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RESEARCH ARTICLE

Downregulation of Runx3 is closely related to the decreased Th1-associated factors in patients with gastric carcinoma

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Abstract Runt-related transcription factor 3 (*Runx3*) is a tumor-suppressor gene and plays an important role in immune regulation, whose reduced expression may play an important role in the development and progression of gastric carcinoma. The aim of this study was to investigate the role of *Runx3* on the levels of transcription factors in patients with gastric carcinoma and analyze the relationship between the expression of *Runx3* and Th1-type cytokines in peripheral blood mononuclear cells (PBMCs). Our results showed that the expression levels of *Runx3*, *T-bet*, and IFN-γ in patients with gastric carcinoma were obviously lower than those in control groups, and there was a positive correlation between the expression of *Runx*3 and *T-bet* or IFN-γ in patients ($p<0.01$). In order to further confirm this result, the *Runx3* gene was constructed into pIRES$_2$-eGFP and the recombined plasmid was transfected into SGC-7901 cells with liposome in vitro, the results obtained from the reverse transcription PCR indicated that the mRNA of *Runx3*, *T-bet*, or IFN-γ was significantly upregulated individually in *Runx*3 gene-transfected SGC-7901 cells. It suggested that the *Runx3* and Th1-associated factors including *T-bet* and IFN-γ synchronization declines in gastric carcinoma may contribute to the development of cancer.

Keywords Runx3 · T-bet · IFN-γ · Immune regulation · Gastric carcinoma

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Introduction

Malignancies of the upper intestinal tract are among the most common cancers. Approximately 700,000 people die of gastric cancer worldwide annually, and it is the fourth most common cancer in the world [1–3]. Immunocytes have long been recognized as a factor promoting antitumor immunity, but recent study shows that $CD4^+$ T subset imbalance will participate in tumor immune microenvironment, with the underlying immune basis remaining largely mysterious [4]. Transcription factors act in concert to induce lineage commitment towards Th1, Th2, Th17, or T regulatory (Treg) cells, and their counter-regulatory mechanisms are shown to be critical for polarization between Th1 and Th2 phenotypes [5–7]. In our previous study, we found that there was a predominant Th2 phenotype in gastric cancer patients, and we noted that the greatest downregulation of the gene encoding IFN-γ was associated with *T-bet* mutation [8, 9].

Runt-related transcription factor 3 (*Runx3*) is associated with genesis and progression of gastric carcinoma. Silencing of *Runx3* in gastric cancer affects the expression of important genes involved in metastases including cell adhesion, proliferation, and apoptosis; such silencing would promote peritoneal metastasis [10–12]. The expression of *Runx3* is greatly reduced in intestinal metaplasia in human stomachs, and *Runx3*−/− mouse gastric epithelial cells have a potential to differentiate into Cdx-2-positive intestinal-type cells [13]. We selected this gene for study in patients with gastric cancer not only because it is the cancer gene but also because of its immunomodulatory effects. In addition, the expression of *T-bet* was required to imprint the Th cell for IFN-γ reexpression. However, the effect of *Runx*3 expression level on Th1-associated factors in patients with gastric carcinoma is poorly understood. In the present study, we investigated the correlation between *Runx*3 and *T-bet* or IFN-γ, as well as the expression level of *T-bet* and IFN-γ in *Runx*3 gene-transfected SGC-

7901 cells, in order to understand the significance of *Runx*3 expression in the pathogenesis of gastric cancer.

Materials and methods

Patients

One hundred patients diagnosed newly with gastric cancer treated at the Affiliated People's Hospital of Jiangsu University were included in this study, of these patients, 42 were female and 58 were male, who age ranged from 28 to 75 years. All the patients were untreated for their condition at the time of blood collection. The diagnosis of gastric cancer was based on commonly accepted clinical and laboratory criteria and had been histologically proven, which included 10 papillary adenocarcinoma, 38 tubular adenocarcinoma, 22 poorly differentiated adenocarcinoma, 9 mucinous adenocarcinoma, 15 signer-ring cell carcinoma, and 6 anaplastic carcinoma, and 15 patients had metastasis through lymph node and 44 patients without lymph node metastasis. Fifty healthy volunteers were studied simultaneously as control, including 21 females and 29 males ranging in age from 25 to 65 years. This study was approved by the ethical committee of Jiangsu University, and written informed consent was obtained from all individuals.

Blood samples

Peripheral blood samples were collected from healthy volunteers and patients. The collection tubes contained 0.2 ml sodium heparin. Peripheral blood mononuclear cells (PBMCs) were obtained by standard Ficoll-Hypaque density centrifugation (T lymphocytes occupy about 70 %), and the $CD8^+$ T cells were isolated from PBMCs by magnetic cell sorting (MACS). The PBMCs were also stored at −70 °C for measurement of cytokines and transcription factors.

Reverse transcription PCR and quantitative real-time PCR

RNA samples were extracted from cancer or control specimen using TRIzol (Invitrogen, Life Technologies, Carlsbad, CA, USA), and total RNA was isolated and reversed-transcribed with ReverTra Ace qPCR-RT Kit (TOYOBO, Osaka, Japan) according to the manufacturer's instructions. Reverse transcription PCR (RT-PCR) and quantitative real-time PCR (qRT-PCR) were performed as described previously [10]. The sequences for the primers used were as follows: β-actin, 5′-CACGAAACTACCTTCAACTCC-3′ (forward), 5′-CATACTCCTGCTTGCTGATC-3′ (reverse); IFN-γ, 5′-TATTCGGTAACTGACTTG-3′ (forward), 5′-AATCACATAGCCTTGC-3′ (reverse); T-bet, 5′-GTTCCCATTCCTGTCSTTTACT-3′ (forward), 5′-TCTCCGTCGTTCACCTCAA-3′ (reverse); and Runx3, 5′-GATGGCAGGCAATGACGA-3′ (forward), 5′-TGCTGAAGTGGCTTGTGGT-3′ (reverse). Relative quantification of mRNA expression was calculated by the comparative threshold cycle (Ct) method.

