RESEARCH ARTICLE

Expression of the Proto-oncogene Pokemon in Colorectal Cancer - Inhibitory Effects of an siRNA

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Abstract

Objective: This study aimed to investigate expression of the proto-oncogene POK erythroid myeloid ontogenic factor (Pokemon) in colorectal cancer (CRC), and assess inhibitory effects of a small interference RNA (siRNA) expression vector in SW480 and SW620 cells. Methods: Semi-quantitative reverse transcription-polymerase chain reaction (PCR) and immunohistochemistry were performed to determine mRNA and protein expression levels of Pokemon in CRC tissues. Indirect immunofluorescence staining was applied to investigate the location of Pokemon in SW480 and SW620 cells. The siRNA expression vectors that were constructed to express a short hairpin RNA against Pokemon were transfected to the SW480 and SW620 cells with a liposome. Expression levels of Pokemon mRNA and protein were examined by real-time quantitative-fluorescent PCR and western blot analysis. The effects of Pokemon silencing on proliferation of SW480 and SW620 cells were evaluated with reference to growth curves with MTT assays. Results: The mRNA expression level of Pokemon in tumor tissues (0.845 ± 0.344) was significantly higher than that in adjacent tumor specimens (0.321 ± 0.197). The positive expression ratio of Pokemon protein in CRC (87.0%) was significantly higher than that in the adjacent tissues (19.6%). Strong fluorescence staining of Pokemon protein was observed in the cytoplasm of the SW480 and SW620 cells. The inhibition ratios of Pokemon mRNA and protein in the SW480 cells were 83.1% and 73.5% at 48 and 72 h, respectively, compared with those of the negative control cells with the siRNA. In the SW620 cells, the inhibition ratios of Pokemon mRNA and protein were 76.3% and 68.7% at 48 and 72 h, respectively. MTT showed that Pokemon gene silencing inhibited the proliferation of SW480 and SW620 cells. Conclusion: Overexpression of Pokemon in CRC may have a function in carcinogenesis and progression. siRNA expression vectors could effectively inhibit mRNA and protein expression of Pokemon in SW480 and SW620 cells, thereby reducing malignant cell proliferation.

Keywords: Pokemon - colorectal cancer - RNA interference - real-time quantitative-fluorescent PCR - Western blot

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Introduction

Colorectal cancer (CRC), one of the leading causes of cancer mortality in industrialized areas and second to lung cancer, accounts for nearly one million new cases worldwide and half a million deaths each year (Bolocan et al., 2012). Thus, understanding the molecular basis of CRC is important for proper management of this disease. CRC is a common malignant tumor with complex, heterogeneous genetic and biochemical backgrounds (Remo et al., 2012). The carcinogenesis and development of CRC involve complex processes with multiple factors and stages. The overexpression of certain oncogenes and/or loss of certain anti-oncogenes may be involved in tumorigenesis of CRC. However, the detailed molecular mechanisms of oncogenes and anti-oncogenes related to CRC remain unclear.

POK erythroid myeloid ontogenic factor (Pokemon), also known as leukemia/lymphoma-related factor and osteoclast-derived zinc finger, is a factor that binds to human immunodeficiency virus type 1 inducer of the short transcripts and a proto-oncogene discovered by Maeda et al. (2005a). Pokemon enhances the ability of cancer cells to resist aging and death. This protein also regulates the activities of other proto- or anti-oncogenes, and is a transcription factor with proto-oncogenic activity (Lunardi et al., 2013). Pokemon contains a POZ domain at the N-terminus and a Krüppel-type (C2H2) zinc finger domain at the C-terminus. The POZ/BTB domain mediates homodimerization and heterodimerization, as well as the recruitment of corepressor/HDAC complexes; whereas, the COOH-terminal zinc fingers mediate specific DNA recognition and binding (Maeda et al., 2005a; Stogios et al., 2007). Pokemon functions as a transcriptional suppressor in cellular differentiation and oncogenesis. Several studies indicated that Pokemon also functions in

