MACC1 INVOLVED IN THE REGULATION OF RESISTANCE OF HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2–POSITIVE GASTRIC CANCER CELLS TO TRASTUZUMAB

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ABSTRACT

Objective: *We sought to analyze whether the mechanism of the MACC1 gene involved in human epidermal growth factor receptor 2 (HER2)-positive gastric cancer cells regulates trastuzumab-resistant gastric cancer.*

Methods: *Cell lines sensitive to trastuzumab and positive for MACC1 and HER2 expression were screened from human gastric cancer cell lines, drug-resistant cell MKN45/TR was established by a stepwise dose-increase method, and the expression level of MACC1 protein in trastuzumab-resistant cells was detected using Western blot. The inhibition rate of cell proliferation after trastuzumab treatment for 72 hours was detected using MTT.*

Results: *Drug-resistant cell MKN45/TR was established using a stepwise dose-increase method. Half-maximal inhibitory concentration and resistance index of cell trastuzumab were detected during the induction period. The induction time was five months and the final induction concentration was 2,500 μg/mL. Additionally, the half-maximal inhibitory concentration of trastuzumab was 295.15 μg/mL and the drug resistance index was 9.67. Western blot detection results showed that, as compared with cells without drug resistance, the MACCI protein level of trastuzumab-resistant cells obviously increased. NCI-N87/TR and MKN45/TR were given transinfection using MACC1 interference and empty vector and were treated using different concentrations of trastuzumab. The cell inhibition rate was detected using the MTT method. Under the function of trastuzumab at concentrations of 20 μg/mL, 80 μg/mL, and 160 μg/mL, there was statistical significance between the interference group and the control group with respect to the inhibition rate of cell proliferation ($P<0.05$).*

Conclusion: *MACC1 participates in the regulation of trastuzumab in gastric cancer cells. The expression level of MACC1 protein cells in transinfection resistance increases, thus interfering MACC1 in resistance cells and reversing the resistance of MACC1.*

Keywords: *MACC1 gene, human HER2-positive gastric cells, trastuzumab, resistance.*

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Introduction

Gastric cancer is one of the common malignant tumors of the digestive system, with an incidence rate placing it in fourth place among all malignant tumors and a death rate placing it in first place among various malignant tumors[1]. Surgery is the optimal choice for the treatment of gastric cancer. However, due to the absence of typical symptoms in the early stage of gastric cancer, most patients reach the advanced stage or where the disease has already invaded the peritoneum and surrounded large blood vessels, thus losing the opportunity for surgery. However, nearly 50% of tumor patients are unable to undergo radical resection, and there is still a risk of recurrence ranging from 40% to 60% after surgery[2]. Chemotherapy incorporated as the main treatment in the perioperative period can prolong the survival of patients with progressive gastric cancer. However, due to factors such as degeneration in some elderly patients, the toxic side effects caused by chemotherapy may not be tolerated, as they may further reduce the immune function of patients and limit the therapeutic effect. Recent studies in molecular biology have suggested that the occurrence of gastric cancer is associated with the abnormality of various oncogenes and tumor suppressor genes, and molecular-targeted therapy has gradually become an effective method in anticancer therapy efforts[3]. Human epidermal growth factor receptor 2 (HER2), a member of the epidermal growth factor receptor family, belongs to the proto-oncogene and

binds to specific receptors to regulate the proliferation and differentiation of tumor cells. Importantly, high expression of HER2 is present in gastric cancer and is associated with its prognosis. Trastuzumab, a humanized monoclonal antibody that targets HER2 recombinant DNA, can inhibit cell growth by antagonizing the HER2 signal transduction pathway and is applied in targeted treatment for breast cancer in clinic, with better effects achieved with earlier usage[4]. The positive effects of trastuzumab for the treatment of patients with HER2-positive gastric cancer are less than 60%. Most patients develop different drug resistance within one year after use. Therefore, more effective indicators are needed in clinic to predict the sensitivity of trastuzumab. Metastasis-associated in colon cancer-1 (MACC1) gene is a gene sequence that is continuously expressed in colon cancer. It has been previously confirmed that it has high expression in gastric cancer tissues, but no reports regarding MACC1 affecting gastric cancer resistance seem to available[6]. This study further explores the mechanism of the MACC1 gene involved in the regulation of trastuzumab resistance to gastric cancer, using HER2-positive gastric cancer cells as study subjects.