Cell culture and Runx3 gene transfection

Human gastric carcinoma cell line SGC-7901 was cultured in DMEM medium, supplemented with 100 U/ml penicillin, 100 mg/L streptomycin, and 10 % FBS (Gibco). For *Runx3* gene transfection, 1×10^5/ml cell suspensions were prepared.

The $CD8^+$ T cells were isolated from human peripheral blood mononuclear cells with MACS, and *Runx3* cDNA was amplified by RT-PCR and cloned into pMD19-T vector and detected by single and double restriction enzyme as well as sequencing. *Runx3* gene was constructed into $pIRES_2$-eGFP (a eukaryotic expression plasmid), and the recombined plasmid was transfected into SGC-7901 cells with liposome according to the manufacturer's instructions ($pIRES_2$-eGFP as a negative control). After transfection, the eGFP as a mark of recombined plasmid was observed by fluorescence microscope.

Cell proliferation and apoptosis assay

The *Runx3* gene-transfected SGC-7901 cells were incubated at 37 °C for 24, 48, or 72 h. At each time point, 20 μl (0.5 mg/mL) of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT; Sigma, USA) was added to each well, and the plate was further incubated for 4 h to deoxidize MTT under light-blocking condition. After removal of the MTT dye solution, cells were treated with 150 μl DMSO, and the absorbance at 490 nm was measured using an ELX 800 UV reader (BioTek, Winooski, VT, USA). This cell proliferation assay was performed in triplicate and repeated at least twice. The proliferation inhibition rate was calculated according to the following formula: proliferation inhibition rate = 1 − the absorbance from transfected group/the absorbance from control group. At the same time, the cell apoptosis was detected with Hoechst 33258 fluorescent dye staining.

Flow cytometric analysis of cell cycle

The cells were collected by centrifugation at 4 °C at 1,000 rpm for 10 min and washed twice with PBS and fixed with 70 % cold ethanol at 4 °C overnight followed by digestion with 0.2 mg/ml of RNaseA for 30 min. Flow cytometry was performed using FACSCalibur Flow Cytometer (Becton Dickinson, Sparks, MD, USA) following 800 μl PI(100 μg/ml) staining, the red fluorescent at 488 nm was recorded.

Western blot

After polyacrylamide gel electrophoresis (PAGE), the protein was transferred to a PVDF membrane (PerkinElmer, USA), and the membrane was forbidden with 3 % BSA for 1 h and Tris-buffered saline and Tween 20 (TBST) washing three times, each time 5 min. The membrane was incubated at 4 °C overnight with anti-His monoclonal antibody, followed by incubating with HRP-conjugated goat anti-mouse IgG (TAKARA Company, Japan) for 1 h. After extensive washing in TBST, the blots were processed for detection of Ag using the ECL Plus Western Blotting Detection system (GE Healthcare Life Sciences, USA). Typhoon molecular imaging system was used for scanning and recording results.

In order to further understand the role of *Runx3* in gastric cancer patient's peripheral blood mononuclear cells, the western blot was also used to detect the expression of *Runx3* proteins after obtaining the cell lysates. In brief, PBMCs were washed twice with cold phosphate-buffered saline (PBS) and lysed in 150 μl of sample buffer (100 mM Tris-HCl, pH 6.8, 10 % glycerol, 4 % sodium dodecyl sulfate, 1 % bromophenol blue, 10 % β-mercaptoethanol). Then, the western blot was performed using monoclonal antibodies against RUNX3 (R3-G54; Abcam, Cambridge, MA, USA) and β-actin (Sigma).

ELISA for plasma cytokines

Plasma levels of IFN-γ protein was measured by ELISA kit (Shanghai ExCell Biology, China) following the manufacturer's protocols. All samples were measured in triplicate, and the mean concentration was calculated from the standard curve.

Statistical analysis

Data were expressed as the mean ± SD. Statistical analysis was performed using SPSS 17.0 software. All experiments were performed at least in triplicate. Correlations between variables were determined by Spearman's correlation coefficient. The rest of the data were analyzed by Student's unpaired or paired *t* test. Differences were considered statistically significant when the *P* value <0.05.