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human carcinogenesis of lymphoma, breast cancer, lung cancer, colon cancer, and so on (Maeda et al., 2005b; Zhao et al., 2008; Aggarwal et al., 2010; Aggarwal et al., 2011a). Furthermore, Pokemon is overexpressed in non-small cell lung carcinoma and malignant gliomas (Apostolopoulou et al., 2007). An aberrant overexpression of Pokemon in human tumors indicates that this protein can undergo oncogenic transformation and tumorigenesis. Pokemon is also expressed in human breast carcinomas, hepatocellular carcinomas (HCCs), and in the nuclei of human colon, renal, and thymoma tumor cells. However, only few studies have assessed the function of Pokemon in CRC.

RNA interference (RNAi) refers to the mechanism by which a small interference RNA (siRNA) effectively interferes with the expression of specific genes by specifically degrading its homologous mRNA. This phenomenon is an efficient tool for studying gene functions, and has been applied in multiple research fields, including functional genomics and gene expression regulation, as well as malignant tumor and cell signaling transduction (Kim and Rossi, 2008; Nakayashiki and Nguyen, 2008). Lin (2012) and Liu (2012) successfully silenced the Pokemon gene by RNAi in human HCC and hepatoma cell QGY7703, respectively. They observed that the silenced Pokemon inhibits cell proliferation and migration of HCC or enhances cellular sensitivity to apoptotic stress in hepatoma cell QGY7703. Thus, Pokemon is possibly an effective target for cancer gene therapy.

This study aims to detect Pokemon expression in CRC and investigate its functions in the carcinogenesis and development of CRC. This study is also designed to observe the inhibitory effects of siRNA expression vector on Pokemon in SW480 and SW620 cells, and investigate the function of Pokemon in the initiation of CRC.

Materials and Methods

Primers

The primers were designed using the DNAMAN software and synthesized by TaKaRa (Dalian, China) based on the cDNA sequences encoding human Pokemon (Accession number: NM-015898) and β-actin (Accession number: NM-001101) recorded in Genbank. The primers used were as follows: Pokemon, forward primer 5'-AAA TCT GGC ACC ACA CC-3' and reverse primer 5'-GTC TTT GCG GAT GTC CA-3'; and β-actin, forward primer 5'-ATC GTC ACC AAC TGG GA-3' and reverse primer 5'-GAT GCC ACA GGA CTC CA-3'. The siRNA sequence of Pokemon was 5'-GCC AGG AGA AGC ACT TTA A-3', which was synthesized by Wuhan Genesil Biotechnology Co., Ltd. (Wuhan, China).

Tissue samples

The samples were collected from tumor tissues of 46 CRC patients who underwent general surgery in Shanxi Provincial Cancer Hospital between October 2009 and July 2010. The tissue, located 5 cm away from the distal margin of the specimen, was also collected as the control. Each sample was divided in two parts: the first part was stored in RNAlater™ at –80 °C for RNA extraction and semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR), whereas the other part was fixed with 4% paraformaldehyde for immunohistochemistry (IHC). The CRC patients comprised 33 males and 13 females (aged 44 years to 62 years; average age = 54.6 years) who did not undergo any anti-cancer therapy before surgery. Postoperative pathology confirmed that the tumor samples manifested adenocarcinoma. The histological grades were as follows: 19 cases of well/moderately differentiated adenocarcinoma and 27 cases of poorly differentiated adenocarcinoma. This study was conducted with approval from the Ethics Committee of Shanxi Medical University. Written informed consent was obtained from all participants.

