Fra-1 is downregulated in cervical cancer tissues and promotes cervical cancer cell apoptosis by p53 signaling pathway *in vitro*

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Abstract. Cervical cancer is a potentially preventable disease; however, it is the third most commonly diagnosed cancer and the fourth leading cause of cancer deaths in women worldwide. Cervical cancer is thought to develop through a multistep process involving virus, tumor suppressor genes, proto-oncogenes and immunological factors. It is known that human papillomavirus (HPV) infection is necessary but insufficient to cause malignancy. At present, the etiology of cervical carcinoma remains poorly understood. In this study, we found that the expression of FOS-like antigen-1 (*Fra-1*) gene was downregulated in cervical cancer compared with the adjacent non-cancerous tissues by RT-qPCR, immunohistochemistry (IHC) and western blotting techniques. To uncover the effect of Fra-1 on cervical cancer, we tested and confirmed that Fra-1 significantly inhibited the proliferation of HeLa cells by MMT assays *in vitro*. At the same time, overexpression of Fra-1 promoted apoptosis of HeLa cells. To explore the possible mechanism of Fra-1 in cervical cancer, we tested the expression levels of key molecules in p53 signaling pathway by western blotting technology. The results showed that p53 was downregulated in cervical cancer compared with the adjacent non-cancerous tissues, but MDM2 proto-oncogene, E3 ubiquitin protein ligase (*MDM2*) was upregulated in cervical cancer. *In vitro*, the p53 was upregulated and MDM2 was downregulated in HeLa cells with Fra-1 overexpression. In summary, our results suggested that Fra-1 expression is low in cervical cancer tissues and promotes apoptosis of cervical cancer cells by p53 signaling pathway.

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Abbreviations: Fra-1, FOS-like antigen-1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HPV, human papillomavirus; *MDM2*, MDM2 proto-oncogene, E3 ubiquitin protein ligase; TP53, tumor protein p53; CI, confidence interval; IHC, immunohistochemistry

Key words: cervical cancer, Fra-1, apoptosis, p53

Introduction

Cervical cancer is a potentially preventable disease; however, it is the third most commonly diagnosed cancer and the fourth leading cause of cancer deaths in women worldwide, accounting for 9% (529,800) of the new cancer cases and 8% (275,100) of the cancer deaths among women in 2008 (1-3). More than 85% of these cases and deaths occur in developing countries, including China (1-3). Cervical cancer is thought to develop through a multistep process involving virus, tumor suppressor genes, proto-oncogenes and immunological factors (4,5). It is known that human papillomavirus (HPV) infection is necessary, but insufficient to cause malignancy indicating the importance of other factors for malignant conversion of high-grade HPV infection (6-9). Key events that drive cancer are influenced by a multitude of factors that still remain to be understood (10-12). The etiology of cervical carcinoma remains poorly understood.

The FOS-like antigen-1 (Fra-1) is a member of the FOS transcription factor family playing important roles in transformation, proliferation, and metastasis (13-18). Fra-1 is extensively phosphorylated in response to serum mitogens or insulin in normal cell types, or in response to oncogenic RAS in transformed thyroid lines (19-22). In addition, the extent of Fra-1 phosphorylation is cell cycle regulated, being further increased in the G2/M cell fraction (13,23-25). The results obtained from various studies show different implications for Fra-1 according to tumor type. Fra-1 overexpression is predominantly associated with a large variety of epithelial tumors, including thyroid, breast, lung, brain, nasopharyngeal, esophageal, endometrial, prostate and colon carcinomas, along with glioblastomas and mesotheliomas (26,27). Fra-1 is downregulated in the tumorigenic cell lines CGL3 and HeLa compared to the non-tumorigenic 444 cells. It inhibits the tumorigenicity of cervical carcinoma cell lines (28). Fra-1 has tumor-suppressing function upon micro-cell transfer in HPV-16- and HPV-18-positive cervical carcinoma cells (29). Thus, it is urgent to explore the relationship between Fra-1 and cervical carcinoma.

