor, while oncogene in advanced neoplasm, promoting to tumor progression and metastasis [13]. In our study before, down-regulation of

Caveolin-1 was found in gastric cancer tissue. And the present study showed that Caveolin-1 can inhibit the proliferation and migration ability of gastric carcinoma cells, but the specific mechanism is still unclear.

The mechanism of Caveolin-1 acting as a tumor suppressor is controversial. Caveolin-1 may inhibit the activity of the cyclinD1 gene promoter, and then inhibit the MAPK pathway and the phosphorylation of the Src tyrosine kinase [12]. Caveolin-1 may promote the activation of gastric cancer-related fibroblasts, resulting in gastric cancer [14]. In leukemia HL-60 cells, caveolin-1 may inhibit the proliferation and induce apoptosis via PI3K/AKT signaling pathway. The metastasis process of tumors is complicated, including the participation of different sets of genes or the cooperation of multiple molecular pathways. The BMI1 gene is a member of the PcG family, acting on the development of bone, hematopoietic, and nerve [15]. Over-expression of BMI1 was found in tumor tissue and is related to tumor invasion, and prognosis. As shown in our study, there is a strong interaction between Caveolin-1 and BMI1, demonstrating that Caveolin-1 inhibited the proliferation and invasion in GC cells line through the BMI1 gene. BMI1 may promote the transformation and formation of tumors via apoptosis, DNA damage repair, cell cycle, and EMT [16].

5. Conclusion

In conclusion, our findings demonstrated that Caveolin-1 inhibited the proliferation and invasion of GC cells line through the BMI1 gene. We hope that our findings could provide new insight into the treatment of gastric cancer. Although the relationship between Caveolin-1 and BMI-1 and the role of Caveolin-1 are needed to be further research in gastric cancer.

Funding

CHENGDE Science and Technology Research and Development Program. NO. 202109A055.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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