Total RNA Preparation and RT-PCR analysis

Total RNA was extracted using Trizol™ reagent (Life Technologies Corporation, NY, USA) according to the manufacturer's protocol. In brief, 30 mg of tissue was homogenized in 1 mL of Trizol™ reagent and incubated at room temperature for 10 min. Chloroform (0.2 mL) was added to the mixture, which was then shaken vigorously and incubated for 3 min at room temperature. The resulting mixture was centrifuged at 12,000 ×g and 4 °C for 15 min. The upper aqueous phase was transferred to a new microfuge tube. RNA was precipitated with 0.5 mL of isopropanol at room temperature for 10 min. The RNA pellet was washed with 75% ethanol, dried with vacuum, and resuspended in diethylpyrocarbonate-treated water. The extracted total RNA was treated with RNase-free DNase (Sigma-Aldrich, CA, USA) at 37 °C for 20 min to remove the contaminating genomic DNA. Total RNA concentration was measured by determining the absorbance (260 and 280 nm). The quality of total RNA was verified by 1% agarose gel electrophoresis (AGE). Only the samples with 28S/18S ratio > 2 and without evidence of RNA degradation were used for cDNA synthesis. Total RNA (1 μg) from each sample was reverse transcribed to synthesize cDNA using Moloney Murine leukemia virus (M-MLV) reverse transcriptase (Promega, WI, USA) at 37 °C, with a random hexamer for 1 h. The optimal conditions of the PCR reaction system (20 μL) were as follows: 94 °C for 5 min; 32 cycles at 94 °C for 30 s; 55 °C for 30 s, 72 °C for 45 s; and a final extension at 72 °C for 10 min. The housekeeping gene β-actin was amplified for 25 cycles and used as an internal reference. The amplified products were separated by 1% AGE and analyzed with a gel imaging system (Bio-Rad, CA, USA). The relative mRNA expression of Pokemon was determined as the ratio of the gray value of a Pokemon band to the gray value of a β-actin band.

IHC

IHC was performed using the avidin biotin complex (ABC) method according to the manufacturer's instructions (Vector Elite ABC Kit, Vector Laboratories, CA, USA). In brief, the tissue samples were removed, fixed in phosphate-buffered 4% paraformaldehyde for 48 h, and embedded in paraffin. The sections were cut to 10 μm using a microtome, and then placed on coated

slides. The tissues were deparaffinized in xylene and rehydrated with a descending concentration of ethanol. Heat-induced epitope was retrieved by boiling the slides in 10 mM citrate buffer (pH 6.0) in a microwave at 100 °C for 10 min. The slides were cooled for 30 min at room temperature in the buffer. Endogenous peroxidase activity was blocked with 3% H2O2 for 10 min, and non-specific staining was blocked by 10% normal goat serum in 0.3% PBS-Triton X-100 for 60 min. The samples were incubated with the primary antibody against Pokemon (1:200 dilution, Abcom, Cambridge, UK) overnight at 4 °C. Each section was washed twice in 1% goat serum and PBS for 5 min. The bound primary antibody was detected by a biotinylated secondary antibody (goat anti-rabbit IgG) for 60 min. The staining sensitivity was increased using the Vector Elite ABC kit according to the manufacturer's instructions. The reaction was visualized by incubating with the liquid substrate 3,3'-diaminobenzidine (DAB) for 5 min. The slides were counterstained with Meyer's hemalaun, mounted, and then covered with a cover slip. PBS was used as a negative control instead of the primary antibody. The IHC staining result of the human ovarian tissue in the kit was used as a positive control reference. After DAB staining, the brown granules were considered as a positive signal. Positive expression was indicated by the presence of pale yellow to brown granules in the cytoplasm and the cell nucleus. The proportion of positive cells to their staining intensities was expressed as a high magnification score. Five random fields were observed in each section. The scoring criteria for positive cell percentage used were as follows: ≤ 10%, 1; 10% to 50%, 2; and > 50%, 3. The grading standard of the staining intensity used was as follows: no color, 0; pale yellow, 1; tan, 2; and brown, 3. The total score was calculated by multiplying the score of the positive cell percentage by the score of the staining intensity. The total scores were as follows: < 3, negative; 3 ≤ total score < 6, positive; and ≥ 6, strongly positive.