Materials and methods

Trastuzumab injections were provided by Shanghai Roche Biotech Co., Ltd.; the human gastric cancer cell lines MKN45 and NCI-N87 were provided by the American Type Culture Collection (Manassas, VA, USA); MACC1 primary antibody was provided by China Abnova Biotech Co., Ltd.; HER2 primary antibody was provided by Abcam (Cambridge, UK); fetal bovine serum (FBS) was provided by Invitrogen (Carlsbad, CA, USA); an automatic microplate reader (Bio-Tek ELX800) and electrophoresis equipment were provided by American Baote Instrument Co., Ltd., a gel imaging analysis system was provided by Shanghai Peiqing Technology Co., Ltd.; an electric thermostatic water tank was provided by Shanghai Yiheng Technology Co., Ltd.; and refrigerated centrifuge oil was provided by Zhuhai Haima Co., Ltd. Based on the screening results, the trastuzumab-resistant cell lines NCI-N87/TR and MKN45/TR were induced and constructed using a stepwise dose-increase method.

Experimental methods

First, the cultivation of the resistance cell lines was performed, as follows: 2,500 μgL trastuzumab, 10% FBS, and 1% penicillin were added into BPMI1640 culture media and cultivated with 5% CO2 at 37°C. Subculturing was performed until the cell density reached 60% to 80%. The half-maximal inhibitory concentration (IC50) values of resistance cells were detected regularly. The final concentrations of trastuzumab were 3,500 μg/mL for NCI-N87 and 256 μg/mL for MKN45, and the cells grew stably in the medium.

Second, the detection of drug resistance index was performed, as follows: cell survival rate = (OD value of drug-containing group and blank group) / (OD value of normal group – blank group) × 100% and cell proliferation inhibition rate = 100% - cell survival rate. The IC50 values of the mother cells and drug-resistant cells were calculated, and the drug resistance index (RI) = IC50 (mother cells)/ IC50 (resistant cells).

Third, small interfering RNA exerted a transient interference effect on the expressions of NCI-N87/TR and MKN45/TR. Drug-resistant cells (MKN45/TR) were established using a stepwise dose-increase method of trastuzumab resistance. The expression level of MACC1 protein in trastuzumab-resistant cells was detected using Western blot.

Fourth, RPMI1640 cells containing no 10% FN were added into the NCI-N87 and MKN45 cell lines for amplification and passage. NCI-N87 and MKN45 cell lines were introduced into liposome fectamine 2000 transfection reagent, respectively, and treated with trastuzumab of different concentrations. The inhibition rate of cell proliferation after 72 hours treated by trastuzumab was detected using MTT.

Statistical methods

The SPSS 12.0 software (IBM Corp., Armonk, NY, USA) was used to perform statistical analysis for all data in this study. Enumeration data were represented as percentages and compared with outcomes of the chi-squared test. Measurement data were presented as "$\bar{x} \pm s$" and compared using a t-test. Results were considered as statistically significant when $P<0.05$.

Results

Expression level of human gastric cancer line MACC1 and HER2 proteins

The expression levels of human gastric cancer line MACC1 and HER2 proteins were positive, as is seen in Figure 1.

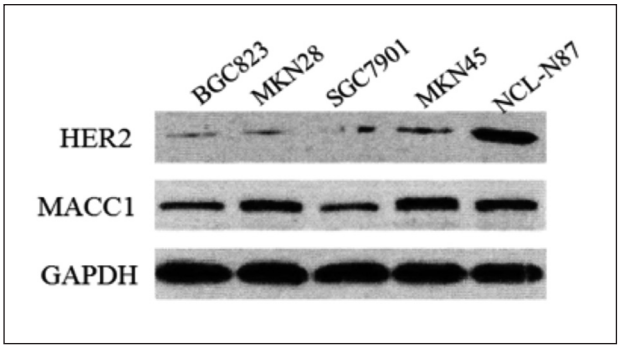

Figure 1: The expression level of human gastric cancer line MACC1 and HER2 proteins.