Tumor suppressor p53 is the central component of a system maintaining the genetic stability of animal and human somatic cells (30-33). One of the important functions of p53 is to recognize when DNA damage has occurred in a cell and arrest the growth of that cell in the G1 period of the cell cycle to allow for DNA repair or, if repair is not possible, to lead that cell into cell-mediated death or suicide, called apoptosis (32-35). The *p53* gene plays the key role in maintaining the genetic homogeneity of somatic cells and is most often affected in cancer (32-37).

We examined the expression levels of Fra-1 and the key molecules of p53 signaling pathway in cervical cancer tissues. At the same time, the effects and possible mechanism of Fra-1 were studied in a cervical cancer cell line.

Materials and methods

Cell culture. A human HeLa cervical cancer cell line was cultured in DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco by Life Technologies™, Grand Island, NY, USA), 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C in the presence of 5% CO_2.

Tumor samples. Twenty participants were recruited at the Third Xiangya Hospital, Central South Uuniversity (Hunan, China). Consent forms were obtained from individual patients, and experimental protocols were approved by the Institutional Review Board of the Third Xiangya Hospital. At the Third Xiangya Hospital, 20 participants were women with histologically confirmed cervical cancer (Table I). All subjects enrolled in the study were Chinese. Cervical cancer tissue and corresponding non-tumor normal tissue were collected, and each biopsy sample was divided into two sections, one was submitted to routine histological diagnosis, and the remaining section was evaluated by qPCR and western blotting.

RNA extraction and quantitative real-time PCR. Total RNA was extracted from the biopsy samples with RNeasy® kit (Qiagen, Carlsbad, CA, USA) according to the manufacture's instructions. The total RNA sample (1 μg) was used to generate cDNA. Reverse transcription was carried out as described previously (38-42). After the RT reaction, the PCR reaction was preceded by 94˚C for 5 min, then 30 cycles for Fra-1 of 94˚C for 45 sec, 55˚C for 45 sec, and 72˚C for 1 min followed by 72˚C for 7 min. All RT-PCR reactions were repeated at least three times at different number of extension cycles to avoid false results of the PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous control for normalization. The sequences of the primers used for RT-PCR were as follows: Fra-1 forward, 5'-cgaaggccttgtgaacagat-3' and reverse, 5'-cttctgcttctgcagctcct-3'; GAPDH forward, 5'-cgaccactttgtcaagctca-3' and reverse, 5'-actgagtgtggcagggactc-3'. Expression of mRNA was assessed by evaluating cycle threshold (CT) values. The CT values were normalized with the expression levels of GAPDH and the relative amount of mRNA specific to each of the target genes was calculated using the $2^{-\Delta\Delta CT}$ method (42,43).

Immunohistochemistry (IHC) and evaluation of staining. IHC was done using the peroxidase-anti-peroxidase technique following a microwave antigen retrieval procedure. Antibody for *Fra-1* was purchased from ImmunoWay Biotechnology Co. (Newark, DE, USA). Antibody against *Fra-1* (1:100) was overlaid on cervical cancer and corresponding non-tumor normal tissue sections and incubated overnight at 4˚C. Secondary antibody incubation (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was performed at room temperature for 30 min.

Sections were blindly evaluated by two investigators in an effort to provide a consensus on staining patterns by light microscopy (Olympus, Tokyo, Japan). *Fra-1* staining was assessed according to the methods described by Hara and Okayasu (44) with minor modifications. Each case was rated according to a score that added a scale of intensity of staining to the area of staining. At least 10 high-power fields were chosen randomly, and >1,000 cells were counted for each section. The intensity of staining was graded on the following scale: 0, no staining; 1+, mild staining; 2+, moderate staining; 3+, intense staining. The area of staining was evaluated as follows: 0, no staining of cells in any microscopic fields; 1+, <30% of tissue stained positive; 2+, 30-60% stained positive; 3+, >60% stained positive. The minimum score when summed (extension + intensity) was, therefore, 0, and the maximum, 6. A combined staining score (extension + intensity) of ≤2 was considered to be a negative staining (low staining); 3-4, a moderate staining; and 5-6, a strong staining.