Indirect immunofluorescence staining

Single-layer cultured SW480 and SW620 cells were slightly washed with 0.01 mol/L PBS (pH 7.4) and fixed with 95% ethanol for 10 min in 60 mm Petri dishes. The Pokemon antibody (1:200 dilution) was subsequently added, and the dishes were incubated at 4 °C overnight. Cy3-labeled goat anti-rabbit IgG (1:500 dilution) was added, and the dishes were incubated at 37 °C for 1 h. Then, the samples were washed twice with PBS, incubated with 4',6'-diamidino-2-phenylindole, and re-dyed for 1 min. An anti-fade mounting medium was added dropwise. The results were immediately observed under fluorescence microscopy (DMRXA/HC type, Leica Co., Berlin, Germany).

Cell culture and transfection

SW480 and SW620 cells were conventionally cultured in Roswell Park Memorial Institute (RPMI)-1640 medium with 10% fetal calf serum, and 2×10^5 cells in the exponential phase were seeded in 35 mm Petri dishes. After the cells reached 90% confluence, 2 μg of the interference plasmid and the negative control plasmid were transfected to SW480 and SW620 cells with Lipofectamine® 2000 (Life Technologies Corporation, NY, USA). Opti-MEMI medium without serum and antibiotics was utilized to dilute the liposomes and the plasmids. The transfection mixture was replaced with RPMI-1640 medium containing the serum at 6 h post-transfection for continuous culture.

Real-time quantitative-fluorescent PCR

The cells in the control and experimental groups were collected at 48 h post-transfection. Total RNA from the cultured cells was extracted using the Trizol™ reagent kit. Reverse transcription was performed using 3 μg of total RNA from each group under catalysis by M-MLV reverse transcriptase. Real-time quantitative-fluorescent PCR was performed in an ABI7700 sequence detection system based on the comparative Ct method, with β-actin as the internal reference. Real-time quantitative PCR was conducted in 20 μL of the total reaction system comprising 10 μL of 2× SYBR Premix Ex Taq™ (Takara, Dalian, China), 2 μL of cDNA template, and 0.4 μL of both forward and reverse primers (10 pmol/L). The cycling conditions were initiated with an initial DNA denaturation step at 95 °C for 10 s, followed by 40 cycles at 94 °C for 15 s, 56 °C for 25 s, and 72 °C for 25 s. All reactions were run as single-plex reactions, and the $2^{-\Delta\Delta Ct}$ value was expressed relative to β-actin. Changes were expressed as relative expression values. The real-time quantitative PCR products were subjected to electrophoresis.

Western blot analysis

The cells in the control and experimental groups were collected at 72 h post-transfection. The total protein from cultured cells was isolated with cell lysis buffer RIPA (Biyuntian Co., Nantong, China) and quantified by bicinchoninic acid assay. Vertical electrophoresis was performed with 30 μg of protein, transferred, and blocked with 5% skim milk. The diluted polyclonal Pokemon (1:200) and the diluted monoclonal β-actin (1:500) were added and incubated at 4 °C overnight. The membrane was washed with Tris-buffered saline containing Tween, incubated with 1:200 diluted secondary antibodies, and stained with an electrochemiluminescence luminescent reagent for 5 min. BioRad ChemiDocXRS imaging analysis system was directly used to analyze the images and calculate the ratio of the integral optical density (IOD) of each sample to the IOD of β-actin, which indicated the protein level changes in Pokemon.

