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Research Article

Molecular Cancer Therapeutics

The Superparamagnetic Nanoparticles Carrying the *E1A* Gene Enhance the Radiosensitivity of Human Cervical Carcinoma in Nude Mice

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Abstract

To explore the effects of *early region 1A* (*E1A*) carried by superparamagnetic dextran iron oxide nanoparticles (SDION) on the radiosensitivity of human cervical cancer. The xenograft mice with cervical cancer received weekly intratumoral SDION-*E1A* injection and a subsequent 50-Gy irradiation. The weekly relative tumor volume and the final tumor volume were compared among different experimental groups. *p53* and *human epidermal growth factor receptor-2 (HER-2)/Neu* expression in final tumor tissue was detected by reverse transcription-PCR and Western blot. The relative tumor volume and the final tissue volume in the SDION-*E1A* group was significantly smaller than that in Sham and SDION-Vector groups at each time points after irradiation ($P < 0.05$). Exogenous *E1A* expression by SDION delivery significantly increased *p53* expression, but inhibited *HER-2/Neu* expression in tumor tissue ($P < 0.05$). The intratumoral delivery of exogenous *E1A* carried by SDION increases *p53* expression but inhibits *HER-2/neu* expression, and enhances the radiosensitivity of human cervical cancer in xenograft mice. *Mol Cancer Ther; 9(7); 2123–30. ©2010 AACR.*

Introduction

Carcinoma of the cervix is a common malignant tumor and a significant cause of morbidity and mortality among women in the world. Despite the widespread use of cytologic screening and improvements in early diagnosis, mortality rates have changed little over the past 25 years (1). Radiotherapy may be applied to all stages of cervical cancer in disease control and survival, and it is the most effective treatment for the middle and advanced stage patients. However, the resistance of tumor to radiation caused by intrinsic factors remains a major therapeutic hurdle (2), and a high proportion (50%) of these patients die from local recurrences as well as regional and distant metastases despite the first-line radiation therapy. There is an unmet need for the exploration of new approaches to enhance response to radiation therapy leading to improved survival rate of cervical cancer (3).

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Given that cancer is ultimately a genetic disease, it has long been the hope of researchers to use gene therapy to enhance the radiosensitivity in which they might be most susceptible. *Human adenovirus type 5 early region 1A (E1A)* can result in an increase of the intracellular concentration of p53 protein (4). Moreover, *E1A* gene can induce transcriptional repression of the *human epidermal growth factor receptor 2 (HER-2) /Neu* oncogene in ovarian cancer (5) and downregulate the expression of the *HER-2/Neu* in human cervical cancer cells (6). Interestingly, *HER-2/Neu* is known as a poor prognostic factor in human cervical cancers and is currently considered as a target for cervical cancer therapy (7–9). In gynecologic malignancies, 36.6% of nontreated human squamous cervical cancers have an amplified or overexpressed *HER-2/Neu* gene (10). Given the close association between *p53/HER-2* and the radiosensitivity in human cancer, as well as the regulation effects of *E1A* on these antioncogene and oncogene, *E1A* could be logically applied as a radiosensitizer to decrease the radioresistance in human cervical cancer.

Although gene therapy provides a promising approach to enhanced radiation sensitivity and abolish acquired resistance to radiation in human cancer (11, 12), the lack of safe and efficient gene delivery vectors has largely reduced the potential of gene therapy in the clinic. Most of the previous attempts at cancer gene therapy had used adenovirus vectors to deliver the therapeutic gene, but nonviral and noninfectious nanoparticle delivery systems are currently under intensive investigation to improve the curative effects and relieve the cytotoxicity (13, 14). The application of superparamagnetic dextran iron oxide nanoparticles (SDION) as gene carrier in gene therapy of tumor has been developed quickly in recent years with great experimental and clinical significance because it is convenient for preparation and can drive target gene to express highly and stably (15). The present study was designed to explore the enhancement effects of *E1A* carried by SDION on the radiosensitivity of human cervical cancer in nude mice. At the same time, the mechanism of *E1A* gene on radiotherapy sensitization by its regulation of *HER-2/Neu* and *p53* expression is also investigated.