The expression level of MACC1 protein in trastuzumab-resistant cells

Drug-resistant cell MKN45/TR was established by a stepwise dose-increase method, and the IC50 and resistance index values of cell trastuzumab were detected during the induction period. The induction time was five months long and the final induction concentration was 2,500 μg/mL. The IC50 value of trastuzumab was 295.15 μg/mL, while the resistance index was 9.67 (Table 1).

The final induction concentration (μg/mL)	IC50 (μg/mL)	Resistance index
0	30.2	
10	32.16	1.08
40	44.36	1.49
160	50.78	1.57
640	67.67	2.16
1,600	197.02	6.43
2,500	295.15	9.67

Table. 1: The expression level of MACC1 protein in trastuzumab-resistant cells.

The expression level of MACC1 protein in trastuzumab-resistant cells

The results of Western blot detection revealed that, as compared with cells without drug resistance, the MACCI protein levels of trastuzumab-resistant cells obviously increased ($P < 0.05$; Figure 2).

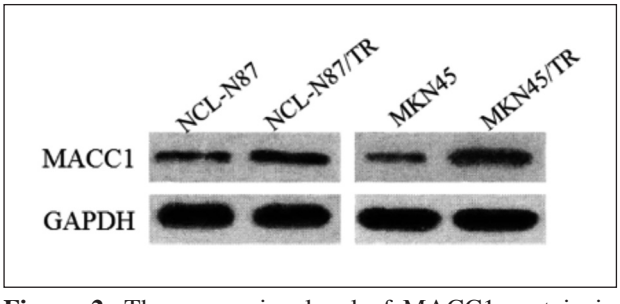

Figure 2: The expression level of MACC1 protein in trastuzumab-resistant cells.

The influences of interference resistance cell MACC1 expression on the sensitivity of trastuzumab

NCI-N87/TR and MKN45/TR were given transinfection using MACC1 interference and empty vector and were treated using different concentrations of trastuzumab. Cell inhibition rate was detected using MTT method. Under the function of trastuzumab of concentrations of 20, 80, and 160 μg/mL, there was statistical significance between the interference group and the control group noted with respect to the inhibition rate of cell proliferation ($P<0.05$; Table 2).

Trastuzumab (μg/mL)	Grouping	Cell activity	*T*	*P*
20	NCI-N87/TR MACC1 interference group	12.08±0.50	73.081	< 0.001
	NCI-N87/TR control interference group	81.20±1.56		
	MKN45/TR MACC1 interference group	58.14±1.11	11.761	< 0.001
	MKN45/TR control interference group	69.14±1.18		
80	NCI-N87/TR MACC1 interference group	24.28±1.13	18.805	< 0.001
	NCI-N87/TR control interference group	61.74±3.26		
	MKN45/TR MACC1 interference group	35.29±2.88	11.019	< 0.001
	MKN45/TR control interference group	58.23±2.17		
160	NCI-N87/TR MACC1 interference group	20.11±1.10	13.752	< 0.001
	NCI-N87/TR control interference group	50.15±3.62		
	MKN45/TR MACC1 interference group	20.26±3.12	15.983	< 0.001
	MKN45/TR control interference group	53.18±1.73		

Table. 2: Changes in activity ratio of trastuzumab of different concentrations in various human gastric cancer cell lines.