Detection of cell proliferation

The proliferation of SW480 and SW620 cells was detected using MTT assay. Pokemon siRNA plasmid-transfected cells, empty vector-transfected cells, and parental cells in logarithmic growth phase cells were inoculated into a 96-well plate (1×10^5 cells/mL), respectively. Approximately 100 μL of 1640 medium containing 10% fetal bovine serum was added to each well, followed by culture at 37 °C with 5% CO_2. After 1, 2, 3, 4, 5, and 6 d, 20 μL of MTT (5 mg/mL) was added to each well, followed by continuous culture for 4 h. The culture fluid supernatant was discarded, and 150 μL of

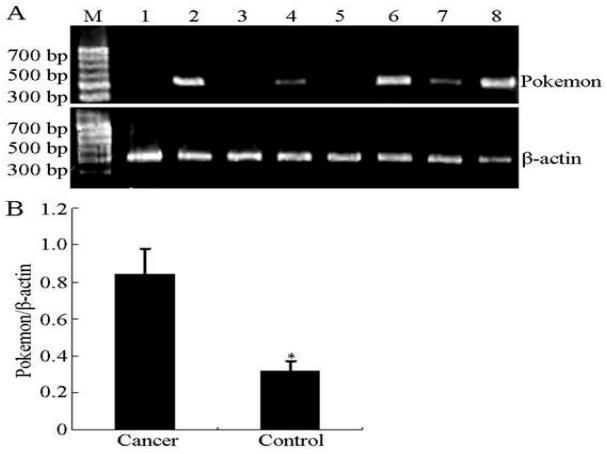

Figure 1. Expression of Pokemon mRNA in Colorectal Cancer and Cancer Surrounding Tissues. A, Representative RT-PCR products of Pokemon mRNA expression in colorectal cancer (2, 4, 6, 8) and pericarcinomatous tissue (1, 3, 5, 7). Housekeeping gene β-actin was used as an internal control. B, integrated absorbance of Pokemon mRNA expression after normalization with β-actin. *$P < 0.05$, compared with the control

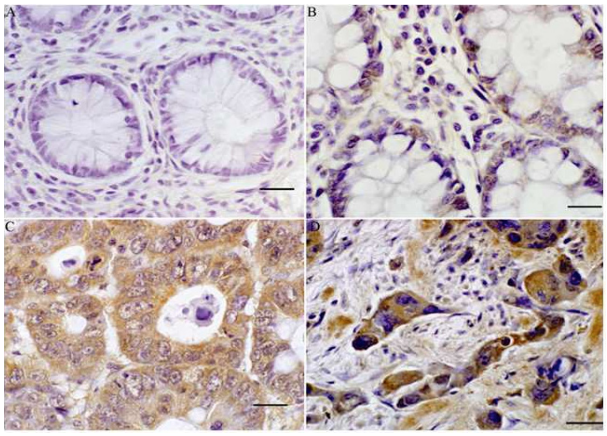

Figure 2. IHC Staining of Pokemon Protein (ABC method, bar=50 μm). Representative slides (×400) of Pokemon protein staining in normal colorectal mucosa (A); Pericarcinomatous tissue (B) and Colorectal adenocarcinoma (C, D)

DMSO was added, followed by shaking and mixing. The optical density (OD) of the culture fluid was determined by ELISA at 490 nm. The growth curve of cells was drawn with culture time as the abscissa and OD value as the ordinate.

Statistical analysis

Experimental data were analyzed with SPSS 11.5. Data were obtained and enumerated by t-test and chi-square test, respectively. $P < 0.05$ and $\alpha = 0.05$ were considered significant.

Results

Pokemon mRNA expression in CRC

Semi-quantitative RT-PCR revealed the Pokemon mRNA amplified band in 42 out of 46 cases of CRC tissues (Figure 1). Among these 46 cases, 23 pericarcinomatous tissues presented the amplified band of Pokemon mRNA (Figure 1). Gray scale analysis showed that their relative

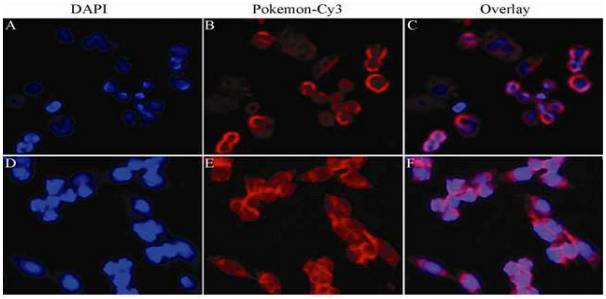

Figure 3. Immunofluorescence Staining of Pokemon Protein in SW480 and SW620 Cells. Immunofluorescence staining in SW480 cells (A, B, C) and SW620 cells (D, E, F). A, D, images show the positions of nucleus stained with DAPI (biue). B, E, the location of Pokemon protein (red) in cells. C, F, show the overlay of nucleus and Pokemon (×400)