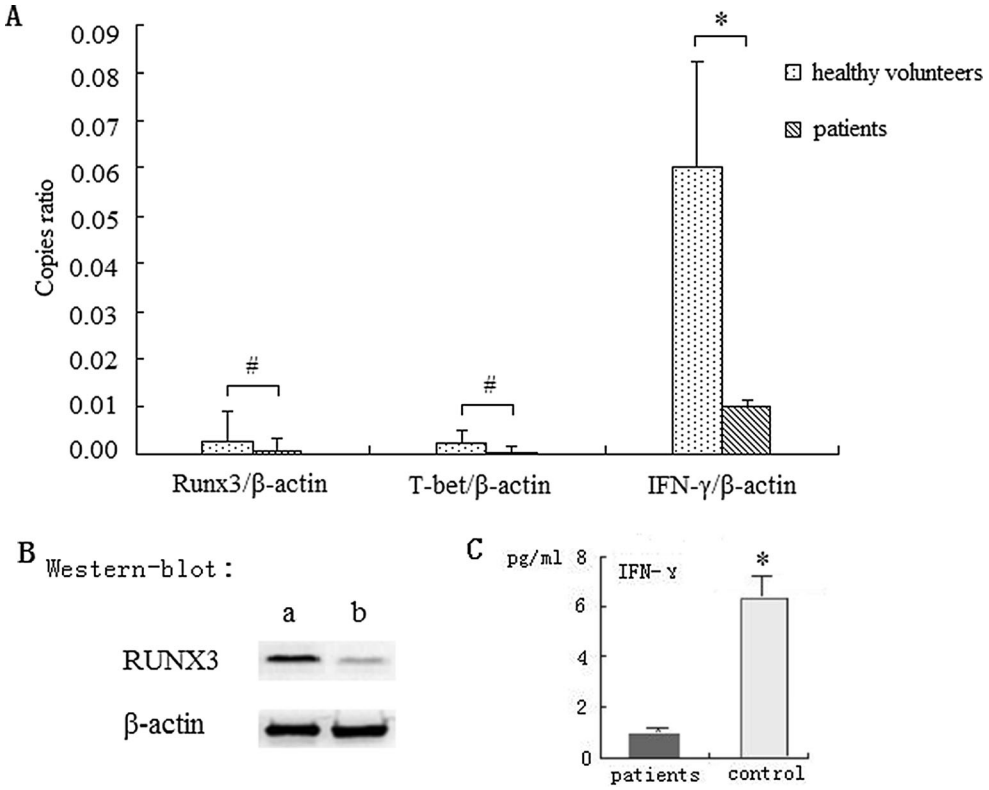

Fig. 1 The expressions of *Runx3*, *T-bet*, and *IFN-γ* in peripheral blood from patients with gastric carcinoma. (*A*) The levels of *Runx3*, *T-bet*, and *IFN-γ* mRNA in PBMC from patients with gastric carcinoma. The values were presented as relative expression ratio (copies ratio) which means expression for the target transcript versus reference transcript (β-actin, a housekeeping gene). The copy ratio showed that the *Runx3*, *T-bet*, and *IFN-γ* expressions were decreased in patients with gastric cancer, compared with healthy control. All samples were measured in triplicate. #$p<0.05$, *$p<0.01$. (*B*) The expressions of Runx3 protein in PBMC from patients with gastric carcinoma by western blot. It was obviously lower than that in healthy control (*a* healthy control, *b* patients with gastric carcinoma). (*C*) The expression levels of IFN-γ protein in plasma from patients with gastric carcinoma by ELISA were significantly decreased, compared with control.*$p<0.01$

Results

Downregulated expression of Runx3, T-bet, and IFN-γ mRNA in patients with gastric carcinoma

Silencing of *Runx3* in gastric cancer affects the expression of important genes involved in metastases and plays an important role in immune regulation. Our previous study found that there was a predominant Th2 phenotype in patients with gastric carcinoma, and it may relate to immunomodulatory effects of *Runx3*. To analyze the relation between the expression of *Runx3* and Th1-associated factors in the patients with gastric carcinoma, the transcription factors *Runx3* and *T-bet* mRNA in PBMC were detected. As shown in Fig. 1, the mRNA expression levels of *Runx3* and *T-bet* were significantly decreased in patients compared with healthy controls.

Correlation analysis between Runx3 and T-bet or IFN-γ expression in patients with gastric carcinoma

Using the method of Spearman's analysis, we have found that there was a positive correlation between the expression level of *Runx3* and *T-bet* or IFN-γ in gastric carcinoma samples. No correlation was found between the expression level of *Runx3* and *T-bet* in healthy volunteers (Fig. 2).

Electrophoresis identification of PCR amplification fragment

The length of the amplified *Runx3* gene was 1,266 bp, and it was consistent with the expected ones. The RT-PCR products of *Runx3* gene are shown in Fig. 3a. Identification of positive clone recombinant plasmids was validated by sequence. These objective gene sequences were in accordance with the ones provided by the National Center of Bioinformatics Institute