Construction of pEGFP-N1-Fra-1 vector and cell transfection. The pEGFP-N1-Fra-1 plasmid constructed to target Fra-1 (RefSeq ID: NM_001300844.1) was obtained from Shanghai Genechem Co., Ltd. (Shanghai, China). pEGFP-N1 plasmid (Shanghai Genechem Co., Ltd.) was cut with *Eco*RI/*Bam*HI and ligated by T4 DNA ligase with gene encoding Fra-1, making the Fra-1-pEGFP construct. The fusion sequences were verified by DNA sequencing using ABI 3730. The empty pEGFP-N1 vector was used as a negative control.

To establish a stable Fra-1-expressing cell line, the plasmid pEGFP-N1/Fra-1 or control empty vector pEGFP-N1 was transfected into HeLa cells, using Lipofectamine (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions, followed by G418 selection. The stable transfectants, HeLa/Fra-1 and HeLa/vector, were isolated and the transcription of Fra-1 protein was determined by western blot experiments.

Cell proliferation assay. The impact of Fra-1 on HeLa cell proliferation was measured by MTT assay as described previously (34). Briefly, HeLa cells (HeLa, HeLa/vector, and HeLa/Fra-1 cells) (10^4 cells/well) were cultured in triplicate with 10% FCS DMEM in 96-well plates, respectively. The cells were then exposed to 5 mg/ml MTT for 4 h. The generated formazan was dissolved with dimethyl sulfoxide and measured at 570 nm using an ELx800 Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA).

The effect of Fra-1 to cervical cancer cell apoptosis. Cell apoptosis was analyzed by flow cytometry analysis using a MoFlo™ XDP High-Performance Cell Sorter (Beckman Coulter, Miami, FL, USA) PI and Hoechst 33342 double staining (Nanjing KeyGen Biotech., Co., Ltd., Jiangsu, China).

Table I. Characteristics of cervical cancer patients.

Samples	Age (years)	HPV type	Histological diagnose	Stage[a]
1	43	33, 58	Cervical poorly differentiated squamous cell cancer	Ib1
2	39	16	Cervical intermediately differentiated squamous cell cancer	IIa1
3	42	16	Cervical intermediately differentiated squamous cell cancer	IIb
4	45	(-)	Cervical poorly differentiated squamous cell cancer	Ib1
5	60	16	Cervical intermediately differentiated squamous cell cancer	IIb
6	60	16	Cervical intermediately differentiated squamous cell cancer	IIb
7	70	16	Cervical intermediately differentiated squamous cell cancer	IIa1
8	49	(-)	Cervical intermediately differentiated squamous cell cancer	IIa2
9	37	16, 58	Cervical intermediately differentiated squamous cell cancer	IIa1
10	44	16	Cervical intermediately differentiated squamous cell cancer	IIa2
11	46	52	Cervical intermediately differentiated squamous cell cancer	IIb
12	42	(-)	Cervical intermediately differentiated squamous cell cancer	IIa2
13	43	45	Cervical intermediately differentiated squamous cell cancer	IIa2
14	61	16	Cervical intermediately differentiated squamous cell cancer	IIb
15	36	59	Cervical poorly differentiated squamous cell cancer	Ib1
16	36	59	Cervical poorly differentiated squamous cell cancer	Ib1
17	57	16	Cervical poorly differentiated squamous cell cancer	Ib1
18	66	16, 33	Cervical intermediately differentiated squamous cell cancer	IIb
19	43	18, 35	Cervical poorly differentiated squamous cell cancer	IIa1
20	43	45	Cervical intermediately differentiated squamous cell cancer	IIa2

[a]The International Federation of Gynecologists and Obstetricians (FIGO) stage: 2009. HPV, human papillomavirus.

Table II. Identification of the mRNA expression level of Fra-1 in cervical cancer and adjacent non-cancerous tissues by qPCR.

