Int J Clin Exp Med 2016;9(7):12553-12562
www.ijcem.com /ISSN:1940-5901/IJCEM0022518

Original Article

Target research on tumor biology characteristics of mir-155-5p regulation on gastric cancer cell

Ning Ma[1], Jun-An Feng[2]

[1]*Department of Oncology, Henan Province People's Hospital, Zhengzhou City, P. R. China;* [2]*Department of Gastroenterology, People's Hospital of Zhengzhou, Zhengzhou 450000, Henan Province, P. R. China*

Received December 22, 2015; Accepted April 3, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Objective: After the mir-155-5p over expressed in gastric cancer cells, the expression profile chip was adopted to screen its target genes. Some of the intersection of target genes were selected based on the bioinformatics prediction, in order to study the mechanism of its function and role of research. Method: Affymetrix eukaryotic gene expression spectrum was conducted to screen mir-155-5p regulated genetic experiment. Western blot technique was employed to detect and screen the protein expression of target genes. Result: Mimics was transfected in BGC-823 of gastric cancer cells. Compared with mimics-nc group and mock group, the mRNA expression quantity of SMAD1, STAT1, CAB39, CXCR4 and CA9 was significantly lower. After the gastric cancer cells BGC-823 and MKN-45 been transfected by mimics, compared with mimics-nc (MNC) group and mock (MOCK) group, it was decreased for the protein expression of SMAD1, STAT1 and CAB39 in mimics (MIMICS) group. Conclusion: The verification of qRT-PCR demonstrated that SMAD1, STAT1, CAB39, CXCR4 and CA9 was the predicted target genes of mir-155-5p, the over expression of mir-155-5p could enable its expression level in gastric cancer cells MKN-45 and BGC-823 decrease. Western Blot verification proved that, the SMAD1, STAT1 and CAB39 were the predicted target protein of mir-155-5p, the over expression of mir-155-5p made its expression level in gastric cancer cells MKN-45 and BGC-823 decrease.

Keywords: mir-155-5p, expression profile chip, target gene, gastric cancer cells, protein expression

Introduction

To detect the action target of miRNA was the hinge content of miRNA action mechanism research. At present, there were a variety of methods used in the miRNA targets of research. Firstly, expression profile chip was applied to screen the target gene of miRNA. The function of miRNA on target gene was processed on the transcription and regulation level. If miRNA was completely complementary with the seed of 3'UTR zone of target gene, it would cause the degradation of mRNA, and lead to the inhibition of translation level [1]. Hence, this method was feasible, and was cited in the literature, which was combined the bioinformatics prediction research of miRNA targets. Secondly, based on the bioinformatics method to predict targets, the directly application of Western Blotting method was adopted to study target validation. This method was currently the most commonly used method as well. However, the shortcoming was unable to confirm whether the protein level changes caused by miRNA regulation was direct function or indirect function. Thirdly, miRNA expression clone and plasmid clone and double transfection of target gene upstream 3'UTR area were studied for target verification through luciferase report gene and Western Blotting detection. This method had become the classic method of the effect target study for miRNA. Fourthly, some scholars adopted quantitative proteomics (also named protein chip, or protein spectrum method) to screen the differential proteins of miRNA over expression or low expression, and conduct the target verification study combined with bioinformatics prediction and Western Blotting method [2, 3]. Fifthly, 3'RACE method was employed to expand the upstream 3'UTR region of miRNA target gene and verify the garget genes of miRNA regulation [4]. Sixthly, some other scholars adopted the

method of combined avidin and streptomycin, and employed magnetic activated flow cytometry sorting method to make miRNA combine target gene after miRNA over expressed, and then study the target of miRNA through the method of RNA extraction, elution, reverse transcription and PCR amplification target gene sequencing verification [5]. Seventhly, RISC co-immunoprecipitation: Some scholars utilized RISE co-immunoprecipitation technology to enable argonaute of RNA-induced silencing complex (RISC) to combine miRNAs and mRNAs, and get miRNA-mRNA through separation. RISC RIP-Chip technology was to co-immunoprecipitate the RISC of protein complex combined with miRNA, and identified the gene after RNA isolation [6-8].

This paper chose the first method. Mir-155-5p mimics was transfected into gastric cancer cells, and screened its target gene through expression profile chip, then combined with the bioinformatics prediction to select target gene so as to target research of gastric cancer biological behavior regulated by mir-155-5p.

Material and method

Material

Gastric cancer cells SGC-7901, MKN-45 and BGC-823 were purchased from Shanghai Cell Bank of Chinese Academy of Sciences and Cell Bank of Peking Union Medical College Tumor Research Institute.