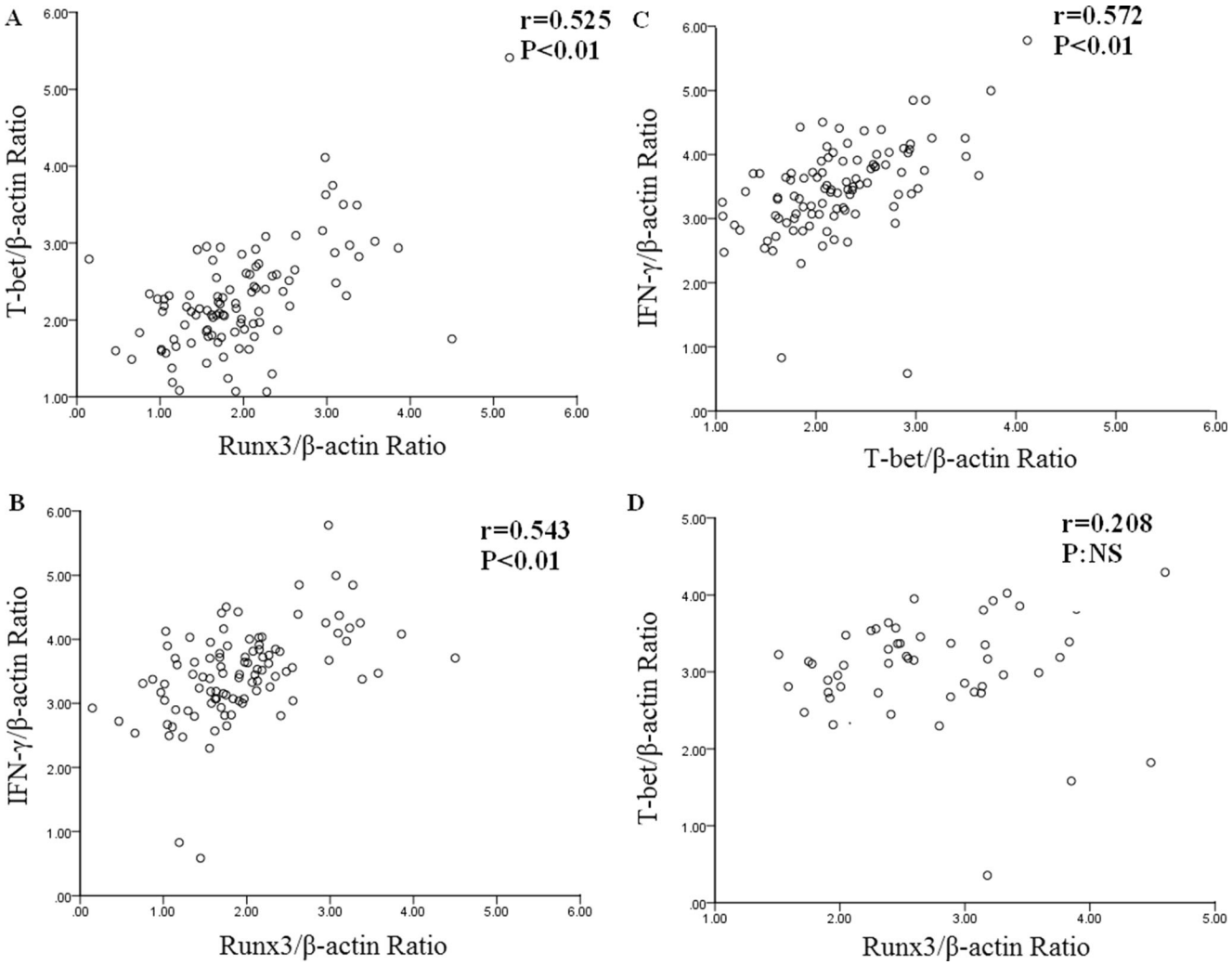

Fig. 2 Correlation between the transcription factor *Runx*3 and related genes' mRNA levels. **a** The correlation between *Runx3* and *T-bet* mRNA expression in gastric carcinoma patients. There was a positive correlation (r=0.525, p<0.01). **b** The correlation between Runx3 and IFN-γ mRNA expression in patients. There was a positive correlation (r=0.543, p<0.01). **c** The correlation between T-bet and IFN-γ mRNA expression in patients (r=0.572, p<0.01). **d** The correlation between T-bet and IFN-γ mRNA expression in healthy volunteers (r=0.208, p>0.05)