Gene	Sample	No.	Fra-1 CT (mean ± SD)	GAPDH CT (mean ± SD)	ΔCT (mean ± SD)	ΔΔCT (mean ± SD)	Fold[a]
Fra-1	Cervical cancer	20	32.70±1.37	19.08±0.79	13.62±0.51	1.61±0.56	0.32
	Non-cancerous tissues	20	33.08±1.65	20.07±0.84	12.01±0.45	1.61±0.56	(0.22-0.48)

[a]Mean fold change in expression of the target gene, *Fra-1*, relative to the internal control gene, *GAPDH*, was calculated using the $2^{-\Delta\Delta CT}$ equation previously adopted by Livak *et al* (43): $\Delta\Delta CT = (CT_{Target} - CT_{GAPDH})$ cervical cancer - $(CT_{Target} - CT_{GAPDH})$ control. At least three replicates of each reaction were performed. Fra-1, FOS-like antigen-1; qPCR, quantitative polymerase chain reaction; CT, cycle threshold; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Briefly, HeLa cells (HeLa, HeLa/vector, and HeLa/Fra-1 cells) were seeded at a density of $3x10^5$ cells/well in 24-well culture plates. Cells were collected in an Eppendorf tube 24 h and washed twice with PBS by centrifugation. The supernatants were discarded. To detect apoptosis, 500 μl PBS, 5 μl Hoechst 33342 and 5 μl PI were added to each tube, and the contents of the tube were mixed in the dark, at room temperature for 15 min, followed by FCM testing. The data acquired were analyzed with Summit v5.2 software.

Western blotting. Proteins of the biopsy samples were prepared by lysis buffer. The protein concentrations were determined using the Bicinchoninic Acid Protein Assay method (Pierce Biotechnology, Rockford, IL, USA). Extracts containing 50 μg of proteins were separated in 10% SDS-PAGE gels and electroblotted onto nitrocellulose membrances (HyClone Laboratories, Inc., Logan, UT, USA). The membranes were blocked using Tris-buffered saline/Tween-20 (25 mM Tris-HCl, 150 mM NaCl, pH 7.5, and 0.05% Tween-20) containing 5% non-fat milk followed by overnight incubation at 4˚C with primary antibodies (rabbit anti-Fra-1 antibody, 1:300, ImmunoWay Biotechnology Co.; rabbit anti-MDM2 antibody, 1:200, and rabbit anti-p53 antibody, 1:200, Wuhan Boster Biological Technology, Ltd., Hubei, China). After three washes, secondary antibodies (anti-horseradish peroxidase antibodies, 1:2,000; Santa Cruz Biotechnology, Inc.) were added, and incubated for 1 h. Then anti-GAPDH antibody (1:3,000; Santa Cruz Biotechnology, Inc.) was used as a loading control.

Statistical analysis. Differences of non-parametric variables were analyzed by the Fisher's exact test using EPI software

Table III. The difference of Fra-1 expression between cervical cancer and the adjacent non-cancerous tissues.

		Score			
	No.	Low (0-2)	Moderate (3-4)	High (5-6)	P
Cervical cancer	20	13 (65.0%)	5 (25.0%)	2 (10.0%)	0.004
Non-cancerous tissues	20	3 (15.0%)	7 (35.0%)	9 (45.0%)	0.004

P<0.05 by Mann-Whitney U test. Fra-1, FOS-like antigen-1.