Materials and Methods

Materials

HeLa cell line of human squamous carcinoma of the cervix was purchased from the American Type Culture Collection. PRMI-1640 culture medium, fetal bovine serum, Trizol Reagent, the plasmid of pcDNA3.1, and DH5α-competent cells were from Invitrogen. Mouse monoclonal $IgG2_a$ against adenovirus-5 *E1A*, *p53*, and *HER-2/Neu* were purchased from Pharmingen Biosciences. Horseradish peroxidase–conjugated anti-mouse IgG and enhanced chemiluminescence kit for Western blot were from Amersham Pharmacia Biotech. Advantage RT-for-PCR kit and Advantage cDNA PCR kit were purchased from Clontech Laboratories, Inc. Mouse monoclonal antibody against β-actin and other chemicals were purchased from Sigma-Aldrich Ltd. unless otherwise indicated.

E1A plasmid construction and SDION-*E1A* preparation

Employing two restriction enzymes of EcoR I and BamH I, the full coding sequence of *E1A* gene was cut from pUC119-*E1A* plasmid (Promega Corp.). The 1,170-bp DNA fragment with the full open reading frame of *E1A* gene was inserted into pcDNA3.1 plasmid by T4 DNA ligase (Invitrogen), cloned into DH5α competent cells, subsequently amplified, and purified with the Wizard Plus Minipreps kit (Promega Corp.).

The *E1A*-carrying vector of SDION was prepared as previously described (16). Briefly, 1.5 g of dextran T-40 (Amersham Pharmacia Biotech), 0.234 g of $FeCl_3{\cdot}6H_2O$, and 0.086 g of $FeCl_2{\cdot}4H_2O$ dissolved in 3 mL of deionized water was mixed with magnetic force stirring at 800 rpm/min. Following an addition of ammonia at 8.5% weight ratio, the temperature was rapidly increased and kept at 60°C for 30 minutes. The pH value was adjusted to 7.0 by glacial acetic acid, and the accumulation of large particles was removed by 1,500 rpm/min centrifugation. The supernatant was loaded on a gel-filtration chromatography column (2.6 cm × 40 cm) of Sephacryl S-300HR (Amersham Pharmacia Biotech). The first PBS-elution peak was collected and dialyzed at 4°C for 24 hours, and the vacuum freeze drying products were dissolved in 10 mL deionized water to make the SDION.

Fifty milligrams of SDION were dissolved in sodium periodate at the final concentration of 20 mmol/L and stirred at 4°C in darkness for1 hour. Following an adequate hemodialysis and vacuum freeze drying, the lyophilized products was dissolved in 10 mL of PBS, mixed thoroughly with 1 mg *E1A* plasmid, and reacted at 4°C in darkness for 24 hours. The mixture was reduced at 4°C for 1 hour by sodium borohydride at the concentration of 1 mol/L and subsequently centrifuged at 1,500 rpm/min for 15 minutes. The supernatant was loaded on a Sephacryl S-300HR gel chromatography column, and the first PBS-elution peak was collected and applied as SDION-*E1A* ready for further use.

Experimental cervical cancer xenograft model

HeLa cells were cultured in PRMI-1640 supplemented with 5% fetal bovine serum, 100 IU penicillin, 100 μg/mL of streptomycin, and 2 mmol/L L-glutamine at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

A suspension of HeLa cells (5×10^6 cells in 200 μL PRMI-1640) in log-phase growth was s.c. inoculated into both lower limbs of 6-week-old *nu/nu* BALB/c mice (Central Animal Care Unit, Central South University, China). The mice were maintained under 12-hour light/dark cycles with food and water *ad libitum*. All animals received humane care in compliance with the university's guidelines. After inoculation, the tumors were measured weekly in two perpendicular diameters using a caliper and the tumor volume was determined by using the following formula: $V = \pi/6 \times$ [large diameter × (short diameter)^2] (17). When the tumor xenografts developed to ~300 mm^3, the tumor-bearing mice were randomly divided into three groups maintaining a similar tumor size distribution: (*a*) the Sham group receiving irradiation with PBS injection in tumor, (*b*) irradiation treatment with blank SDION vector intratumoral injection, and (*c*) irradiation with intratumoral SDION-*E1A* injection.