Discussion

In China, the number of newly diagnosed patients with gastric cancer is 300,000 per year, accounting for 23% of the total number of malignant tumor deaths. Although the mortality rate has decreased in recent years, the annual incidence rate remains high[7]. At present, the treatment for advanced gastric cancer is mainly surgery and chemotherapy. The five-year survival rate after radical gastrectomy for early gastric cancer can reach 90%, but about 80% of patients progress to the middle and advanced stages, and the cancer cells are distantly transferred and surround the surrounding large blood vessels, thus making surgical treatment difficult to implement. In recent years, with the advancement of medical molecular biology techniques and theories, targeted therapies for molecular events in the pathogenesis of gastric cancer have received increasing attention[8-9]. Furthermore, because traditional cytotoxic drugs face bottlenecks in improving patient prognosis and prolonging sur-

vival, currently, cancer molecular targeted drugs have brought new hope to patients with gastric cancer as a result of the limitations of traditional cytotoxic drugs in the improvement of patient prognosis and the prolongation of survival.

HER2 boasts tyrosine protein kinase activity, which plays an important role in the activation of whole family receptors and regulates cell division, differentiation, and proliferation. Its overexpression usually indicates that the cancer is highly malignant and resistant to conventional chemotherapy drugs. Relevant data show that[10] HER2 overexpression is related with the expansion of the ERBB2 gene: its mechanism of action is mainly to inhibit cell apoptosis, enhance cancer cell invasion, and improve angiogenesis and lymphangiogenesis. Trastuzumab can specifically act on HER2, mainly inhibiting the biological function of HER2 in promoting tumor growth and development by antagonizing the HER2 signaling pathway, and is nontoxic or low-toxic to normal tissues, and so has been widely applied in breast cancer patients with HER2 overexpression. Previous studies have considered trastuzumab for the treatment of HER2-positive breast cancer and have achieved significant results in improving the survival rate of breast cancer patients[11-12]. Some scholars have used trastuzumab as adjuvant therapy in patients with gastric cancer. The existing results show that only 12% of patients with HER2-positive gastric cancer experience an immune response, which indicates that these patients with gastric cancer have high levels of resistance to trastuzumab. Drug resistance is also gradually occurring in patients who are sensitive to trastuzumab. MACC1 is a gene closely related with colon cancer metastasis and invasion, which is mainly located on chromosome 7p21.1 with a structure composed by seven exons and six introns[13]. The MACC1 gene is a key regulator of the HGF/MET signaling pathway. When the hepatocyte growth factor receptor HGF specifically binds to its receptor, it can bind to the MET promoter to activate the HGF/MET signaling pathway. Under the physiological conditions of repair, it plays an important role in embryonic development and tissue damage repair. Meanwhile, the HGF/MET signaling pathway can also transmit intracellular signals through the MAPK and p13K-Akt pathways, which are closely related with the occurrence of malignant tumors, especially the invasion and metastasis of malignant tumors[14]. MACC1 has the highest expression level among various cancer tissues, especially colon cancer tissues. Some scholars have found that MACC1 is highly expressed in colon cancer tissues by analyzing the expression of MACC1 in colon cancer tissues and healthy human colon tissues, which can represent the prognostic index for the metastasis of colon cancer. Separately, some foreign scholars have detected the expression level of MACC1 in gastric cancer patients. It was found that the expression of MACC1 in gastric cancer tissues is significantly higher than that in adjacent tissues, and the expression frequency is especially higher in peritoneal diffuse gastric cancer and positively correlated with the malignant degree of gastric cancer, which can be used as a predictor of prognosis in patients with gastric cancer[15].

This study adopts NCI-N87 and MKN45 as positive HER2 gastric cancer cells, and the trastuzumab cell lines NCI-N87/TR and MKN45/TR were successfully constructed. Drug-resistant cell MKN45/TR was established using a stepwise dose-increase method. IC50 and resistance index values of cell trastuzumab were detected during induction period. The induction time was five months and the final induction concentration was 2,500 μg/mL, while the IC50 value of trastuzumab was 295.15 μg/mL and the drug resistance index was 9.67. Western blot detection results revealed that, as compared with cells without drug resistance, the MACCI protein level of trastuzumab-resistant cells obviously increased. In this study, NCI-N87/TR and MKN45/TR were further given transinfection using MACC1 interference and empty vector and were treated using different concentrations of trastuzumab. Cell inhibition rate was detected using the MTT method. Under the function of trastuzumab of concentrations of 20, 80, and 160 μg/mL, there was statistical significance of various human gastric cell lines between the interference group and the control group with respect to the inhibition rate of cell proliferation ($P<0.05$). Therefore, we inferred that MACC1 may participate in the formation of HER2-positive gastric cancer cells on trastuzumab.

