Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Expression of MACC1 protein in gastric cancer and its effect on proliferation and invasion of gastric cancer cells

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Received January 12, 2020; **Accepted** May 5, 2020; **Published** May 15, 2020

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Doi: http://dx.doi.org/10.14715/cmb/2020.66.2.18

Abstract: To detect the expression of metastasis-associated colon cancer gene 1 (MACC1) protein in gastric cancer tissues, and analyze its relationship with clinicopathological parameters of gastric cancer and its effect on proliferation and invasion of gastric cancer cells. Methods: 71 patients with gastric cancer in Fifth Hospital in Wuhan from June 2014 to March 2018 were selected as research subjects. Western blot was used to detect the expression of MACC1 in gastric cancer tissue and normal gastric mucosa tissue, and gastric cancer cell SGC7901 was transfected. Transfection group (transfected with MACC1-siRNA), negative control group (transfected with siRNA-NC) and blank control group (untreated cells) were set up. After transfection, the expressions of MACC1 protein and mRNA in the 3 groups were detected by Western blot and qRT-PCR methods, the cell proliferation was detected by MTT method, and the invasion ability of cells in vitro was detected by Transwell chamber. Results: The expression of MACC1 protein in gastric cancer tissue was higher than the control group (P< 0.05). The expression of MACC1 protein in gastric cancer was related to the differentiation degree, infiltration depth, lymph node metastasis and different stages of gastric cancer (P< 0.05). After transfection, the expressions of MACC1 protein and mRNA in the transfection group was significantly lower than the negative control group and blank group (P< 0.05). There was no significant difference in cell viability between the blank group and negative control group at each time point (P> 0.05). Conclusion: MACC1 was highly expressed in gastric cancer tissues. The expression of MACC1 was related to the differentiation degree, infiltration depth, lymph node metastasis and staging of gastric cancer. Down-regulation of MACC1 could inhibit the proliferation and invasion of gastric cancer cells. This study provided a certain biological basis for early clinical prediction, diagnosis and treatment of gastric cancer.

Key words: MACC1 protein; Gastric cancer; Proliferation; Invasion.

Introduction

Gastric cancer is known as one of the common malignant tumors with high morbidity and mortality. It has been controlled in morbidity and prognosis in recent years, but the development trend of gastric cancer patients is still not optimistic (1). The incidence and mortality of gastric cancer are high in our country, only after lung cancer. Most of the patients had metastasis when they went to the hospital for physical examination or were discomfort. The poor curative effects of conventional drug surgery and radiotherapy and chemotherapy lead to a low survival rate (2, 3). The high mortality rate of gastric cancer patients is mainly due to the recurrence and metastasis of tumor caused by the strong apoptosis resistance of gastric cancer cells (4), and this process is closely related to abnormal gene expression, cell proliferation, invasion and migration (5). Therefore, it is urgent to explore the influence of genes on cell proliferation, invasion and migration, to provide a new diagnosis and treatment direction for gastric cancer patients, to improve the diagnosis and treatment effect of gastric cancer, and to prolong the survival time of gastric cancer patients.

Metastasis-associated colon cancer gene 1 (MACC1) has the function of regulating signal transduction pathway, cancer invasion and metastasis. When MACC1 is highly expressed, it can activate the signal transduction pathway of hepatocyte growth factor/hepatocyte growth factor receptor tyrosine kinase c-Met (HGF/c-Met). After HGF is combined with c-Met, it activates tyrosine kinase activity, promotes mitosis, strengthens cell proliferation, differentiation and migration, and induces tumor invasion and metastasis (6-8). Many experts and scholars have shown that MACC1 is highly expressed in many malignant tumors such as liver cancer, nasopharyngeal carcinoma, breast cancer, osteosarcoma and glioma (9-13).

At the present, there are few reports on the regulatory effect of MACC1 on gastric cancer. This study explores the relationship between the expression of MACC1 in gastric cancer patients and the proliferation and invasion of gastric cancer cells, as well as the clinic pathological parameters of gastric cancer, so as to provide more effective biological basic data for clinical early prediction, diagnosis and treatment of gastric cancer.