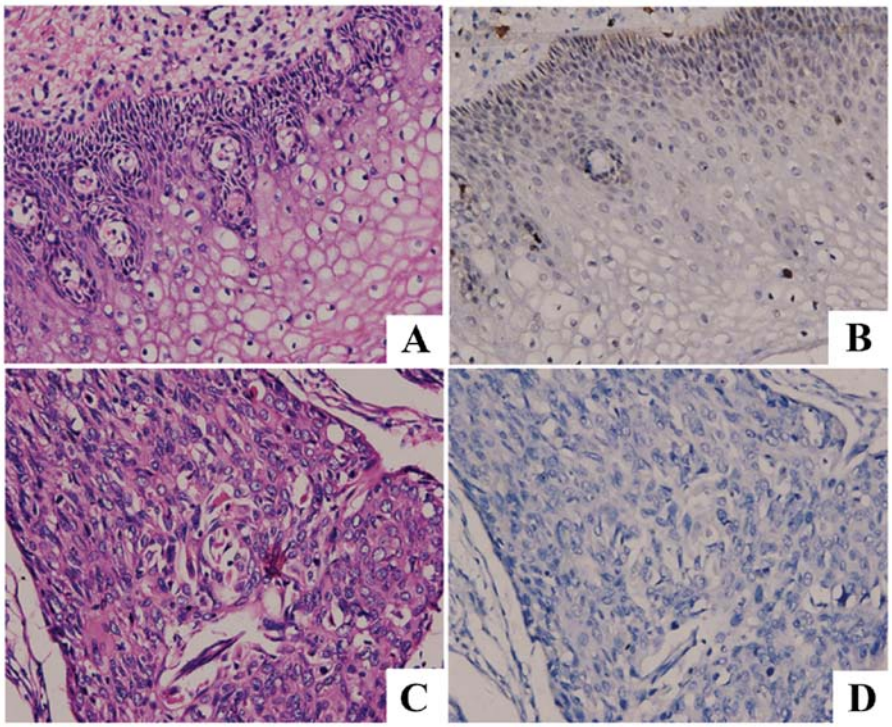

Figure 1. Immunohistochemistry (IHC) analysis of the expression of FOS-like antigen-1 (Fra-1) protein in the cervical cancer and the adjacent non-cancerous tissues. Antibody of Fra-1 protein was used; brown grains denote positive signal. (A) H&E staining of cervical epithelial tissue, (B) Fra-1 staining of cervical epithelial tissue, (C) H&E staining of cervical cancer tissue, (D) Fra-1 staining of cervical cancer tissue. Original magnification, x200.

(EPI Info, version 3.2.2, www.CDC.gov/epiinfo/). Differences of the quantitative variables between groups were analyzed by Student's t-test using SPSS 13.0 program (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered statistically significant.

Results

Detection of mRNA expression levels of Fra-1 gene in cervical cancer. To detect the mRNA expression levels of *Fra-1* gene in cervical cancer and the adjacent non-cancerous tissues, we chose 20 cervical cancer tissues and the adjacent non-cancerous tissues to perform real-time quantitative RT-PCR of *Fra-1* genes. Sample spreadsheet of data analysis was constructed by the $2^{-\Delta\Delta CT}$ method. The fold change in the expression of the *Fra-1* gene relative to the internal control gene (*GAPDH*) was studied. The expression of *Fra-1* gene was downregulated in cervical cancer (Table II). Compared with the control samples, the normalized *Fra-1* gene expression in cervical cancer was 0.32 times, 95% confidence interval (CI) was 0.22-0.48.

IHC analysis of protein expression levels of Fra-1 in cervical cancer. IHC was carried out with antibodies against Fra-1 protein in cervical cancer and the adjacent non-cancerous tissues. Fra-1 was identified as differentially expressed between cervical cancer tissues versus the adjacent non-cancerous tissues. IHC showed a similar pattern in protein expression with RT-qPCR results. There was 10.0% (2/10) high score of Fra-1 in cervical cancer tissues and 45% (9/20) in the adjacent non-cancerous tissues. The distribution of low score was 65.0% (13/20) and 15.0% (3/20) in cervical cancer and the adjacent non-cancerous tissues, respectively (p=0.004 <0.05) (Fig. 1 and Table III).

Analysis of protein expression levels of Fra-1 in cervical cancer by western blotting. To determine whether the Fra-1 had lower expression level in cervical cancer than the adjacent non-cancerous tissues, we further examined the protein expression levels of Fra-1 in cervical cancer and the adjacent non-cancerous tissues by western blotting. In comparison with

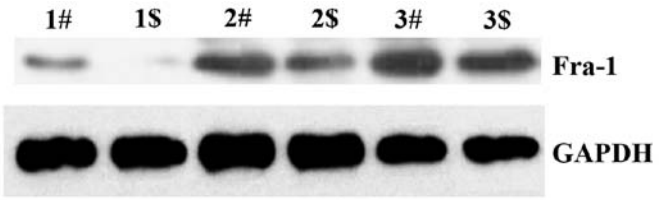

Figure 2. Expression levels of the FOS-like antigen-1 (Fra-1) protein in cervical cancer and the adjacent non-cancerous tissues. In total, 1, 2, and 3 tissues which were used in the detection of mRNA expression levels by qPCR were selected to detect the expression levels of Fra-1 protein by western blotting. $Cervical cancer and #adjacent non-cancerous tissues. Data are representative of three independent experiments.