In conclusion, MACC1 participates in the regulation of trastuzumab in gastric cancer cells. The expression level of MACC1 protein cells in transinfection resistance increases, thus interfering MACC1 in resistance cells and reversing the resistance of MACC1.

References

1) Li Z, Wang Y, Ji J. [Prognostic and influential factors of gastric stump cancer]. Zhonghua Wei Chang Wai Ke Za Zhi 2018; 21: 491-497.
2) Kobayashi J, Uchida H, Ito J. Long-term proton pump inhibitor use after Helicobacter pylori eradication may create a gastric environment for N-nitrosamine formation and gastric cancer development. Gut 2019; 68: 1131.

3) Szász AM, Lánczky A, Nagy Á, Förster S, Hark K, et al. Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients. Oncotarget 2016; 7: 49322-49333.
4) Brandão M, Pondé NF, Poggio F, Kotecki N, Salis M, et al. Combination therapies for the treatment of HER2-positive breast cancer: current and future prospects. Expert Rev Anticancer Ther 2018; 18: 629-649
5) Yu H, Xia H, Tang Q, Xu H, Wei G, et al. Acetylcholine acts through M3 muscarinic receptor to activate the EGFR signaling and promotes gastric cancer cell proliferation. Sci Rep 2017; 7: 40802.
6) Koh YW, Hur H, Lee D. Increased MACC1 expression indicates a poor prognosis independent of MET expression in gastric adenocarcinoma. Pathol Res Pract 2016; 212: 93-100.
7) Lorenzen S, Riera Knorrenschild J, Haag GM, Pohl M, Thuss-Patience P, et al. Lapatinib versus lapatinib plus capecitabine as second-line treatment in human epidermal growth factor receptor 2-amplified metastatic gastro-oesophageal cancer: a randomised phase II trial of the Arbeitsgemeinschaft Internistische Onkologie. Eur J Cancer 2015; 51: 569-576.
8) Inokuchi M, Nakagawa M, Tanioka T, Okuno K, Gokita K, et al. Long- and short-term outcomes of laparoscopic gastrectomy versus open gastrectomy in patients with clinically and pathological locally advanced gastric cancer: a propensity-score matching analysis. Surg Endosc 2018; 32: 735-742.
9) Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. Lancet Oncol 2016; 17: 717-726.
10) Le QA, Bae YH, Kang JH. Cost-effectiveness analysis of trastuzumab emtansine (T-DM1) in human epidermal growth factor receptor 2 (HER2): positive advanced breast cancer. Breast Cancer Res Treat 2016; 159: 565-573.
11) Chen G, Gupta R, Petrik S, Laiko M, Leatherman JM, et al. A feasibility study of cyclophosphamide, trastuzumab, and an allogeneic GM-CSF-secreting breast tumor vaccine for HER2+ metastatic breast cancer. Cancer Immunol Res 2014; 2: 949-961.
12) Gambardella V, Fleitas T, Tarazona N, Cejalvo JM, Gimeno-Valiente F, et al. Towards precision oncology for anti-HER2 blockade in gastroesophageal adenocarcinoma. Ann Oncol 2019; 2: 143.
13) Mohammadi M, Goudarzi PK, Rahmani O, Kaghazian P, Yahaghi E, et al. Evaluation of gene expression level of CDC5L and MACC1 in poor prognosis and progression of osteosarcoma. Tumor Biol 2016; 37: 8153-8157.
14) Ma L, Zhou Y, Luo X, Gao H, Deng X, et al. Long non-coding RNA XIST promotes cell growth and invasion through regulating miR-497/MACC1 axis in gastric cancer. Oncotarget 2017; 8: 4125.
15) Lang F, Pelzl L, Hauser S, Hermann A, Stournaras C, et al. To die or not to die SGK1-sensitive ORAI/STIM in cell survival. Cell Calcium 2018; 74: 29-34.

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