Method

Experiment of screening mir-155-5p regulation gene through Affymetrix eukaryotic gene expression spectrum: Total RNA was extracted, reverse transcribed and synthesized first strand cDNA. Total RNA was set as the start. T7 Oligo(dT) Primer containing T7 promoter sequence was set as the primer. First strand enzyme was utilized to synthesize first strand cDNA. Second strand enzyme mix was adopted to enable RNA strand in DNA-RNA heterozygote converse into second strand cDNA and synthesize double-stranded DNA. In the process of cRNA transcription synthesis in vitro, the Second Strand cDNA was set as the template. T7 enzyme mix was utilized to synthesize cRNA and mix biotin biotin. During the cRNA purification, magnetic beads was adopted to purify cRNA, remove impurities such as salt and enzyme, and quantitate cRNA. In the cRNA fragmentation, the cRNA segment was fragmentated to fit the size of hybridization. The below methods were conducted, such as chip hybridization, washing, dyeing and scanning.

qRT-PCR verification experiment of screening target gene: Cells in logarithmic growth phase were collected and performed bellow experiment. Firstly, total RNA was extracted according to the TRIzol reagent, 1% agarose denatured gel electrophoresis was used to detect the RNA integrity. The UV-Vis spectrophotometer was adopted to detect the purity and concentration of RNA. Secondly, cDNA was reverse transcribed according to reverse transcription kid. The reaction condition were as follows: 37°C and 15 minutes for reverse transcription reaction, 85°C and 5 seconds for inactivation of enzyme reaction. Thirdly, qRT-PCR reaction parameters settings were below, 95°C for 15 seconds, 60°C for 20 seconds, 70°C for 30 seconds, 95°C for 15 seconds, and 60°C for 30 seconds. Totally, it required 40 times, each sample needed 3 parallel pipes. Each experiment should repeat 3 times at least.

The analysis of the experiment results were below, dissolution curve was analyzed after the PCR reaction, in order to detect whether the amplification products have nonspecific amplification, analyze the amplification curve, calculate Ct value and relative quantity of each mRNA based on quantitative methods and machine with package.

Protein expression of target gene through Western Blot technical inspection and screening: Cell supernatant was collected, and extracted total protein, which were conducted concentration detection and protein denaturation. During the protein denaturation, 1 μl protein buffer (5X) was added into each protein sample. Based on the proportion, it required to mix the protein sample and protein buffer, then bathed in boiling water for 5 minutes. During the SDS-PAGE electrophoresis, the protein sample was added into ready gel eletrophoresis hole, then preform the electrophoresis, 80 Voltage for concentrated gel, 120 Voltage for separation adhesive gel. Electrophoresis should be terminated when bromophenol blue just ran out of the glass plate, then transferred the film. In the process of transforming film, the below steps should be followed. Firstly, the distance between the protein bands on the glass board. The suitable 0.22 μm of PVDF

Table 1. Quality detection of RNA extracted from cells in each group

Sample No.	Group	A260/280	A260/230	Concentration (μg/μl)	Gross (μg)
1	Mkn45-mimics	2.06	1.52	0.577	23.1
2	Mkn45-mock	2.02	1.50	0.674	26.9
3	BGC-823-mimics	2.07	1.41	0.542	21.7
4	BGC-823-mock	2.09	1.94	1.060	42.4

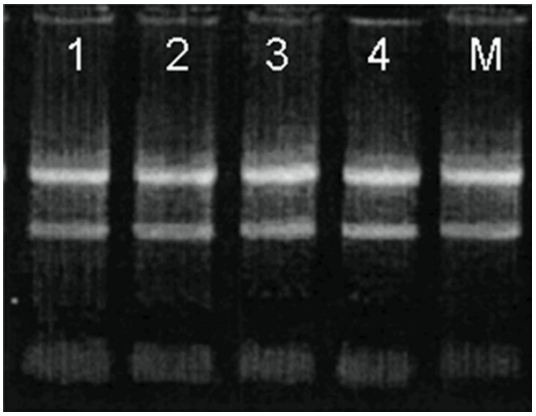

Figure 1. RNA electrophoresis figure of gastric cancer cells sample.

membrane were prepared to add into methanol to activate. Secondly, the membrane transformed clips were added into basin with transformed membrane. Four pieces of filter paper, 2 pack of sponge, glass rod, and activated PVDF membrane should be ready to use at any time. Thirdly, the transformed membrane clip should keep the black side on horizontal level. A layer of sponge and two layers of filter paper should be placed on the mat. Forthly, the separation plastic cover was carefully peeled and placed on the filter paper. The membrane was cover on the gel and eliminated the bubbles. Two layers of filter paper were covered on the membrane, then the bubbles was eliminated. Another sponge cushion was covered at last. Finally, transformed membrane was placed into the transformed membrane tank. Then sufficient transform membrane liquid was added. Ice bath has preformed for 30 minutes, then went for transforming membrane process, transforming membrane condition were 100 Voltage, 300 mA, and 90 minutes.

In immune reaction process, the transformed membrane was set in the room temperature, and placed on the bed wave to rock after added 5% of skimmed milk (TBST based). Then sealed for 2 hours. Secondly, the first antigen (1:2000 proportion) was diluted by TBST dissolved 5% skimmed milk to the proportion 1:1000, then kept at 4°C for the night. At temperature, TBST was employed to wash 3 times, each time 10 minutes. The second antigen was diluted with the proportion based on TBST. In room temperature, it was incubated 2 hours in the shaking table. Then TBST was used to wash 3 times on the shaking table in room temperature, each time 10 minutes.