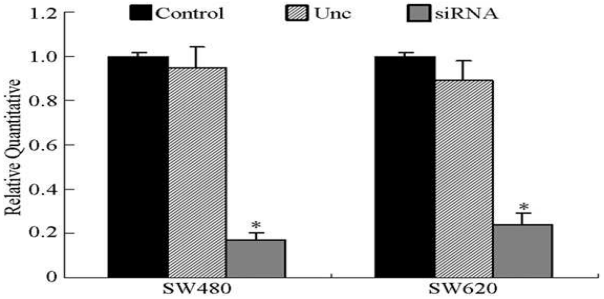

Figure 4. Expression of Pokemon mRNA after Transfected with siRNA Expression Vectors by Real Time Quantitative PCR

gene expressions were 0.845 ± 0.344 and 0.321 ± 0.197, indicating that the mRNA expression level of Pokemon significantly increased in tumor tissues than that in pericarcinomatous tissues ($P < 0.05$). This result suggests that the Pokemon gene was actively expressed in cancer tissues with abundant Pokemon mRNA.

Pokemon protein expression in CRC

IHC staining showed that the positive signal of the Pokemon protein was stained with brown granules mainly located in the cytoplasm and the nucleus of CRC cells (Figure 2). The Pokemon protein was highly expressed mostly in the cytoplasm and partly in the nucleus of CRC cells, whereas the Pokemon protein was predominantly expressed in the nucleus of proliferative epithelial cells in pericarcinomatous tissues. Pokemon protein expression was not observed in the normal colorectal mucosa. The positive expression ratio of Pokemon protein in CRC was 87% (40/46), which was significantly higher than that in adjacent tumor tissues (9/46, 19.6%, $P < 0.005$).

Pokemon protein expressions in SW480 and SW620 cells

Indirect immunofluorescence staining showed that the Pokemon protein was stained with strong red fluorescence mainly located in the cytoplasm of the SW480 and SW620 cells, particularly around the nucleus (Figure 3). These results demonstrate that SW480 and SW620 cells contained expressed Pokemon.

Inhibitory effect of siRNA on Pokemon mRNA expression

Real-time quantitative-fluorescent PCR was performed to detect the mRNA expression of Pokemon at 48 h post-

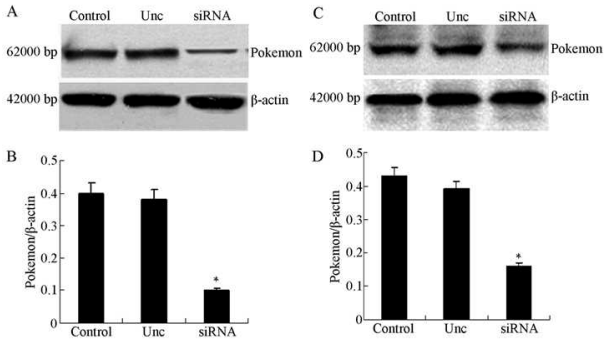

Figure 5. Expression of Pokemon Protein after Transfected with siRNA Expression Vectors by Western Blotting. A, Pokemon protein expression in SW480 cells. β-actin is the house-keeping gene used as an internal control; B, integrated absorbance of Pokemon expression in SW480 cells after normalization with β-actin. C, Pokemon protein expression in SW620 cells; D, integrated absorbance of Pokemon expression in SW620 cells after normalization with β-actin. *$P < 0.05$, compared with the control