Materials and Methods

General information

Seventy-one patients with gastric cancer who admitted to Fifth Hospital in Wuhan from June 2014 to March 2018 were selected as research subjects, including 37 males and 34 females, with an average age of (63.19±3.31) years. During the operation, 71 gastric cancer tissues and 71 normal gastric mucosa tissues were excised and collected with the consent of the patient (the distance from the tumor site of gastric cancer was more than 6.0 cm, and no cancer cells were confirmed). After excision, the tissues were immediately put into a liquid nitrogen tank for preservation. The inclusion criterion was as follow: Patients diagnosed with gastric cancer by pathological diagnosis; Exclusion criteria were as follows: Patients with other malignant tumors, severe liver and kidney dysfunction; before the operation, the patients received definite treatment such as radiotherapy, chemotherapy or immunotherapy; patients with cognitive or communication disorders; patients with poor compliance. All patients and their families agreed to participate in the experiment and signed a conscious consent. The experiment had been approved by the Medical Ethics Committee.

Experimental reagents and materials

SGC7901 gastric cancer cell was purchased from Shanghai Shuaiyue Industry Co., Ltd.; RIPA lysate and BCA kit were purchased from Beyotime Biotechnology. The centrifuge was purchased from Hunan Xiangyi Centrifuge Instrument Co., Ltd.; the 10% of SDS-PAGE gel kit, TBST, ECL luminescent agent and MTT solution were purchased from Beijing solarbio science&technology Co., Ltd.; the PVDF membrane was purchased from Shandong Chuanyi Water Treatment Technology Co., Ltd.; the MACC1 antibody was purchased from Zhen Shanghai and Shanghai Industrial Co., Ltd.; Horseradish peroxidase was purchased from Huzhou InnoReagents Co., Ltd. Goat anti-rabbit IgG, Lipofectamine 2000 transfection kit, Trizol kit, microplate reader and DMEM culture medium were purchased from Thermo Fisher Scientific (China) Co., Ltd. RPMI 1640 medium was purchased from Shanghai Bio Sun Sci & Tech Co., Ltd.; The UV spectrophotometer was purchased from Shanghai Metash Instruments Co., Ltd.; cDNA reverse transcription kit was purchased from Beijing Think-Far Technology Co., Ltd.; Dimethyl sulfoxide was purchased from Guangzhou Xinyuan Chemical Co., Ltd.; FBS was purchased from Shanghai Chuanqiu Biotechnology Co., Ltd.; PBS buffer was purchased from Lubang Technology Co., Ltd. All primers and transfection plasmids were synthesized and designed by Shanghai Weiao Biotechnology Co., Ltd.

Detection of MACC1 protein expression by western blot

The gastric cancer tissue and normal gastric mucosa tissue were taken, RIPA lysate was added on ice for the reaction for 30min, the reaction solution was centrifuged at 7500r/min for 20 min at 4 ℃, and BCA kit was used to detect the concentration of protein supernatant. After lysis, the tissue was boiled in 100 ℃ water for 5min to denature. Every 30μL of denatured lysate was added into sample wells of 10% of SDS-PAGE gel to separate the protein. Then the tissue was transferred to the PVDF membrane, sealed with 5% of skimmed milk powder at room temperature for 2 hours, added with MACC1 polyclonal antibody (1:1000 dilution) and GAPDH (1: 1000), incubated at 4 ℃ for 12 hours. The horseradish peroxidase-labeled goat anti-rabbit IgG (1:1000 dilution) was added after TBST membrane rinsing and incubated at 37 ℃ for 2 hours. After rinsing, it was developed with ECL luminescent agent, and Image J was used to calculate the grayscale or whiteness of each band, so as to semi-quantify the protein expression level.

Cell Culture, passage and transfection

The gastric cancer cell SGC7901 was inoculated into a 24-well plate, cultured in RPMI 1640 medium with 10% of FBS at 37℃ and 5% of CO_2 environment. When the cell reached 80% of fusion, 0.25% of the pancreas was added for continuous culture for 24 hours to complete the passage. Before transfection, a transfection group (transfected with MACC1-siRNA), a negative control group (transfected with siRNA-NC) and a blank control group (untreated cells) were set up. Cells were inoculated in a 6-well plate with a density of 1×10^4 cells/well. Lipofectamine 2000 and DNA were diluted and mixed according to the instructions of the Lipofectamine 2000 transfection kit, and the mixture was placed at room temperature for 5 minutes. Then, the mixed solution was mixed with the cells evenly and transfected for 48 hours at 37 ℃ and with 5% of CO_2.