During the chemiluminescence, reagen A and B in ECL ELISA kit were mixed in the same volume in centrifuge tube. The membrane protein faced up. Then the luminous fluid was pure on the membrane, and enabled it fully touch with membrane. Then it was wrapped well and exposed in the gel imaging system. Based on the different light intensity, it required to adjust the exposure condition.

In the gel image analysis, the file was scanned and kept in archive, Image J2x software processiong system was conducted to analyze gray value of the target. The ratio of each group target protein gray value and GAPDH density was analyzed to be the relative expression of quantitative analysis for protein.

Statistical method

Experimental data was processed by SPSS 16.0 statistical software. The measurement data was marked as mean ± s and analyzed by One-Way ONOVA. *q* test was adopted for the difference between each group. $P<0.05$ was for statistically significant. $P<0.01$ was for the significantly statistically significant.

Result

Affymetrix eukaryotic gene expression spectrum experiment

Quality detection of RNA extracted from cells in each group: Cells in each group had been extracted for the RNA. The ratio of A260/280 had been compared, and the concentration had been detected, as seen in **Table 1**.

The RNA extracted from each group were detected by formaldehyde denaturing gel electrophoresis. The RNA sample electrophoresis

Table 2. Bioinformatics prediction of target gene SMAD1, STAT1, CAB39, CXCR4 and CA9 (y for support)

Gene	DIANAmT	miRanda	miRDB	miRWalk	RNAhybrid	P1CTAR4	P1CTAR5	Targetscan	Sum
SMAD1	y	y	y	y	0	0	y	y	6
STAT1	y	y	0	y	0	0	y	y	5
CAB39	y	y	0	y	y	y	0	y	6
CXCR4	0	0	0	y	0	0	0	0	1
CA9	0	0	0	y	0	0	0	0	1

Table 3. mRNA target gene expression in gastric cancer cells (2-ΔΔ value (*means* ± s), n=3)

Cell	SMAD1	STAT1	CAB39	CXCR4	CA9
GES-1	1	1	1	1	1
MKN-45	42.96±2.23▲▲	1.00±0.01	1.33±0.06▲	2.73±0.31▲▲	12.2±0.75▲▲
MGC-803	453.5±1.78▲▲	1.43±0.15▲▲	2.14±0.12▲▲	3.53±0.56▲▲	60.15±2.36▲▲
SGC-7901	126.1±11.06▲▲	4.08±0.48▲▲	6.59±0.88▲▲	1.51±0.44▲▲	69.32±3.08▲▲
BGC-823	105.8±7.58▲▲	3.60±0.34▲▲	1.27±0.34▲	1.99±0.16▲▲	96.83±5.72▲▲

Note: ▲P<0.05, ▲▲P<0.01. MKN-45, MGC-803, SGC-7901, BGC-823 of gastric cancer cells group and GES-1 group.

Table 4. mRNA expression of SMAD1, STAT1, CAB39, CXCR4 and CA9 in gastric cancer cells BGC-823

Team	CA9	CAB39	SMAD1	STAT1	CXCR4
Mimics team	0.62±0.04▲	0.51±0.03▲	0.35±0.02▲	0.27±0.03▲	0.34±0.11▲
Mimics-nc team	0.95±0.04	0.81±0.03	0.64±0.05	0.59±0.07	0.63±0.15
Mock team	1.00	1.00	1.00	1.00	1.00

Note: ▲P<0.05. Mimics group versus mimics-nc group and mock group.

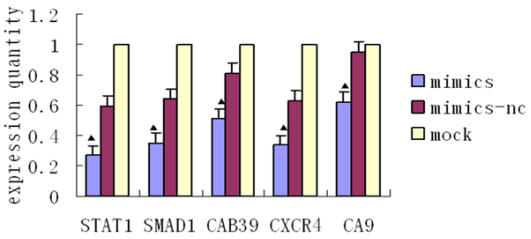

Figure 2. Target gene of mRNA expression after gastric cancer gene BGC-823 over-expressed in mir-155-5p. Note: ▲P<0.05, mimics group versus mimics-nc group and mock group.

bands were clear. The band lightness of 28S:18SrRNA was greater than or close to 2:1. The results demonstrated the qualified RNA could conduct the expression profile chip screening experiment. As seen in **Figure 1**.

The differential expressed gene after expression profile chip screened mir-155-5p mimics transfected gastric cancer cells: This paper adopted differential expressed gene that human genome-wide gene expression profile chip screened mir-155-5p mimics transfected gastric cancer cells MKN-45 and BGC-823. The results had proved that there were 983 genes differentially expressed under the screening criteria of cut-off>1.5, 48 hours after mir-155-5p mimics being transfected in BGC-823 gastric cancer cells. Among these 983 differential expressed genes, there were 685 up-regulated genes and 298 down-regulated genes.