SDION-*E1A* treatment and radiotherapy

One hundred micrograms of *E1A* plasmid carried by SDION were injected directly into the tumor mass weekly. The tumors were exposed to nominal 2.5 Gy entrance doses on a 6MV X-ray linear accelerator (Varian Clinac 2100 C/D System, Varian Medical Systems), 5 d/wk, to complete 50 Gy in accordance with an irradiation protocol similar to clinical patient treatment (17). The lower limbs were selected as the tumor xenograft sites, and all the tumor-bearing mice were shielded with a specially designed lead apparatus allowing local irradiation to minimize irradiation to other body organs. The tumor volumes were measured weekly as described above, and a relative tumor volume (RTV) was determined using the relation: RTV = Vi/V_0, in which Vi was the weekly measured tumor volume and V_0 was the initial

Table 1. Primers and reaction conditions of regular and real-time RT-PCR

Genes	Genbank accession no.	Primers	Temperature (°C)	Size (bp)
E1A				
Sense	AY339865	5′-TATGATTTAGACGTGACG-3′	60	267
Anti-sense		5′-CTCGTCGTCACTGGG-3′		
p53				
Sense	M14694	5′-CATCTACAAGCAGTCACAGCA-3′	61	186
Anti-sense		5′-GGCGGCTCATAGGGCACCACCACA-3′		
HER-2/Neu				
Sense	M12036	5′-CATGGGAGAACTCTGAGTG-3′	62	182
Anti-sense		5′-CTCCTCACCCACCCCTCCCAACAC-3′		
β-Actin				
Sense	EF156276	5′-ACCACACTTTCTACAATGA-3	60	178
Anti-sense		5′-CCAGAGGCATACAGGGACAACACA-3′		

tumor volume at the beginning of the treatment. All the mice were sacrificed 30 days after the initial irradiation treatment and the tumor mass was collected.

RNA extraction and real-time reverse transcription-PCR quantitation

Total RNA was isolated from the tumor tissue using Trizol reagent per the manufacturer's instruction. The first strand cDNA synthesis was done using the Advantage RT-for-PCR kit as previously described (18). The specific primers for *p53, HER-2/Neu*, and β-actin were designed from the respective Genbank sequence, synthesized by Bio Basic, Inc., and listed in Table 1. The regular PCR amplification for *E1A* was carried out by applying 28 cycles comprising the following: denaturation at 94°C for 20 seconds, annealing at 60°C for 30 seconds, elongation at 72°C for 1 minute, followed by a final elongation at 72°C for 4 minutes on an Eppendorf MasterCycler (Eppendorf). The real-time reverse transcription-PCR (RT-PCR) quantitation for *p53* and *HER-2/Neu* mRNA expression was done on an ABI Model 7500 Sequence Detector (Applied Biosystems) using a TaKaRa real-time PCR kit (TaKaRa Biotechnology). The amplified PCR products were quantified by measuring the target and β-actin mRNA calculated cycle thresholds. The amount of specific mRNA in each sample was calculated from the standard curve and normalized with the β-actin mRNA. The comparative $2^{-\Delta\Delta CT}$ method was used for relative quantification and statistical analysis, and the results were expressed as a *n*-fold difference relative to Sham controls (19).

Western blot analyses of *E1A, p53*, and *HER-2/Neu* protein expression

Total protein was lysed in protein extract solution, and the protein concentration in lysates was calculated by the Lowry method. Twenty five micrograms of total protein extracted from the tumor tissue were separated on 10% SDS-polyacrylamide gel electrophoresis under reducing conditions and were transferred to Nitroplus-2000 membrane (Micron Separations, Inc.). Nonspecific antibody binding was blocked by preincubation of the membranes with 5% skim milk in 1× TBS for 1 hour at room temperature. Membranes were then incubated overnight at 4°C with primary antibodies against respective target protein at a dilution of 1:1,000 in 1× TBS containing 2% skim milk, and were subsequently incubated with horseradish peroxidase–conjugated sheep anti-mouse IgG at 1:1,000 dilutions for 1 hour at room temperature. Bands were visualized by using the enhanced chemiluminescence kit according to the manufacturer's instruction (20).

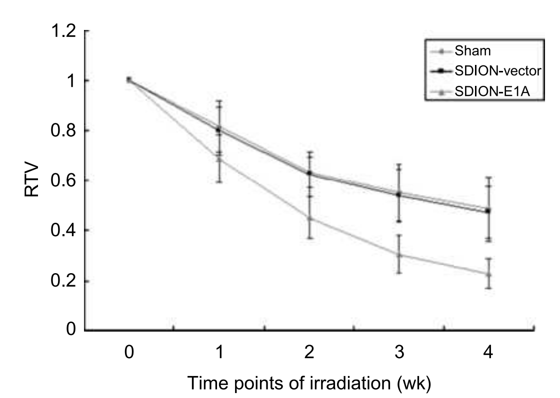

Figure 1. The RTV in xenograft mice following intratumoral injection of SDION-*E1A*. The mice tumors in the groups SDION-Vector and SDION-*E1A* were respectively injected with SDION carrying blank plasmid without *E1A* insert and SDION carrying *E1A* plasmid, and followed by radiotherapy. The mice in Sham group received PBS intratumoral injection and subsequent irradiation as the control. The entrance radiotherapy was applied 72 h after the first SDION-*E1A* injection. The value 0 on the abscissa indicates the time point of the first irradiation treatment.