Detection of expression of MACC1 protein and mRNA in transfected cells

Expression of MACC1 protein in transfected cells

The expression of MACC1 protein in each group of cells, 48 h after transfection was strictly detected.

Expression of MACC1mRNA in transfected cells

After 48 h of transfection, all groups of cells were taken and the total RNA was extracted according to the instructions of the Trizol Kit. The purity and concentration of RNA were detected by UV spectrophotometer. cDNA was reverse transcribed by reverse transcription kit. The reverse transcription reaction conditions were as follows: 75 ℃ for 45 min, 37 ℃ for 50min, 70 ℃ for 10 min. Amplification was carried out by real-time quantitative polymerase, and the amplification conditions were as follows: after 9 min at 88℃, denaturing 30s at 92℃; annealing for 50s at 68℃; extension was carried out for 2min at 72℃ with 35 cycles. GAPDH was used as an internal reference for PCR, and 35 cycles were carried out, and real-time quantitative PCR deter-

Table 1. Sequence table of related primers.

Factor	Upstream primer	Downstream primer
MACC1	5'-AACCCCAAACCTAAAAAGACTC-3'	5'-ACCCAGGACATCAGCTAAAACT-3'
GAPDH	5'-AGCCACATCGCTCAGACA-3'	5'-TGGACTCCACGACGTACT-3'

mination was carried out to finally obtain the expression level of MACC1mRNA.

The primer sequence table was shown in Table 1.

Detection of cell proliferation rate by MTT assay

Three groups of cells 0, 24, 48, and 72 h after transfection were inoculated into 96-well plates with 6 control wells respectively. The density was 1×10^4 cells /m L. MTT solution with a concentration of 5 mg/mL was added to each control well. The adherent culture was carried out in 5% of the CO_2 incubator at 37℃ for 1.5h. Dimethylsulfoxide was added to the supernatant and shaken for 10min. The absorbance (OD) value at 500 nm wavelength was measured with a microplate reader. The experiment was repeated 3 times. Cell proliferation rate = absorbance value in the experimental group/blank group ×100%.

Detection of cell invasion by transwell chamber

SGC7901 gastric cancer cells in the logarithmic phase were starved for 24 h and then inoculated into 24-well plates with about 1,000 cells per well. A 200μl of DMEM medium without FBS was added to the upper chamber to dilute the cells to a density of $2x10^4$ cells / ml. A 600 μl of DMEM medium with 20% of FBS was added to the lower chamber. The chamber was taken out and rinsed with PBS after being cultured at 37 ℃ for 24 h. The chamber was fixed with 95% ethanol solution for 10min, then taken out and rinsed again with PBS, dyed with 0.3% crystal violet for 10min, taken out and washed again with PBS. Finally, the invasion of the migration number of cells in 6 random wells was calculated by a microscope, and the average value was taken. The experiment was repeated 3 times.

Statistical methods

In this study, SPSS 19.0 software (Beijing NDTimes Technology Co., Ltd.) was used for statistical analysis of experimental data. The measured data were expressed by mean±standard deviation. The comparison between the two groups was performed using t-test, and the comparison between multiple groups was performed using one-way variance. Graphpad Prism8 is used to visualize images. A P-value of less than 0.05 was considered statistically significant.

Results

Expression of MACC1 protein in gastric cancer and normal gastric mucosa

The expression of MACC1 protein in gastric cancer tissues and normal gastric mucosa tissues were (3.62±0.21) and (1.38±0.17), respectively. the expression of MACC1 protein in gastric cancer tissues was significantly higher than normal gastric mucosa tissues (P< 0.05). See Figure 1 for details.

Effect of MACC1 protein expression on clinicopathological parameters in gastric carcinoma

The expression of MACC1 protein in gastric cancer tissues had no significant difference in sex and age (P> 0.05). There were significant differences in the degree of differentiation, depth of invasion, lymph node metastasis and different stages of gastric cancer (P< 0.05). See Table 2 for details.