In mirwalk website, mir-155-5p target gene were predicted by integrated prediction database of DIANAmt, miRanda, miRDB, miWalk, RNAhybrid, PICTAR4, PICTAR5 and Targetscan. At least one database support was taken as interception standard. There were totally 6771 predicted target genes and 1044 experimental verification genes. In mirfocus website, mir-155-5p target genes were predicted by integrated prediction database of DIANAmT, miRanda, miRDB, miRWalk, RNAhybrid, PICTAR4, PICTAR5 and Targetscan. At least one database support was taken as the interception standard. There were total 1888 prediction and experimental verification of target genes. Then,

Table 5. mRNA expression of SMAD1, STAT1, CAB39, CXCR4 and CA9 in gastric cancer cells MKN-45 (2-ΔΔ value (*means* ± s), n=3)

Team	CA9	CAB39	SMAD1	STAT1	CXCR4
Mimics team	0.35±0.05▲	0.01±0.00▲▲	0.05±0.02▲▲	0.03±0.01▲▲	0.12±0.04▲▲
Mimics-nc team	0.75±0.04	1.20±0.03	1.03±0.03	0.94±0.02	0.64±0.03
Mock team	1.00	1.00	1.00	1.00	1.00

Note: ▲P<0.05, ▲▲P<0.01. Mimics group versus mimics-nc group and mock group.

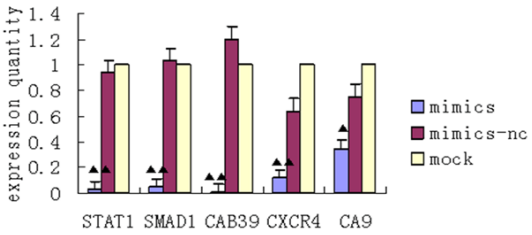

Figure 3. mRNA expression of target gene after mir-155-5p over-expressed in gastric cancer genes MKN-45. Note: ▲P<0.05, ▲▲P<0.01, mimics group versus mimics-nc group and mock group.

the human genome-wide gene expression profile chip was adopted to screen the differentially expressed genes and predicted target genes of mir-155-5p transfection gastric cancer cells MKN-45 and BGC-823 cells, which were performed the intersection analysis. The results demonstrated that, there were 790 differentially expressed genes supported by predicted software. There were 540 up-regulated genes and 250 down-regulated genes. There were 1384 differentially expressed genes that predict software supported in BGC-823 cells, of which there were 86 up-regulated genes and 1298 down-regulated genes.

Bioinformatics analysis of expression profile chip screened and differentially expressed genes: MAS system from Beijing Boao biological chip company was employed to signaling pathway analyze and GO analyze the above mentioned target genes.

In order to further verify the results of expression profile chip, target genes screened by expression profile chip and predict database were conducted for intersection. The down-regulated gastric cancer transfected target gene SMAD1, STAT1, CAB39, CXCR4 and CA9 were performed the qRT-PCR verification and Western Blotting test. The miRNA target genes database was employed to predict the results of mir-155 target gene, such as SMAD1, STAT1, CAB39, CXCR4 and CA9 of mir-155 target genes. The results proved that SMAD1, STAT1 and CAB39 received at least 5 predict software support. While, CXCR4 and CA9 only received the support of miRWalk website target verification database, as seen in **Table 2**.

Expression of SMAD1, STAT1, CAB39, CXCR4 and CA9mRNA in gastric cancer cells

qRT-PCR verification were conducted on the down-regulated expressed gene SMAD1, STAT1, CAB39, CXCR4 and CA9 in gastric cancer cells MKN-45 and BGC-823 with at least 5 predict software support. First of all, qRT-PCH method were conducted respectively on mRNA of SMAD1, STATK CAB39, CXCR4 and CA9 in gastric cancer cells SGC-7901, MKN-45 and BGC-823, compared to GES-I expression of normal gastric mucosa epithelial cells. qRT-PCR experiment illustrated that the expression level of SMAD1, STAT1, CAB39, CXCR4 and CA9 mRNA in gastric cancer cells MKN-45, MGC-803, SGC-7901 and BGC-823 were significantly higher than GES-1 cells, as seen in **Table 3**.

qRT-PCR results of down-regulated gene SMAD1, STATK CAB39, CXCR4 and CA9: Mimics transfectted gastric cancer cell BGC-823. Compared with mimics-nc group and mock group, the mRNA expression quantity of SMAD1, STAT1, CAB39, CXCR4 and CA9 in mimics group were significantly down-expressed, as seen in **Table 4** and **Figure 2**.

Mimics transfected gastric cancer cells MKN-45. Compared to mimics-nc group and mock group, the mRNA expression quantity of SMAD1, STAT1, CAB39, CXCR4 and CA9 in mimics group were significantly down-regulated, as seen in **Table 5** and **Figure 3**.

Western Blot results of down-regulated target gene SMAD1, STAT1 and CAB39: Western Blotting verification were performed on down-regulated target gene SMAD1, STAT1 and

Table 6. Expression quantity of SMAD1, STAT1 and CAB39 in gastric cancer cells MKN-45, compared to GAPDH, (*means* ± s), n=3

Team	CAB39	SMAD1	STAT1
Mimics team	0.24±0.02▲▲	0.40±0.06▲	0.31±0.03▲
Mimics-nc team	0.77±0.05	0.72±0.08	0.58±0.05
Mock team	1.21±0.06	0.85±0.10	0.70±0.08

Note: ▲P<0.05, ▲▲P<0.01, mimics group versus mimics-nc group and mock group.