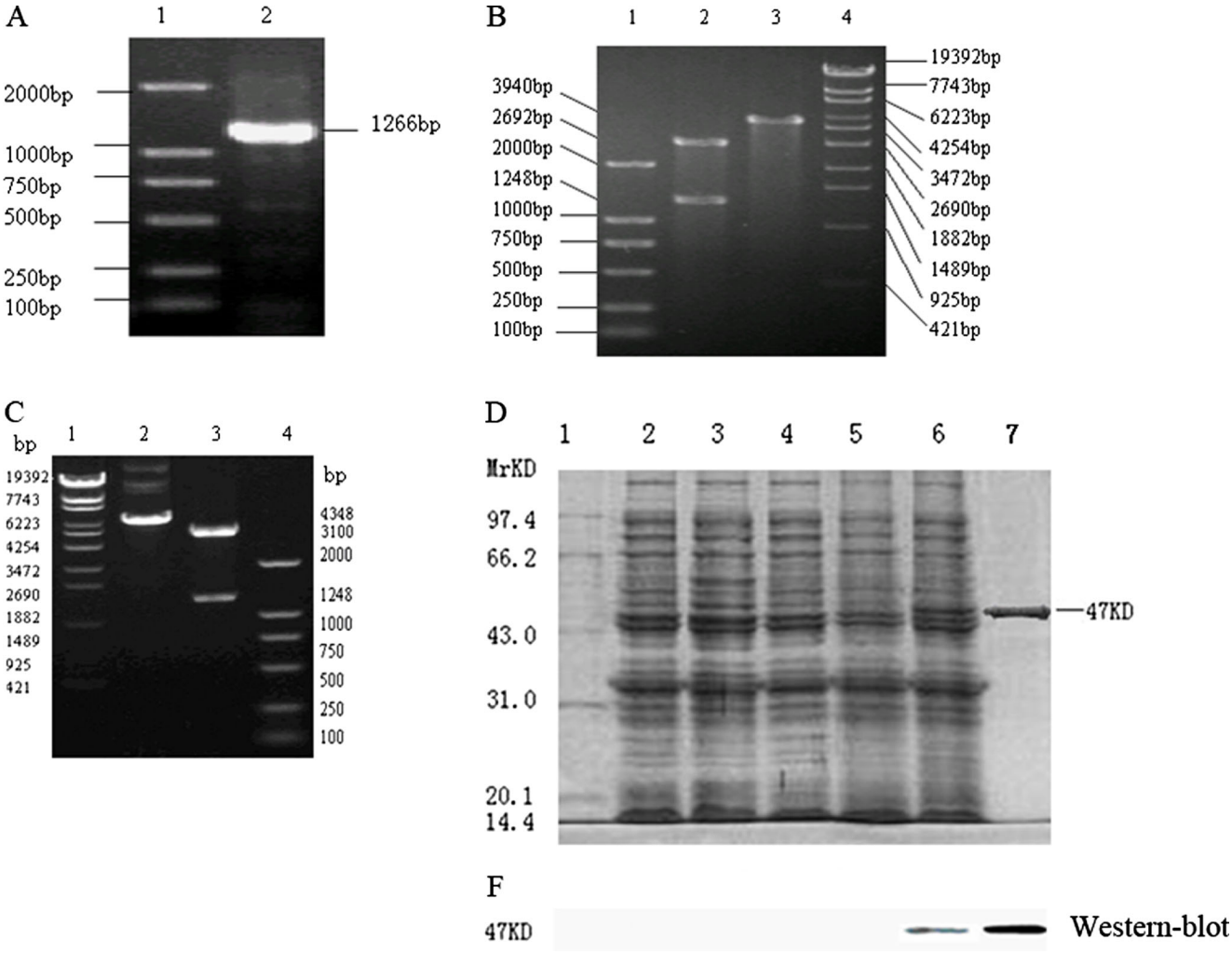

Fig. 3 Cloning and expression of human *Runx*3 gene. **a** *Runx3* gene amplification by RT-PCR. *Lane 1*, DNA marker DL2000; *lane 2*, the PCR products of *Runx3*. **b** Identification of recombinant plasmid containing *Runx3* gene. *Lane 1*, DNA marker DL2000; *lane 2*, recombinant plasmid pMD19-T-*Runx3* identified by double enzyme digestion (*Xho*I and *Bam*HI); *lane 3*, recombinant plasmid pMD19-T-*Runx3* identified by single enzyme digestion (*Bam*HI); *lane 4*, DNA markerλEcoT14 I. **c** Identification of recombinant plasmid containing pQE30-*Runx3*. *Lane 1*, DNA markerλEcoT14 I; *lane 2*, recombinant plasmid pQE30-*Runx3* identified by single enzyme digestion (BamH I); *lane 3*, recombinant plasmid pQE30-*Runx3* identified by double enzyme digestion (*Bam*HI and *Hin*dIII); *lane 4*, DNA marker DL2000. **d** The expression and identification of *Runx3* fusion protein in *E. coli* M15. *Lane 1*, unstained protein marker; *lane 2*, the protein of pQE30 M15 without IPTG induction; *lane 3*, the protein of pQE30 M15 induced by IPTG for 4 h; *lane 4*, the protein of pQE30-Runx3 M15 (without IPTG induction); *lane 5*, the supernatant protein of pQE30-Runx3 M15 induced by IPTG for 4 h; *lane 6*, the pellet protein of pQE30-Runx3 M15 induced by IPTG for 4 h; *lane 7*, the protein of pQE30-Runx3 purified by Ni^{2+} IMAC column. **f** Runx3 protein detected by western blotting

(NCBI) (detailed data not shown). The PCR product was connected with pMD19-T vector, the ligation products were transformed to the DH5α strain of *Escherichia coli*, and the positive clone (white) was selected. The plasmid from positive clone was extracted and identified by single/double enzyme digestion and electrophoresis; the positive clone strip was respectively 2,692 and 1,248 bp (Fig. 3b).

Using recombinant plasmid pMD19-T/*Runx*3 as a template and the full-length *Runx*3 gene(1248bP) amplified, the gene was subcloned into prokaryotic expression vector pQE30 with multiple cloning sites (*Bam*HI and *Hin*dIII). The recombinant clones were analyzed by restriction enzyme digestion and PCR identification and size fragment was determined (Fig. 3c). The result of SDS-PAGE electrophoresis showed that the PQE30/*Runx*3-positive strains were induced by isopropyl β-D-1-thiogalactopyranoside (IPTG) at 30 C for 4 h and the expression level of *Runx3* recombinant protein reached the peak. Western blot identification was performed with anti-HisAb (Fig. 3d, f).

Runx3 gene transfection and associated factors' expression in SGC-7901

Runx3 is considered to be a potential tumor-suppressor gene in gastric carcinoma, but the role of *Runx3* in the regulation of cell proliferation and immune response is not clear. In this