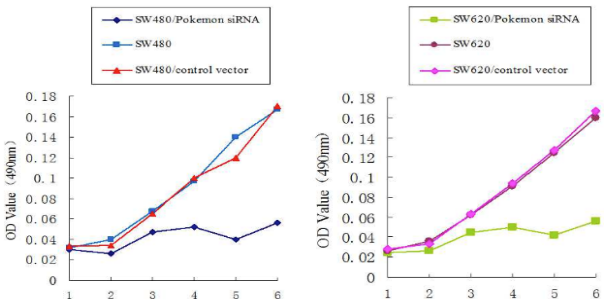

Figure 6. Growth curves of SW480 and SW620 cell

transfection. Quantitative analysis showed that siRNA transfection greatly inhibited the gene expression of Pokemon. The inhibition ratio in SW480 cells was 83.1% at 48 h post-transfection than that in the control group. In SW620 cells, the inhibition ratio was 76.3% at 48 h post-transfection (Figure 4).

Inhibitory effect of siRNA on Pokemon protein

The cells transfected with Pokemon siRNA were analyzed by western blot at 72 h post-transfection. OD scanning was performed to determine the gray value of each band. After transfection, the inhibitory effect on the Pokemon protein was analyzed. The results show that the protein expression level of Pokemon in the transfection group decreased at all time points. The inhibition ratios were 73.5% and 68.7% in SW480 and SW620 cells, respectively (Figure 5).

Effect of Pokemon gene silencing on the proliferation of SW480 and SW620 cells

Compared with parental cells and empty vector-transfected cells, the growth of Pokemon siRNA plasmid-transfected cells significantly decreased, as shown in Figure 6. This result indicates that the proliferation of SW480 and SW620 cells was inhibited after transfection with Pokemon siRNA plasmids.

Discussion

CRC is one of the most prevalent and lethal cancer types worldwide, particularly in Asia, where its incidence and mortality are high. Late diagnosis and high recurrence are currently the major causes of the poor overall survival of CRC patients. Thus, the molecular mechanisms responsible for CRC progression, as well as the efficient therapeutic targets, should be identified. Several studies demonstrated that the Pokemon gene is an important proto-oncogene extensively present in human cancers, including breast, lung, colon, prostate, bladder, and HCC (Maeda et al., 2005a, b; Zhao et al., 2008; Aggarwal et al., 2010; Aggarwal et al., 2011a). These reports showed that Pokemon functions in the development, progression, and metastatic transformation of cancer. Transgenic mouse models also confirmed that the overexpression of Pokemon can cause malignant lymphoma and the deficiency of Pokemon genes suppresses the proliferation of liver cancer cells in vitro (Maeda et al., 2005a; Lin et al., 2012; Liu et al., 2012). Thus, the proto-oncogene Pokemon is an important factor in carcinogenesis and progression of cancer. In this study, the expression level of Pokemon in CRC and the inhibitory effects of siRNA expression vectors on Pokemon in the SW480 and SW620 CRC cells were investigated. Our results indicated that the expression level of Pokemon in CRC tissues was higher than that in adjacent pericarcinomatous tissues. We further confirmed the protein expression level of Pokemon and verified the high expression of Pokemon protein in human SW480 and SW620 cells. We tested the inhibitory effects of the siRNA expression vector constructed on Pokemon in SW480 and SW620 cells. The results show that the Pokemon expression levels in SW480 and SW620 cells were significantly inhibited at mRNA and protein levels, respectively. After Pokemon gene silencing, the malignant proliferation of SW480 and SW620 cells decreased significantly.