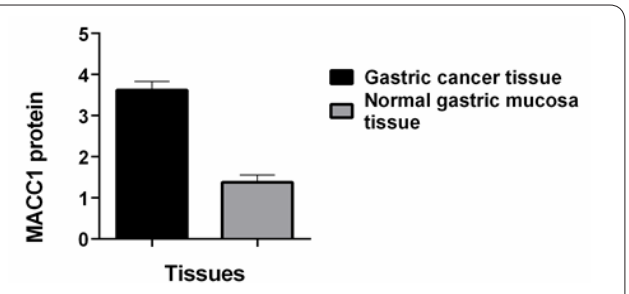

Figure 1. Expression comparison of MACC1 protein in gastric cancer tissue and normal gastric mucosa tissue. Western blot results showed that the relative expression of MACC1 protein in gastric cancer tissues was significantly higher than normal gastric mucosa tissues (P< 0.05).

Table 2. Relationship between expression of MACC1 protein and clinicopathological parameters in gastric cancer.

Group	n	MACC1 protein	t	P
Sex			0.596	0.553
Male	37	3.60±0.23		
Female	34	3.63±0.19		
Age (years)			0.748	0.457
>63	35	3.60±0.24		
<63	36	3.64±0.21		
Degree of differentiation			18.040	<0.001
High-moderate differentiation	28	2.67±0.16		
Poor differentiation	43	3.57±0.23		
Depth of invasion			10.830	<0.001
Submucous muscle layer	32	3.21±0.17		
Under the serious film, outside the serosa	39	3.74±0.23		
Lymph node metastasis			5.556	<0.001
No	25	3.42±0.19		
Yes	46	3.71±0.22		
Different stage			7.059	<0.001
I~II	30	3.29±0.20		
III~IV	41	3.67±0.24		

Relative expression of MACC1 protein in cells of each group after transfection

After transfection, the expression of MACC1 protein in a blank group, negative control group and transfection group were (3.72±0.21), (3.63±0.17), (2.73±0.12), respectively. The expression of MACC1 protein in the transfection group was significantly lower than the negative control group and blank group ($P< 0.05$). The expression of MACC1 protein in the negative control group was not significantly different from that in the blank group ($P> 0.05$). See Figure 2 for details.

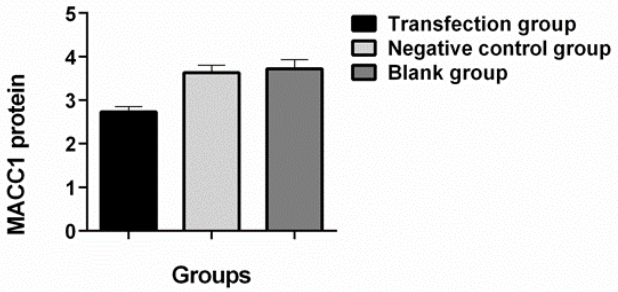

Figure 2. Relative expression comparison of MACC1 protein in each group of cells after transfection. Western blot results showed that the expression of MACC1 protein in the transfected group was significantly lower than the negative control group and blank group ($P< 0.05$), while the expression of MACC1 protein in the negative control group was not significantly different from the blank group ($P> 0.05$).

Relative expression of MACC1mRNA in transfected cells

After transfection, the expression of MACC1mRNA in blank group, negative control group and transfection group were (4.55±0.27), (4.43±0.22), (3.15±0.19), respectively. The expression of MACC1mRNA in the transfection group was significantly lower than the negative control group and blank group ($P< 0.05$). The expression of MACC1mRNA in the negative control group was not significantly different from that in the blank group ($P> 0.05$). See Figure 3 for details.

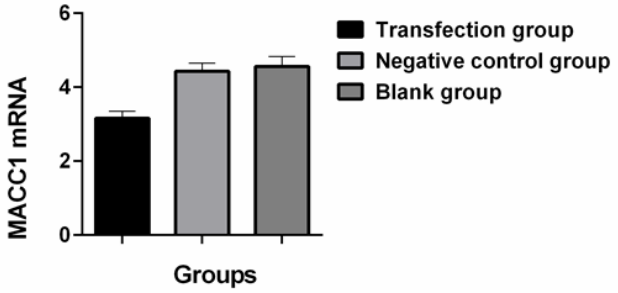

Figure 3. Relative expression of MACC1mRNA in transfected cells. Qrt-PCR results showed that the expression of MACC1mRNA in the transfected group was significantly lower than the negative control group and blank group ($P< 0.05$). The expression of MACC1mRNA in the negative control group was not significantly different from the blank group ($P> 0.05$).