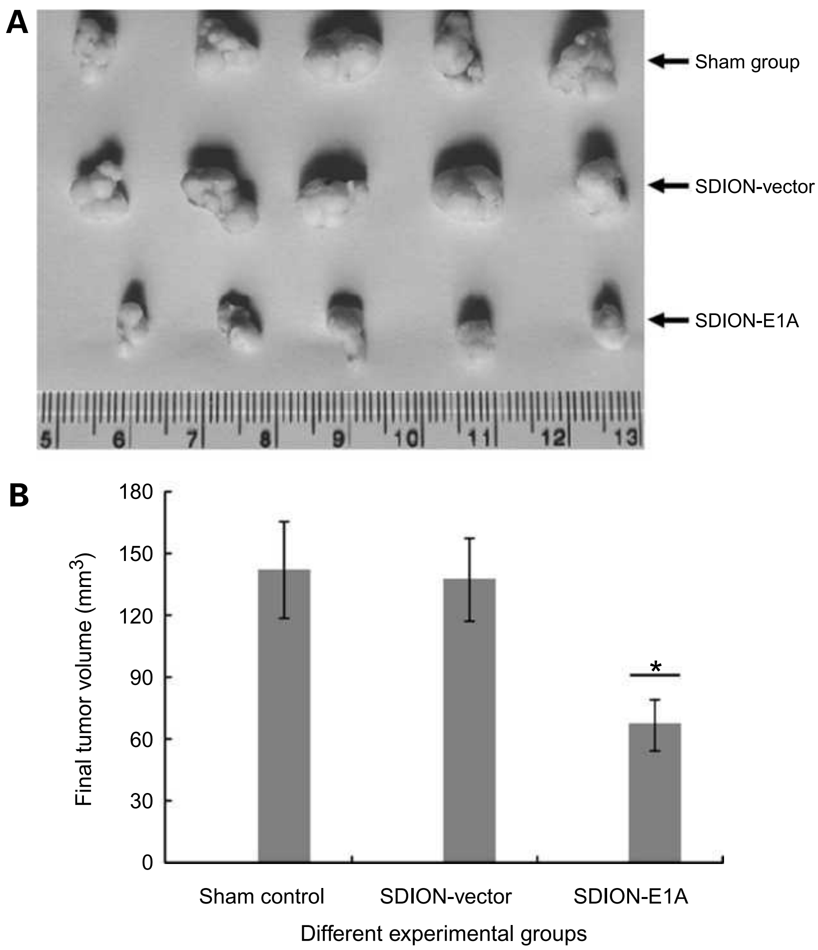

Figure 2. The final tumor volume in cervical cancer xenograft mice 30 days after the initial irradiation treatment. A, representative fresh tumor mass; B, the statistical histogram of the real tumor volume in different groups of sacrificed mice (n = 10). *, the statistical expression difference of final xenograft tumor volume between Sham control and SDION-*E1A* (P < 0.05).

Statistical analyses

To evaluate the value differences given as means ± SEM among different groups, the ANOVA and Fisher's protected least significant difference test were done using StatView software (version 5.0, SAS Institute, Inc.). The differences with P values of <0.05 were defined as statistically significant.

Results

The effects of SDION-*E1A* on tumor growth of xenografts treated with irradiation

Ten to 12 days after HeLa inoculation, most of the xenograft mice developed tumor mass that reached ~300 mm^3, and were administered the first SDION-*E1A* intratumor injection and a subsequent entrance irradiation 72 hours later. The index of RTV was used to reflect the effects of SDION-*E1A* on tumor size reduction in the nude mice. As shown in Fig. 1, the RTV in Sham group injected with PBS in tumor at 1, 2, 3, and 4 weeks after the first radiotherapy was 0.816 ± 0.103, 0.6333 ± 0.061, 0.554 ± 0.115, and 0.486 ± 0.128; 0.798 ± 0.096, 0.626 ± 0.089, 0.539 ± 0.106, and 0.472 ± 0.105 the in SDION-Vector group; and 0.687 ± 0.094, 0.452 ± 0.083, 0.305 ± 0.077, and 0.227 ± 0.058 in the SDION-*E1A* group, respectively. The statistical analyses did not show any significant difference of RTV between the groups of Sham and SDION-Vector at different time points (P > 0.05). However, the RTV in SDION-*E1A* group was significant lower than that in Sham and SDION-Vector groups at each time points from the 1st to the 4th week of irradiation, respectively (P < 0.05).