the control, the expression level was low in cervical cancer tissues (Fig. 2). It corresponded to the results of RT-qPCR and IHC. It confirmed that Fra-1 expression is low in cervical cancer.

Fra-1 inhibits the growth of cervical cancer cells in vitro. To elucidate the function of Fra-1 in the growth of cervical cancer cells, the HeLa cells were transfected with the plasmid pEGFP-N1/Fra-1 or control vector to generate Fra-1-stable expressing HeLa/Fra-1, control HeLa/vector cell lines. After demonstrating Fra-1 protein by western blotting, the spontaneous proliferation of HeLa, HeLa/vector, and HeLa/Fra-1 cells was determined by the MTT assays, respectively. Clearly, Fra-1 significantly inhibited the proliferation of HeLa cells (Fig. 3). Therefore, endogenous Fra-1 overexpression inhibited the proliferation of cervical cancer cells *in vitro.*

Fra-1 induces cervical cancer cell apoptosis. Inhibition of cell proliferation usually is mediated by inducing cell apoptosis. To determine whether apoptosis mediated the growth in HeLa, HeLa/vector, and HeLa/Fra-1 cells, we performed a Hoechst 33342/PI double staining experiment. A considerable increase in apoptotic cells was observed for HeLa/Fra-1 cells (15.36±0.48%), HeLa cells (8.97±0.91%), and HeLa/vector cells (9.22±0.85%) (Fig. 4).

Fra-1 is correlated with dysregulation of p53 signaling pathway in cervical cancer tissues in vitro. To uncover the possible mechanism of Fra-1 in cervical cancer, we tested the expression levels of key molecules in p53 signaling pathway by western blotting technology. p53 was downregulated in cervical cancer compared with the adjacent non-cancerous tissues, whereas, MDM2 proto-oncogene, E3 ubiquitin protein ligase (*MDM2*) was upregulated in cervical cancer (Fig. 5). Combined with the above result showing low Fra-1 expression in cervical cancer, we inferred that Fra-1 is correlated with dysregulation of p53 signaling pathway in cervical cancer tissues *in vitro.*

Fra-1 overexpression affects the expression of p53 and MDM2 in vivo. To confirm whether Fra-1 affects the expression of p53 and MDM2 *in vivo*, the HeLa cells were transfected with the plasmid pEGFP-N1/Fra-1 or control vector to generate Fra-1-stable expressing HeLa/Fra-1, control HeLa/vector cell lines. We harvested the cells and tested the

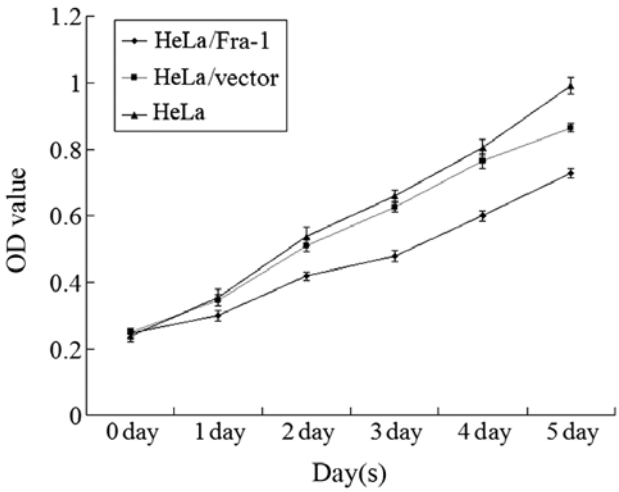

Figure 3. FOS-like antigen-1 (Fra-1) inhibits cervical cancer cell proliferation. The kinetics of Fra-1-expressing cervical cancer cell growth *in vitro*. The HeLa/Fra-1, HeLa/vector, and HeLa cells ($2x10^4$ cells/well) were cultured in duplicate in DMEM up to 5 days. The cell numbers were longitudinally counted daily with a hemocytometer. Data are expressed as the mean ± SEM of living cells for each cell line from three independent experiments.