Comparison of proliferation of SGC7901 gastric cancer cells in each group

There was no significant difference in the cell proliferation of the three groups at the 0th and 24th hour ($P> 0.05$). At the 48th and 72nd hour, the cell proliferation of the transfected group was significantly lower than the blank group and negative control group ($P< 0.05$). There was no significant difference in cell viability between the blank group and negative control group at each time point ($P> 0.05$). See Table 3 and Figure 4 for details.

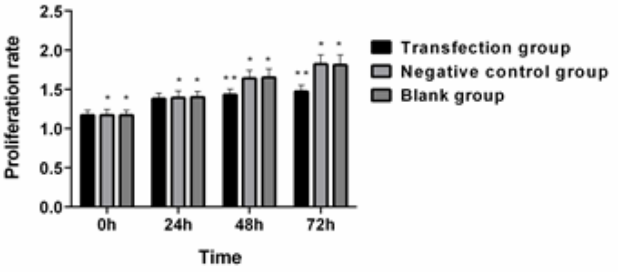

Figure 4. Comparison of the proliferation of SGC7901 gastric cancer cells in each group. MTT results showed that the cell viability of the three groups of cells had no significant difference at 0th and 24th hour ($P> 0.05$), but at 48th and 72nd hour, the cell viability of the transfected group was significantly lower than the blank group and negative control group ($P< 0.05$). There was no significant difference in cell viability between the blank group and negative control group at each time point ($P> 0.05$). Note: Comparison of * at the same period indicates $P> 0.05$; the comparison of * and ** at the same period indicates $P< 0.05$.

Comparison of invasive ability of SGC7901 gastric cancer cells in each group

After 24 hours of transfection, the number of cell penetrations in the transfection group, negative control group and the blank group were (111.45±11.64), (168.59±31.44), (166.71±32.49), respectively. The number of cell penetrations in the transfection group was significantly lower than the negative control group and blank group ($P< 0.05$). There was no significant difference in the number of cell penetrations between the blank group and negative control group ($P> 0.05$). See Figure 5 for details.

Discussion

Due to the popularization and diagnosis of endoscopic examination, the prognosis of patients with early gastric cancer is obviously improved. However, gastric cancer shows only concealed clinical symptoms such as

Table 3. Comparison of Cell Proliferation Rates in Each Group (%).

Time	Transfected group	Negative control group	Blank group	F	P
0h	1.17±0.06	1.17±0.07*	1.17±0.06*	1.000	0.370
24h	1.38±0.07	1.39±0.09*	1.40±0.07*	1.190	0.306
48h	1.43±0.07**	1.64±0.10*	1.65±0.11*	121.800	<0.001
72h	1.47±0.08**	1.82±0.12*	1.81±0.13*	224.300	<0.001

Note: Comparison of * at the same period indicates $P> 0.05$; the comparison of * and ** at the same period indicates $P< 0.05$.

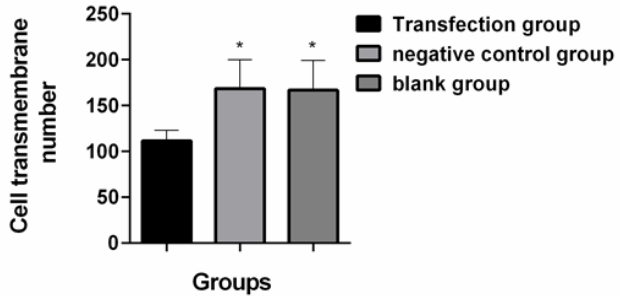

Figure 5. Comparison of invasive ability of SGC7901 gastric cancer cells in each group. Transwell experiment results showed that the number of cells penetrating membrane in the transfected group was lower than the negative control group and blank group (P< 0.05). There was no significant difference between the blank group and negative control group (P> 0.05). Note: Comparison of * indicates P> 0.05.