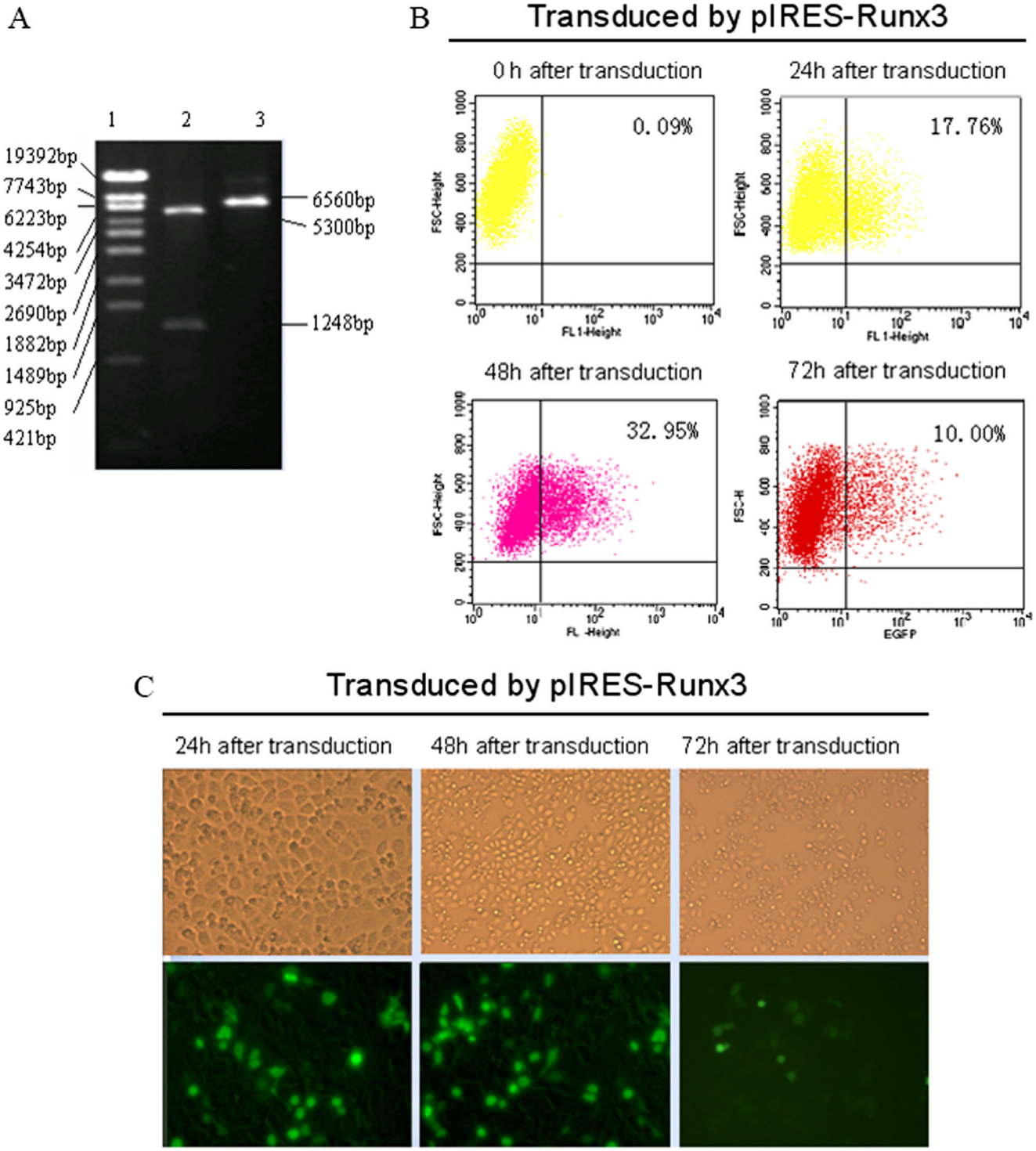

Fig. 4 *Runx3* gene transfection in SGC-7901. **a** Identification of recombinant plasmid containing *Runx3* gene. *Lane 1*, DNA markerλEcoT14 I; *lane 2*, recombinant plasmid pIRES-Runx3 identified by double enzyme digestion (*Xho*I and *Bam*HI); *lane 3*, recombinant plasmid pIRES-Runx3 identified by single enzyme digestion (*Bam*HI). **b** EGFP was detected by flow cytometry after transduction with pIRES-*Runx3*. The transfection at 48 h was the highest, up to 30 % or more; the values gradually reduced after 72 h. **c** The photomicrograph was taken by an ultraviolet laser microscope after transduction with pIRES-*Runx3*. The results showed that the green fluorescence was found in *Runx3*-transfected SGC-7901 cells, and fluorescence intensity was the strongest at 48 h. The positive rate of fluorescence was about 30~40 %. The fluorescence intensity was gradually decreased after 72 h

study, the *Runx*3 gene was transfected into SGC-7901 cells in vitro to investigate the changes of cell biological action in the gastric carcinoma cell line. The PCR product of *Runx*3 was constructed into pIRES$_2$-eGFP, and the recombinant eukaryotic vector was transfected into SGC-7901 cells with liposome in vitro. The results showed that the green fluorescence was found in *Runx*3-transfected SGC-7901 cells under a fluorescence microscope, and fluorescence intensity was the strongest at 48 h. The positive rate of fluorescence was about 30~40 %. Results from flow cytometry analysis showed that the transfection rate was more than 30 % after 48 h of transfection (Fig. 4).

The expression of *Runx*3, *T-bet*, and IFN-γ mRNA in *Runx*3-transfected SGC-7901 cells was detected by qRT-PCR. The results are shown in Fig. 5a. The expression of *Runx*3 mRNA in pIRES-*Runx*3-transfected cells was significantly increased at 48 h ($p<0.01$). In addition, the expression of *T-bet* and IFN-γ was also upregulated in pIRES-*Runx*3-carrying cells at 48 and 72 h after transfection (Fig. 5b, c).

The changes of biological action in Runx3-transfected SGC-7901 cells

Hoechst 33258 staining showed that the number of cells with apoptotic morphology was increased. After transfection, the apoptosis rate was 39.8 and 52.6 % at 48 and 72 h, respectively (Fig. 6a). The MTT results indicated that the ability of cell proliferation was decreased in *Run3*-transfected SGC-7901 cells, and the proliferation inhibition rate was 17.5, 31.8, and 46.6 %, respectively, at 24, 48, and 72 h after transfection (Fig. 6b).

The flow cytometry analysis displayed a phenomenon of increased G0/G1 phase and decreased S phase in *Run3*-transfected SGC-7901 cells. It prompted that the DNA synthesis was inhibited after *Runx*3 expression in SGC-7901 cells, and the cell cycle arrested in the G0/G1 phase (Fig. 6c, Table 1).