expression levels of p53 and MDM2 proteins *in vivo*. The p53 was upregulated in HeLa cells with Fra-1 overexpression, but MDM2 was downregulated (Fig. 6). Our results suggested that Fra-1 overexpression affected the expression of p53 and MDM2 *in vivo.*

Discussion

Cervical cancer that has been proven to be associated with HPV is the second most common cancer in women worldwide and is a leading cause of cancer deaths in women in developing countries (45,46). Therefore, it is necessary and urgent to study the etiology of cervical cancer.

In this study, we chose 20 cervical cancer tissues and the adjacent non-cancerous tissues to perform real-time quantitative RT-PCR of *Fra-1* gene. The results showed that the expression of *Fra-1* gene was downregulated in cervical cancer. The normalized *Fra-1* gene expression in cervical cancer was 0.32-fold compared with the control samples. Results of IHC and western blotting showed a similar pattern in protein expression with RT-qPCR results. Thus, we confirmed low Fra-1 expression in cervical cancer tissues. Kehrmann *et al* found that Fra-1 was downregulated in the tumorigenic cell lines CGL3 and HeLa compared to the non-tumorigenic 444 cells (28). The results of Soto *et al* showed that Fra-1 has tumor-suppressing function upon micro-cell transfer in HPV-16- and HPV-18-positive cervical-carcinoma cells (29). Our data are consistent with the above observations and suggest that Fra-1 may play an important role in cervical cancer.

To elucidate the function of Fra-1 in the growth of cervical cancer cells, our results showed that Fra-1 significantly inhibited the proliferation of HeLa cells by MTT assay. Inhibition of cell proliferation is usually mediated by inducing cell apoptosis. Therefore, we tested apoptosis of Fra-1 overexpression in HeLa cell lines and a considerable increase in apoptotic cells was observed. Our data suggested

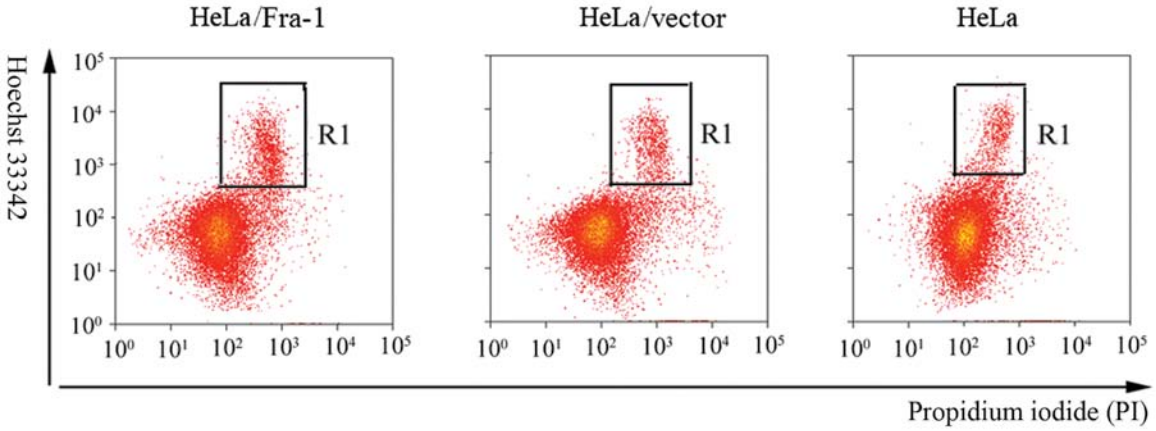

Figure 4. Overexpression of FOS-like antigen-1 (Fra-1) exhibits altered cell apoptosis profile. Cell apoptosis analysis of HeLa/Fra-1, HeLa/vector, and HeLa cells was tested by flow cytometry.