Table 7. The expression quantity of SMAD1, STAT1 and CAB39 in gastric cancer cells BGC-823 compared to GAPDH (*means* ± s), n=3

Team	CAB39	SMAD1	STAT1
Mimics team	1.37±0.10▲▲	0.92±0.05▲▲	0.70±0.05▲▲
Mimics-nc team	2.43±0.12	1.30±0.06	1.41±0.11
Mock team	2.24±0.09	1.16±0.09	2.06±0.16

Note: ▲▲P<0.01, mimics group versus mimics-nc group and mock group.

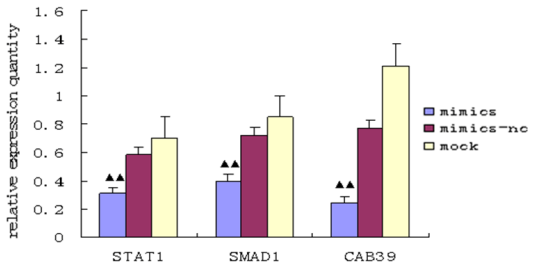

Figure 4. Target protein expression of over-expressed mir-155-5p in gastric cancer cells MKN45. Note: ▲▲P<0.01, mimics group versus mimics-nc group and mock group.

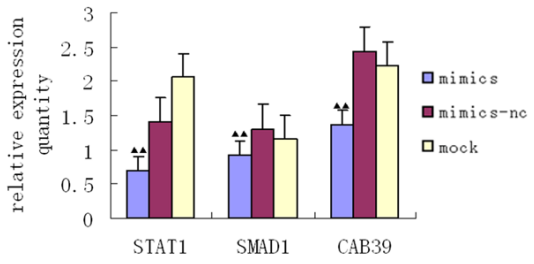

Figure 5. Target protein expression of over-expressed mir-155-5p in gastric cancer cells BGC-823. Note: ▲▲P<0.01, mimics group versus mimics-nc group and mock group.

CAB39 and gastric cancer cells MKN-45 and BGC39 with at least predict software support. Results were below, after gastric cancer cells BGC-823 and MKN-45 been transfected, compared to mimics-nc (MNC) group and mock (MOCK) group, the protein expression of SMAD1, STAT1 and CAB39 in mimics (MIMICS) group were down-regulated, as seen in **Tables 6**, **7** and **Figures 4**, **5**.

The stable converter strain cells were verficated on the constructed gastric cancer cells BGC-823 and MKN-45 infected by Hsa-mir-155-5p chronic virus vector. The EMPTY VECTOR group was compared to CONTROL group, the expression had down-regulated for the protein of SMAD1, STAT1 and CAB39 in Hsa-mir-155-5p VECTOR group, as seen in **Tables 8**, **9**; **Figures 6** and **7**.

Discussion

In order to avoid the blindness of verification experiment after mir-155-5p target gene prediction through bioinformatics software, this study adopt bioinformatics software to screen the differentially expressed genes of over-expressed mir-155-5p in gastric cancer cells MKN-45 and BGC-823, then analyze the intersection of differentially expressed gene and target genes that bioinformatics software predicted. The genes obtained were conducted qRT-PCR and Western Blot verification.

This study applied qRT-PCR verification to research the mRNA of above obtained genes SMAD1, STAT1, CAB39, CXCR4 and CA9. The SMAD1, STAT1, CAB39 had obtained five prediction software support, and was conducted Western Blot verification. Results demonstrated that the mRNA and protein level decreased significantly for SMAD1, STAT1, CAB39, CXCR4 and CA9 after mir-155-5p over-expressed in gastric cancer cells. That proved that SMAD1, STAT1, CAB39, CXCR4 and CA9 were the target genes of mir-155-5p. The mir-155-5p regulated gastric cancer biological behavior might through the function of SMAD1, STAT1, CAB39, CXCR4 and CA9. Before the verification study, qRT-PCR detection were performed on mRNA expression quantity of SMAD1, STAT1, CAB39, CXCR4 and CA9 in gastric cancer cell strain SGC-7901, MKN-45, MGC-803 and BGC-823. The mRNA expression quantity was the expression quantity compared to GES-1. The results

Table 8. Expression quantity of SMAD1, STAT1 and CAB39 in gastric cancer cell MKN-45, compared to GAPDH (*means* ± s), n=3

Team	CAB39	SMAD1	STAT1
mir-155-5p vector team	0.24±0.02▲	0.20±0.01▲	0.65±0.05▲▲
Empty vector team	0.49±0.06	0.48±0.02	1.09±0.06
Control team	0.62±0.04	0.49±0.04	1.69±0.10

Note: ▲P<0.05, ▲▲P<0.01, mir-155-5p vector group versus empty vector group and control group.