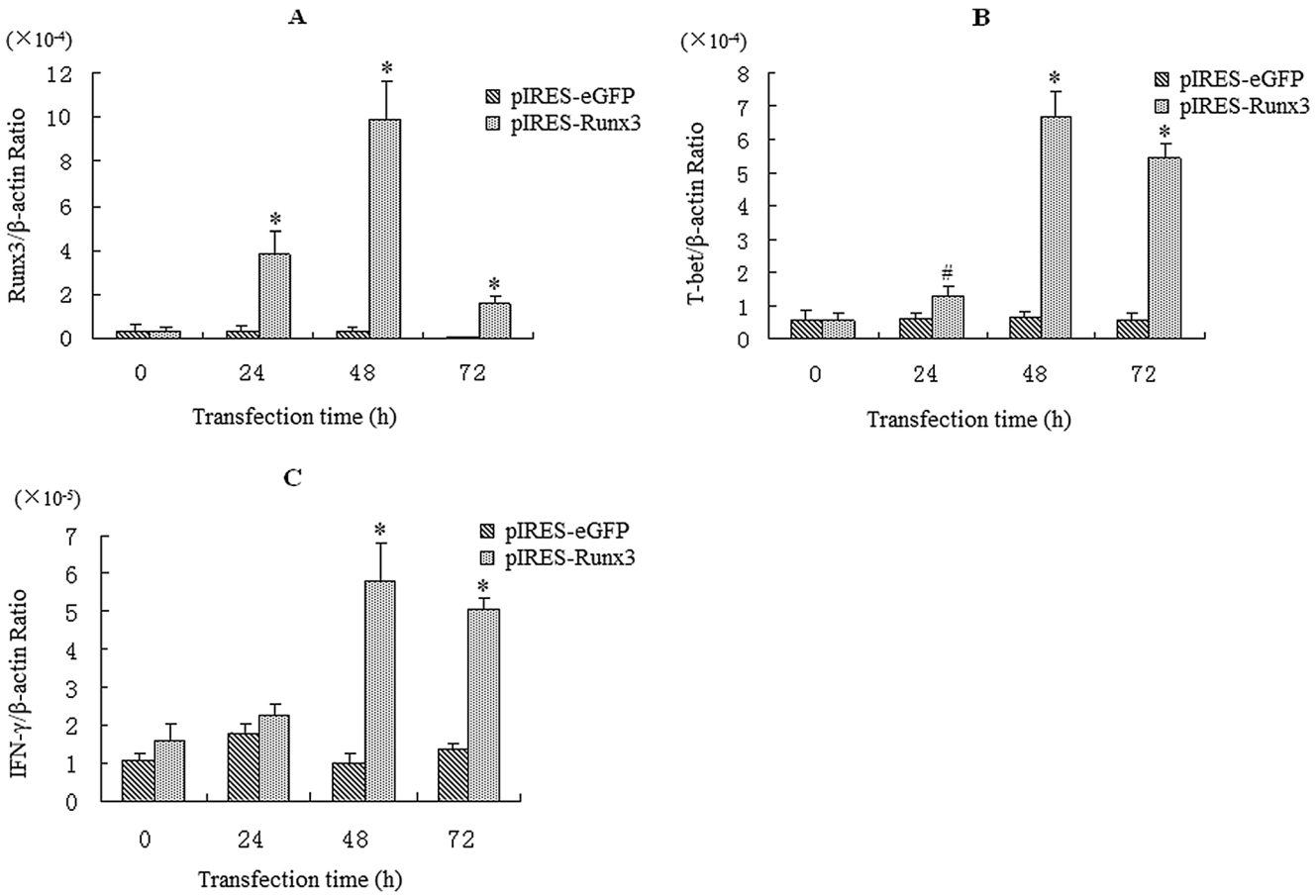

Fig. 5 Expression levels of *Runx*3 and Th1-associated factors in pIRES-*Runx*3-transfected SGC-7901. *Runx3*, *T-bet*, and *IFN-γ* expressions were increased in pIRES-Runx3-transfected SGC-7901, compared with control. **a** The expression of *Runx3* mRNA in pIRES-Runx3-transfected cells was significantly increased, which started from 24 h. **b** The expression of *T-bet* mRNA in pIRES-Runx3-transfected cells was significantly increased at 48 and 72 h. **c** *IFN-γ* was upregulated in pIRES-Runx3-carrying cells at 48 and 72 h after transfection. *$p<0.01$; #$p<0.05$

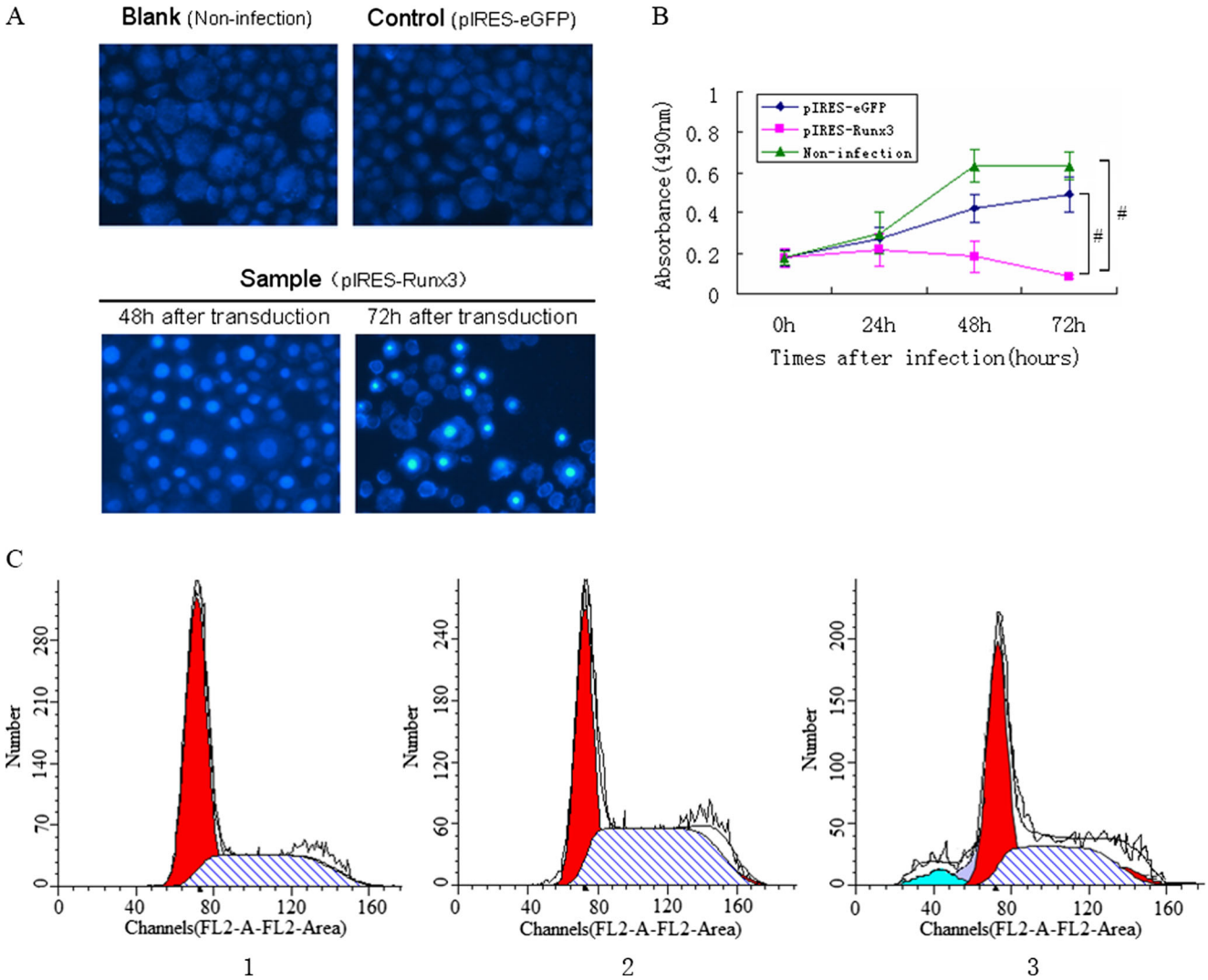

Fig. 6 **a** Hoechst 33258 staining showed that the nuclear of *Runx*3-transfected cells was bright blue and nuclear chromatin condensation and edge set. It was condensed into blocks of different sizes or overflowed and dispersed into the cytoplasm, but the cell morphology was still intact and showed the characteristics of apoptotic cells. **b** The influence of *Runx3* on cell proliferation was analyzed using MTT assay. The cell proliferation in pIRES-*Runx3*-transfected group was significantly suppressed compared to those in pIRES-eGFP-transfected group or uninfected group in a time-dependent manner. #$p<0.05$. The values were represented as mean ± SD (all samples were measured in triplicate). **c** Apoptotic cells were detected by flow cytometry. *1*, the group without transfection; *2*, the group transfected with pIRES-eGFP; *3*, the group transfected with pIRES-*Runx3*. At 72 h after transfection, the cells were centrifuged and fixed in 70 % ethanol. The samples were then treated with RNase, stained with propidium iodide, and analyzed by flow cytometry. A sub-G1 peak was clearly detected when *Runx3* was transduced in SGC-7901 cells