abdominal pain and loss of appetite in the early stage, resulting in the poor overall effect of early diagnosis. The current clinical treatment level is not perfect, and there is still no significant improvement in indicators such as mortality and survival time (14-16). The pathogenesis of gastric cancer is a pathophysiological process involving multiple gene changes and multiple steps (17). Previous studies conducted by many researchers have found that abnormal expression in gastric cancer-related genes is an important factor affecting the malignant process of gastric cancer during the onset and development of gastric cancer. Many abnormal expression genes are related to molecular diagnosis or prognosis of gastric cancer (18-21). Therefore, this article discussed the expression of MACC1 in gastric cancer and its effect on the proliferation and invasion of gastric cancer cells, thus providing more possibilities for improving the prognosis of gastric cancer and survival rate of patients.

Our study found that the expression of MACC1 protein in gastric cancer tissues was significantly higher than normal gastric mucosa tissues (P< 0.05), suggesting that MACC1 protein is usually highly expressed in gastric cancer cells. Previous studies have also shown that (22, 23) MACC1 protein is highly expressed in tumor cells of colon cancer and gastric cancer. These conclusions are consistent with the results of the current study. Then we deeply studied the relationship between MACC1 protein and clinical-pathological data, which showed that MACC1 protein was related to the invasion depth, lymph node metastasis, differentiation degree and different stages of gastric cancer (P< 0.05). Some studies have shown that pathological features such as tumor tissue invasion, staging and lymph node metastasis are related to MACC1 expression (24-26). The conclusion that high expression of MACC1 can have different effects on common clinicopathological features and play a role in predicting the development of gastric cancer is consistent with reports of other scholars. In order to explore the expression of MACC1 in gastric cancer and its effect on proliferation and invasion of gastric cancer cells, we detected the expression of MACC1 protein and mRNA in the three groups after transfection. It was found that the expression of MACC1 protein and mRNA in the transfected group was significantly lower than the negative control group and blank group (P< 0.05). There was no significant difference in cell viability between the three groups at the 0th and 24th hour (P> 0.05). The cell viability of the transfected group was significantly lower than the blank group and negative control group at 48th and 72th hour (P< 0.05).

After transfection, the number of cell penetrations in the transfection group was significantly lower than the negative control group and blank group (P< 0.05), and there was no significant difference between the number of cell penetrations in blank group and negative control group (P> 0.05). Studies have shown that interference with the expression of MACC1 in tumor cells of nasopharyngeal carcinoma patients can inhibit the expression of Protein-Beta (PB) and phosphorylated protein kinase (PPK) and play a role in inhibiting the proliferation and invasion of tumor cells (27). The study (28) concluded that lowering the expression of MACC1 has an inhibitory effect on the proliferation and invasion of human bladder urothelial cancer cells. These studies have confirmed that the expression of MACC1 can affect the biological ability of tumor cells such as proliferation and invasion. As one of the common tumors, gastric cancer laterally reflects the expression of MACC1, which can be used for the development and change of gastric cancer such as proliferation and invasion (29-34).

Based on previous studies and the results of this study, we predict that MACC1 may be a new marker gene for gastric cancer in terms of the proliferation and invasion of gastric cancer cells and participate in the malignant transformation. There are still some limitations in this article, for example, the number of gastric cancer patients is not enough, and how MACC1 promotes malignant biological behaviors such as proliferation and invasion of gastric cancer is not clear, which is also the topic of our next research. However, our research results have initially shown that MACC1 plays an important role in the progression of gastric cancer, providing a new potential molecular marker and theoretical basis for the diagnosis and prognosis evaluation of gastric cancer. There is other research in this area (35-41).

Acknowledgements
Not applicable.

Funding
No funding was received.

Availability of data and materials
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions
GZ, CY and XZ conceived and designed the study.GZ, CY and ZQ were responsible for the collection, analysis and interpretation of the data. GZ drafted the manuscript. XZ revised the manuscript critically for important intellectual content. GZ wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Fifth Hospital in Wuhan. Signed written informed

consent was obtained from the patients and/or guardians.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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