Table 9. Expression quantity of SMAD1, STAT1 and CAB39 in gastric cancer cell BGC-823, compared to GAPDH (*means* ± s), n=3

Team	CAB39	SMAD1	STAT1
mir-155-5p vector team	0.25±0.01▲	0.01±0.05▲▲	0.11±0.05▲▲
Empty vector team	0.48±0.02	0.41±0.03	0.50±0.04
Control team	0.52±0.06	0.60±0.05	0.55±0.06

Note: ▲P<0.05, ▲▲P<0.01, mir-155-5p vector group versus empty vector group and control group.

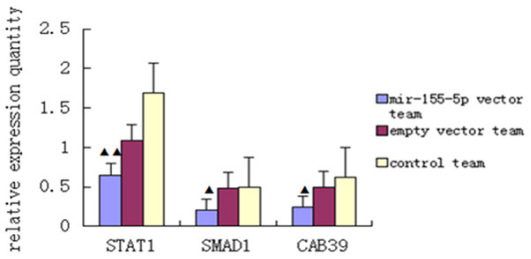

Figure 6. Each target gene expression in mir-155-5p stable converter strain of gastric cancer cell MKN45. Note: ▲P<0.05, ▲▲P<0.01, mir-155-5p vector group versus empty vector group and control group.

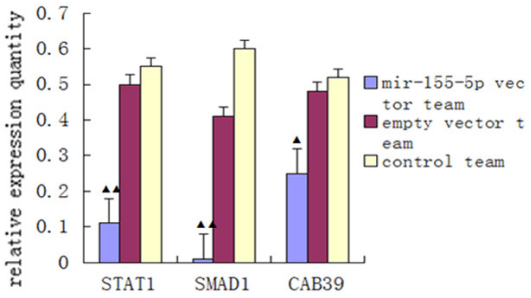

Figure 7. Each target gene expression in mir-155-5p stable converter strain of gastric cancer cell BGC-823. Note: ▲P<0.05, ▲▲P<0.01, mir-155-5p vector group versus empty vector group and control group.

illustrated that the varying degrees of increased expression of SMAD1, STAT1, CAB39, CXCR4 and CA9 in gastric cancer cell strains, and demonstrated its biological behavior function in gastric cancer cells.

SMAD1 belonged to Smad family, that mediated transcriptional regulation of multiply signaling pathways. The protein mediated BMPs involved the scope of its biological activity, including cell growth, cell apoptosis, morphogenesis, development and immune response signal. After the stimulation of BMP ligand, the protein could be phosphated and activated through BMP receptor kinase. The phosphorylation form of the protein could form complexes with SMAD4 and played a vital role in the function of transcriptional regulation and control, and lead its function in DNA damage response and tumorigenesis in BMP-SMAD1 pathway. Under the attack of toxic stimulation, ATM phosphorylation bone formed the intracellular S239 of protein activated SMAD1, and disrupted the complexes of SMAD1 and protein phosphatase PPM1A, thus improved the activation and up-regulation of nucleus SMAD1. Then, Smad1 interacted with p53, and restrained and degradation of the ubiquitination of p53 mediated Mdrn2, thus regulated cell proliferation and survival. Therefore, BMP-SMAD1 signal involved in DNA damage response through ATM-P53 pathway. The BMP-SMAD1 afunction could lead to tumorigenesis [9]. In addition, SMAD1, the protein of TGF-p signaling pathways, had a significant role in the stomach precancerous lesion [10].

STAT1, also named signal transducers and activators of transcription 1, was the member of STAT protein family that gene encoded. In response to cytokines and growth factors, STAT family members were receptor kinase phosphorylation, then formed homopolymer, or transfered the nucleus, as the transcription activator dimers. All kinds of ligands, including α interferon, γ interferon, epidermal growth factor, platelet-derived growth factor and IL-6, could activate the protein, further mediated variety of gene expression. STAT1 was regarded as the important response to different cell

stimulation and the expression of pathogens. Studies have proposed that the high expression of STAT1 in gastric cancer [11], and was the advanced gastric cancer biomarkers [12]. Study found that STAT1 involved in the biological behavior of gastric cancer, such as IL-26 increased level in gastric cancer. The regulated STAT1/STAT3 signal conduction promoted cell proliferation and survival [13].

CAB39, also named binding protein 39 or MO25, was the core structure of MO25/STRAD/LKB1. As the upstream kinase of AMPK [14-16], they constituted the compounds of CAB39/LKB1/STRAD/AMPK. In regulating metabolic stress condition, CAB39 played a vital role in biochemical balance of rapid proliferation and invasion. Studies reported miR-195 and miR-451 could regulate CAB39 and inhibit the proliferation of glioblastoma cell. Through CAB39, miR-451 down-regulated the PI3K/AKT signal pathway expression of brain glioma [17]. Colon cancer cells SW620 and miR451 could regulate CAB39 and inhibit the epxression of colon cancer cell PI3K/AKT signal pathway and the proliferation [18].