Table 1 The changes of cell cycle and apoptosis after transfection

Groups	G0/G1(%)	S(%)	G2/M(%)	Apoptosis rate(%)
Non transfected	37.26±1.82	51.69±1.30	11.04±1.44	4.54±0.67
pIRES-eGFP	40.99±1.53	53.33±1.65	5.68±0.49	5.84±0.53
pIRES-Runx3	52.49±1.41#	40.30±1.53#	7.20±0.63	7.04±0.41#

#$p<0.05$

Discussion

Gastric carcinoma is the fourth most common malignant carcinoma and the second leading cause of cancer-related death. Because of its high incidence and great harm, it is in the first place of morbidity and mortality in gastrointestinal cancer. The precise mechanism of the development of gastric carcinoma is not very clear yet. But it is accepted that the reason was synergistic effect of extrinsic and intrinsic factors, and great attention is paid to the switch of immune state as one of the explanation of the development of gastric carcinoma [14–17]. It is reported that patients with gastric carcinoma had significantly lower absolute numbers of $CD3^+$ $CD4^+$ and $CD4^+/CD8^+$ T lymphocytes in PMBC than normal controls, while $CD8^+$ T lymphocytes are contrarily higher. Th1 plays an important role in enhancing response in cell-mediated immunity. In addition, the effect of Th2 cells is to mediate humoral immunity. The cross regulation and inter restraint between the Th1 and Th2 cells are very essential to maintain the balance of immune system, but a typical Th2 cells drifting has happened in gastric carcinoma [18]. The immunity against humor is mainly in cell-mediated immunity, especially, IFN-γ, the specific factors secreted by Th1 cells, not only regulates the function of target T cells but also stimulates or enhances the effect of killing tumor by NK cells.

Runx3 is a tumor-suppressor gene whose reduced expression may play an important role in the development and progression of gastric carcinoma. Runt domain family, consisting of *Runx1*, *Runx2*, and *Runx3*, are master regulators of gene expression in cell proliferation and differentiation. All three *Runx* family members play important roles in normal developmental processes and tumotigenesis [5, 19]. *Runx3* expression is induced during development of both $CD8^-$ T cell and NK and plays a role in regulating certain immune-associated functions in these cytotoxic cells including proliferation and expression of various maturation and activation-associated markers. It is also reported that *Runx3* initially induces and then cooperates with *T-bet* transcription factors to regulate expression of cardinal markers of the effector CTL program [20–22]. Interaction of Runx3 and T-bet proteins contributed to regulating activity of effector T cells at the level of transcription factor, such as IFN-γ, TNF, and other cytokines. *Runx3* participates in regulating and controlling the final results of the immune response, based on upregulation of the immune protection effect and avoidance of immune inflammation damage during immune responses. It is well known that the decreased Th1 cell-mediated immunity is related to immune escape of tumor. *Runx3* is not only an important transcription factor in the immune regulation, especially in the process of CD^+ T cell differentiation, but also a tumor-suppressor gene [23–25]. In addition, *Runx3* plays an important role in regulating biological effect of cell growth, development, apoptosis, signal transduction, etc.

Because *Runx3* has the dual effects of tumor suppressor and immune adjusting and Th1 cell plays an important role in enhancing response in cell-mediated immunity which appears low state in cancer patients, we hypothesized that the *Runx3*-dependent transcriptional programs may participate in the regulation of Th1/Th2 cells balance. In the present study, we tried to explain the drifting of CD^+ T cells by analyzing the expression of transcription factors and associated cytokines in gastric carcinoma and analyzed the relationship between them. Our results showed that gastric cancer generation and metastasis were due to the imbalance of immunity regulation. There was a typical Th2 cells drifting in the patients who suffered gastric carcinoma. The expression of *T-bet*, *Runx3*, and IFN-γ was significantly lower in gastric carcinoma group than that in control group, but the results of *GATA3* and IL-4 were opposite, which further supported the findings of previous studies and indicated that the expression of *Runx3* was closely related to the generation and development of gastric cancer. The correlation analysis indicated that there was a positive correlation between the expression of *Runx3* and *T-bet* or IFN-γ in patients, which could demonstrate that *Runx3* as a Th1 secondary transcription factor played an important role in the differentiation of $CD4^+$ T cells. We also transfected the *Runx3* gene into SGC-7901 cells in vitro, in order to investigate the changes of cell biological action in the gastric carcinoma cell line. After transfection, the number of cells with apoptotic morphology was increased, the ability of cell proliferation was decreased, and the DNA synthesis was inhibited after *Runx3* expression in SGC-7901 cells. These data all proved that exogenous *Runx3* could inhibit SGC-7901 cell proliferation and promote apoptosis. In addition, the expression of *Runx3* mRNA was significantly increased, and the expression of *T-bet* and IFN-γ was also upregulated in pIRES-Runx3-transfected cells. We thought that *Ifng* might be in silence or low-expression state before *Runx3* transfection, and after transfecting, the exogenous *Runx3* cooperated with *T-bet* and bound to the *cis* element of IFN- γ, which led to the activation of *Ifng*. In this experiment, a SGC-7901 cell line was selected, which was a very low-*Runx3*-expressed cell. Compared with *Runx3* nontransfected cells, the changes of IFN-γ expression level in *Runx3*-transfected SGC-7901 cells could preliminarily indicate the role of *Runx3* gene. In the next study, we will design a *Runx3*-specific shRNA or *Runx3* knockdown tumor cell line for studying tumor intervention in vivo, in order to further confirm the role *Runx3* gene.

Taken together, we report here that downregulation of *Runx3* is closely related to the decreased Th1-associated

factors in patients with gastric carcinoma may contribute to the development of cancer. Moreover, *Runx3* as a new biological marker reveals an application prospect in the diagnosis and treatment of gastric cancer.

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Conflicts of interest None

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