The p14ARF-Mdm2-p53 pathway, commonly referred to as the p53 pathway, is disrupted in the development of cancer and has been the focus of many investigations. P14ARF is a negative regulator of ubiquitin–protein isopeptide ligase Mdm2, which participates in the degradation of p53 by ubiquitin-dependent degradation by the proteasome. P14ARF can also inhibit Mdm2 activity, and this inhibition also indirectly hinders the degradation of p53 (Agrawal et al., 2006). The repression of auxin response factor (ARF) expression can stimulate cell proliferation by decreasing the stability or activity of p53 via Mdm2. When Pokemon is overexpressed, it can suppress the transcription of p14ARF so that Mdm2 is reactivated to alleviate p14ARF suppression, which reduces p53 expression and leads to tumorigenesis (Apostolopoulou et al., 2007; He et al., 2010). Thus, Pokemon initiates tumor formation by inhibiting the negative regulation of the ARF-p53 pathway in malignant cells. Recent studies showed that the Pokemon protein can specifically bind to the upstream regulatory sequence of the ARF gene transcription sites and inhibit the initiation of ARF transcription (Agrawal et al., 2006; Choi et al., 2009a; He et al., 2010).

Other studies also suggested that Pokemon can induce tumorigenesis by inhibiting the expression of anti-

oncogene p21, which inhibits the activities of cyclin-cdk2 complexes, and is a key regulator of mammalian cell cycle arrest. The p21 gene is one of the transcriptional targets of p53. The principal target of Pokemon may be p21. Choi et al. assumed that Pokemon can be a master controller of the ARF-Mdm2-p53-p21 pathway, ultimately impinging on cell cycle arrest factor p21, by inhibiting upstream regulators at the transcriptional and protein levels (Choi et al., 2009a, b).

Several researchers reported that the Pokemon protein can bind to the promoter region of the Rb gene via its POZ domain, and causes the histones H3 and H4 in the promoter sequence nucleosome to initiate deacetylation by activating histone deacetylase, resulting in inhibition of Rb transcription, uncontrollable cell cycle, and cell malignant mutation (Jeon et al., 2008). Pokemon can also influence signal transduction pathways, such as nuclear factor kappa-B (NF-κB), PI3K/Akt, and cell apoptosis (Choi et al., 2009b; Aggarwal et al., 2011b; Zhang et al., 2011; Zhao et al., 2011). The POZ domain of Pokemon interacts with the Rel homology domain of the p65 subunit of NF-κB to enhance NF-κB-mediated transcription (Zu et al., 2011; Zhang et al., 2013).

Although the precise function of Pokemon in oncogenesis is unknown, previous studies showed that Pokemon may regulate a set of genes important in cell growth and proliferation, which may be important in cancer development and cancer cell proliferation. Thus, Pokemon is involved in the upstream of the molecular mechanism of neoplastic transformation. Pokemon can also control the activity of other oncogenes and anti-oncogenes, thereby affecting some important characteristics of tumor cells. The function of Pokemon as a master controller of the p53 pathway and other pathways makes it an attractive therapeutic target in cancer therapy.

Some studies have used RNAi to silence Pokemon gene expression in human liver cancer cell QGY7703 and HepG2 lines. These studies also revealed that Pokemon silencing can inhibit the proliferation of liver cancer cells and promote apoptosis (Lin et al., 2012; Liu et al., 2012). Recent studies also showed increased apoptosis of prostate cancer cells and decreased proliferation after Pokemon silencing by RNAi. These results indicated that Pokemon is an effective target in cancer gene therapy (Tian et al., 2010; Li et al., 2013). To detect the inhibitory effects of the siRNA expression vector that we constructed and investigated, whether or not Pokemon gene silencing can inhibit CRC cell proliferation and simultaneously promote cancer cell apoptosis, we transiently transfected the siRNA eukaryotic expression vectors of Pokemon to human CRC SW480 and SW620 cells using a liposome. We performed real-time quantitative-fluorescent PCR and western blot analysis to detect the inhibitory effects of the interference vectors on mRNA and protein expressions of Pokemon. The results showed that the constructed siRNA expression plasmid elicited an inhibitory effect on the expression of the proto-oncogene Pokemon in SW480 and SW620 cells. Thus, siRNA expression vectors can effectively inhibit the expression of Pokemon in SW480 and SW620 cells. This study provides a scientific basis for the molecular mechanism of Pokemon as a target gene in CRC therapy.

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