CXCR4 was the specific receptor of CXCL12. CXCR4 expressed in most of the tissues and organs of human body. It was the GPCR composed by 352 amino acids, with seven times membrane-penetrating structure. CXCR4 involved in variety of physiological and pathological mechanism, including virus infection, hematopoietic function, embryonic development, invasion and tumor metastasis. Many literature proposed that the high expression of CXCR4 in gastric cancer [19-26] related to gastric cancer invasion metastasis and vascular invasion. The gastric cancer patients with high expressed CXCR4 had poor survival rates, and closely related to prognosis. CA9 also named carbonic anhydrase IX. Literature reported that high expressed CA9 of gastric cancer intimate connected to occurrence and progression of gastric cancer, and was an important marker of prognosis [27]. CA9 was the tumor associated protein related to anoxia, and was the key marker of cancer development. Moreover, CA9 expression associated with tumor invasion and metastasis [28]. Other studies reported that CA9 expression in cell strains strongly depended on methylation status. The state of methylation in gastric cancer tissue was associated with significant CA protein expression. The CA9 expression in gastric cancer was regulated by CpG methylation [29].

The above analysis was through the expression profile chip screening and target gene of mir-155-5p of predicted IE gene, and achieved the support of qRT-PCR and Western Blot verification. The the gastric cancer biological behabior regulated mir-155-5p related to the regulation of above targets. In addition, plenty of target gene of mir-155-5p predicted by bioinformatics combined expression profile chip screening could have the manifestation of up-regulated or down-regulated. The mechanism participating gastric cancer biological behavior was profound and complex, which still remained to be further studied in the future.

Conclusion

qRT-PCR verification stated that SMAD1, STAT1, CAB39, CXCR4 and CA9 was the predict target gene of mir-155-5p. The over expressed mir-155-5p could down-regulate the expression level of gastric cancer cells MKN-45 and BGC-823. Western Blot verification stated that SMAD1, STAT1, CAB39 were the predict target gene of mir-155-5p. The over expression of mir-155-5p could down regulate the expression level of gastric cancer cells MKN-45 and BGC-823.

Disclosure of conflict of interest

None.

Address correspondence to: Jun-An Feng, Department of Gastroenterology, People's Hospital of Zhengzhou, Zhengzhou 450000, He'nan Province, P. R. China. E-mail: junanfengju@sina.com

References

[1] Wang K, Li P, Dong Y, Cai X, Hou D, Guo J, Yin Y, Zhang Y, Li J, Liang H, Yu B, Chen J, Zen K, Zhang J, Zhang CY, Chen X. A microarray-based approach identifies ADP ribosylation factor-like protein 2 as a target of microRNA-16. J Biol Chem 2011; 286: 9468-76.

[2] Li C, Xiong Q, Zhang J, Ge F, Bi LJ. Quantitative proteomic strategies for the identification of microRNA targets. Expert Rev Proteomics 2012; 9: 549-59.

[3] Zhu Y, Xiao X, Dong L, Liu Z. Investigation and identification of let-7a related functional proteins in gastric carcinoma by proteomics. Anal Cell Pathol (Amst) 2012; 35: 285-95.