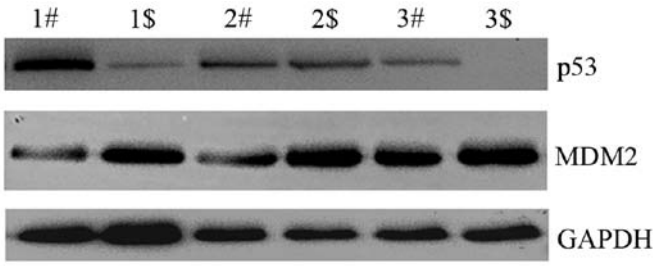

Figure 5. Expression levels of p53 and MDM2 proto-oncogene, E3 ubiquitin protein ligase (MDM2) protein in cervical cancer and the adjacent non-cancerous tissues. In total, 1, 2, and 3 tissues were used in the detection of mRNA expression levels by qPCR selected to detect the expression levels of p53 and MDM2 protein by western blotting. $^{\$}$Cervical cancer and $^{\#}$adjacent non-cancerous tissues. Data are representative of three independent experiments.

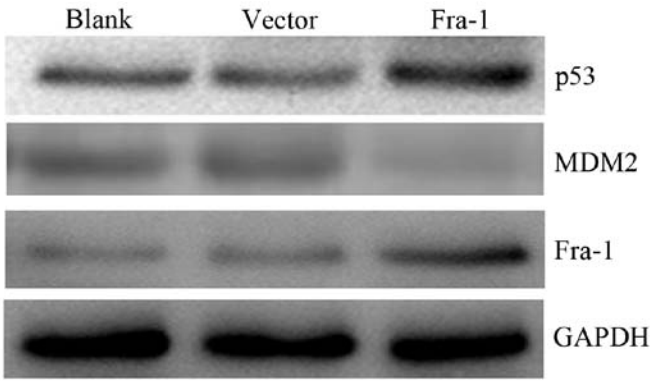

Figure 6. Expression levels of p53 and MDM2 proto-oncogene, E3 ubiquitin protein ligase (MDM2) protein in cervical cancer cell line, HeLa. Blank, HeLa cells not transfected with plasmid; vector, HeLa cells transfected with pEGFP-N1; FOS-like antigen-1 (Fra-1), HeLa cells transfected with pEGFP-N1-Fra-1. Data are representative of three independent experiments.

that Fra-1 may affect the proliferation of cervical cancer cells by mediated cell apoptosis. Song *et al* found that Irisin promoted human umbilical vein endothelial cell proliferation by partly suppressing cell apoptosis (47). Yang *et al* confirmed that downregulation of SIRT3 expression affected the proliferation and apoptosis in esophageal squamous cell carcinoma EC9706 cells (48). Above all, Fra-1 can affect proliferation and apoptosis of HeLa cells.

To uncover the possible mechanism of Fra-1 in cervical cancer, we detected the expression levels of p53 and MDM2 in cervical cancer tissues and in HeLa cells with Fra-1 overexpression by western blotting technology. We found that p53 was downregulated and MDM2 was upregulated in cervical cancer compared with the adjacent non-cancerous tissues, whereas, the p53 was upregulated and MDM2 was downregulated in HeLa cells with Fra-1 overexpression. Degradation of p53 is regulated by its interaction with specific E3 ubiquitin ligases, the best known one being encoded by MDM2 (49). A greater increase in p53 content and activation of p53 via additional modification occur when the cell is exposed to various stress factors, such as irradiation or DNA damage (50). Damage to p53-dependent mechanism is often caused by overexpression of MDM2, which codes for a p53-regulating protein (51). Combined with the above result where Fra-1 expression was low in cervical cancer, we inferred Fra-1 was correlated with dysregulation of p53 signaling pathway in cervical cancer tissues *in vitro* and Fra-1 overexpression affected the expression of p53 and MDM2 *in vivo*.

In summary, our results showed that Fra-1 expression was low in cervical carcinoma tissues and it plays an important role in dysregulation of the p53 signaling pathway.

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