[4] Thomson DW, Bracken CP and Goodall GJ. Experimental strategies for microRNA target identification. Nucleic Acids Res 2011; 39: 6845-53.
[5] Bai JX, Wang T, Zhao ZN, et al. Study on the application of immune magnetic bead separation method for the identification of the miRNA gene. Chinese Journal of Cellular and Molecular Immunology 2011; 27: 101-03.
[6] Dahm GM, Gubin MM, Magee JD, Techasintana P, Calaluce R, Atasoy U. Method for the isolation and identification of mRNAs, microRNAs and protein components of ribonucleoprotein complexes from cell extracts using RIP-Chip. J Vis Exp 2012.
[7] Hafner M, Landthaler M, Burger L, Khorshid M, Hausser J, Berninger P, Rothballer A, Ascano M, Jungkamp AC, Munschauer M, Ulrich A, Wardle GS, Dewell S,Zavolan M, Tuschl T. PAR-CliP--a method to identify transcriptome-wide the binding sites of RNA binding proteins. J Vis Exp 2010.
[8] Tan LP, Seinen E, Duns G, de Jong D, Sibon OC, Poppema S, Kroesen BJ, Kok K, van den Berg A. A high throughput experimental approach to identify miRNA targets in human cells. Nucleic Acids Res 2009; 37: el37.
[9] Chau JF, Jia D, Wang Z, Liu Z, Hu Y, Zhang X, Jia H, Lai KP, Leong WF, Au BJ, Mishina Y, Chen YG, Biondi C, Robertson E, Xie D, Liu H, He L, Wang X, Yu Q,Li B. A crucial role for bone morphogenetic protein-Smadl signalling in the DNA damage response. Nat Commun 2012; 3: 836.
[10] Kim SH, Lee SH, Choi YL, Wang LH, Park CK, Shin YK. Extensive alteration in the expression profiles of TGFB sequence. Histol Histopathol 2008; 23: 1439-52.
[11] Deng H, Zhen H, Fu Z, Huang X, Zhou H, Liu L. The antagonistic effect between STATI and Survivin and its clinical significance in gastric cancer. Oncol Lett 2012; 3: 1 93-99.
[12] Deng H, Wu RL, Chen Y, et al. The expression study of STATI and Survivin in gastric cancer lymph modes in all inspection data with the application of tissue microarray technology (English Version). The Chinese-German Journal of Clinical Oncology 2006; 4: 006.
[13] You W, Tang Q, Zhang C, Wu J, Gu C, Wu Z, Li X. IL-26 promotes the proliferation and survival of human gastric cancer cells by regulating the balance of STATI and STAT3 activation. PLoS One 2013; 8: e63588.
[14] Chen MB, Wei MX, Han JY, Wu XY, Li C, Wang J, Shen W, Lu PH. MicroRNA-451 regulates AMPK/mTORC1 signaling and fascini expression in HT-29 colorectal cancer. Cell Signal 2014; 26: 102-9.
[15] Ponce-Coria J, Gagnon KB and Delpire E. Calcium-binding protein 39 facilitates oleculer-interaction between Ste20p proline alanine-rich kinase and oxidative stress response 1 monomers. Am J Physiol Cell Physiol 2012; 303: CI198-205.
[16] Filippi BM, de los Heros P, Mehellou Y, Navratilova I, Gourlay R, Deak M, Plater L, Toth R, Zeqiraj E, Alessi DR. MO25 is a master regulator of SPAK/OSRI and MST3/MST4/YSK1 protein kinases. EMBO J 2011; 30: 1730-41.
[17] Tian Y, Nan Y, Han L, Zhang A, Wang G, Jia Z, Hao J, Pu P, Zhong Y, Kang C. MicroRNA miR-451 downregulates the PI3K/AKT pathway through CAB39 in human glioma [J]. Int J Oncol 2012; 40; 1105-12.
[18] Li HY, Zhang Y, Cai JH, Bian HL. MicroRNA-451 inhibits growth of human colorectal carcinoma cells via downregulation of Pi3k/Akt pathway. Asian Pac J Cancer Prev 2013; 14: 3631-4.
[19] Chen G, Chen SM, Wang X, Ding XF, Ding J, Meng LH. Inhibition of chemokine (CXC motif) ligand 12/chemokine (CXC motif) receptor 4 axis (CXCLI 2/CXCR4)-mediated cell migration by targeting mammalian target of rapamycin (mTOR) pathway in human gastric carcinoma cells. J Biol Chem 2012; 287: 12132-41.
[20] Deutsch AJ, Steinbauer E, Hofmann NA, Strunk D, Gerlza T, Beham-Schmid C, Schaider H, Neumeister P. Chemokine receptors in gastric MALT lymphoma: loss of CXCR4 and upregulation of CXCR7 is associated with progression to diffuse large B-cell lymphoma. Mod Pathol 2013; 26: 182-94.
[21] Nikzaban M, Hakhamaneshi MS, Fakhari S, Sheikhesmaili F, Roshani D, Ahsan B, Kamali F, Jalili A. The chemokine receptor CXCR4 is associated with the staging of gastric cancer. Adv Biomed Res 2014; 3: 16.
[22] Han M, Lv S, Zhang Y, Yi R, Huang B, Fu H, Bian R, Li X. The prognosis and clinicopathology of CXCR4 in gastric cancer patients: a meta-analysis. Tumour Biol 2014; 35: 4589-97.
[23] He H, Wang C, Shen Z, Fang Y, Wang X, Chen W, Liu F, Qin X, Sun Y. Upregulated expression of C-X-C chemokine receptor 4 is an independent prognostic predictor for patients with gastric cancer. PLoS One 2013; 8: e71864.
[24] Iwanaga T, Iwasaki Y, Ohashi M, Ohinata R, Takahashi K, Yamaguchi T, Matsumoto H, Nakano D. Inhibitory effect of CXCR4 blockers on a CXCR4-expressing gastric cancer cell line in nude mice. Gan To Kagaku Ryoho 2012; 39: 1788-90.
[25] Lee HJ and Jo DY. The role of the CXCR4/CXCL12 axis and its clinical implications in gastric cancer. Histol Histopathol 2012; 27: 1155-61.
[26] Nikkhoo B, Jalili A, Fakhari S, Sheikhesmaili F, Fathi F, Rooshani D, Hoseinpour Feizi MA, Nikzaban M. Nuclear pattern of CXCR4 expres-

sion is associated with a better overall survival in patients with gastric cancer. J Oncol 2014; 2014: 808012.
[27] Fidan E, Mentese A, Ozdemir F, Deger O, Kavgaci H, Caner Karahan S, Aydin F. Diagnostic and prognostic significance of CA IX and suPAR in gastric cancer. Med Oncol 2013; 30: 540.
[28] Chen J, Rocken C, Hoffmann J, Krüger S, Lendeckel U, Rocco A, Pastorekova S, Malfertheiner P, Ebert MP. Expression of carbonic anhydrase 9 at the invasion front of gastric cancers. Gut 2005; 54: 920-7.
[29] Nakamura J, Kitajima Y, Kai K, Hashiguchi K, Hiraki M, Noshiro H, Miyazaki K. Expression of hypoxic marker CA IX is regulated by site-specific DNA methylation and is associated with the histology of gastric cancer. Am J Pathol 2011; 178: 515-24.