Thirty days after the first radiotreatment, the final tumor mass in xenograft mice was dissected and the representative fresh samples were presented in Fig. 2A. As shown in the histogram of Fig. 2B, the final tumor volume in the groups of Sham, SDION-Vector, and SDION-*E1A* was 141.9 ± 23.4 mm^3, 137.3 ± 19.8 mm^3, and 66.7 ± 12.1 mm^3, respectively. The mass volume in SDION-*E1A* group was significantly smaller than that in Sham and SDION-Vector groups (P < 0.05). However, there was no statistical difference of the final tumor volume between the groups of Sham and SDION-Vector ($P > 0.05$).

The effects of SDION-*E1A* on the mRNA and protein expression of *p53* and *HER-2/Neu* in tumor tissue

The mRNA expression of *p53* and *HER-2/Neu* in fresh tumor tissue, which was collected 30 days after the initial radiotherapy, was quantitatively analyzed by real-time RT-PCR, and the statistical results were shown in Fig. 3. As compared with that in Sham group (setting at 1), *p53* mRNA levels were 1.04 ± 0.17 and 1.89 ± 0.37, and *HER-2/Neu* mRNA levels were 0.97 ± 0.21 and 0.29 ± 0.11 in the groups of SDION-Vector and SDION-E1A, respectively. No statistical difference of *p53* and *HER-2/Neu* mRNA expression was shown between the Sham and SDION-Vector groups ($P > 0.05$). However, *p53* and *HER-2/Neu* mRNA levels in SDION-*E1A* group, respectively, were significantly higher and lower than that in control groups of Sham and SDION-Vector ($P < 0.05$).

As shown in Fig. 4, both mRNA and protein expression was shown to some extent in Sham and SDION-Vector groups applied as the experimental control. However, much more efficient E1A mRNA and protein was stably expressed in SDION-*E1A* using SDION as the *E1A* gene carrier (Fig. 4A). As compared with the gray scales of the electrophoretic gels representing target protein expression levels in the Sham group (setting at 1), that of *p53* were 1.42 ± 0.69 and 2.57 ± 0.73, and that of *HER-2/Neu* were 1.05 ± 0.41 and 0.36 ± 0.13 in the groups of SDION-Vector and SDION-*E1A*, respectively. No statistical difference of p53 and HER-2/Neu protein expression was identified between Sham and SDION-Vector groups ($P > 0.05$). However, *p53* and *HER-2/Neu* protein levels in the SDION-*E1A* group, respectively, were significantly higher and lower than that in the control groups of Sham and SDION-Vector ($P < 0.05$; Fig. 4B).

Discussion

Currently, gene therapy is a major focus in many kinds of cancer therapy, including cervical cancer (21). The goal of gene therapy is to alter the expression of a given protein resulting in therapeutic benefit by the delivery of polynucleotides to the cells and target tissue. However, no great progress and true treatment benefits can be realized until effective gene carrier and delivery systems have been developed. The viral vectors used as the gene carrier, which account for ~75% of all conducted clinical trials, are highly efficient as viruses have a highly evolved and specific mechanism for inserting their genome into that of host cell. On the other hand, incidences of severe adverse reactions using viral vectors during clinical trials, which are induced by viral pathogenicity, immunogenicity, and potential for insertional mutagenesis, have caused a gradual shift toward nonviral vectors in gene therapy (21).

Nanomaterials have been extensively investigated and have been at the forefront of gene delivery in the last few years. Meanwhile, more and more evidence has shown the nanogene delivery systems' advantages in cancer treatment due to their particular physicochemical and biological properties, including sustained gene delivery and prolonged release, high transfection efficiency, highly inert, low toxicity, and easy and cheap preparation (22). SDION is a kind of nonpolymeric but superparamagnetic dextran iron nanoparticles, which has 45% transfection efficiency as gene carrier *in vitro* higher than 30% of liposome. The large gene loading of SDION is implemented by the oxidation-reduction reaction in preparation and the tight connection with plasmid by the polymeric dextran surrounding SDION

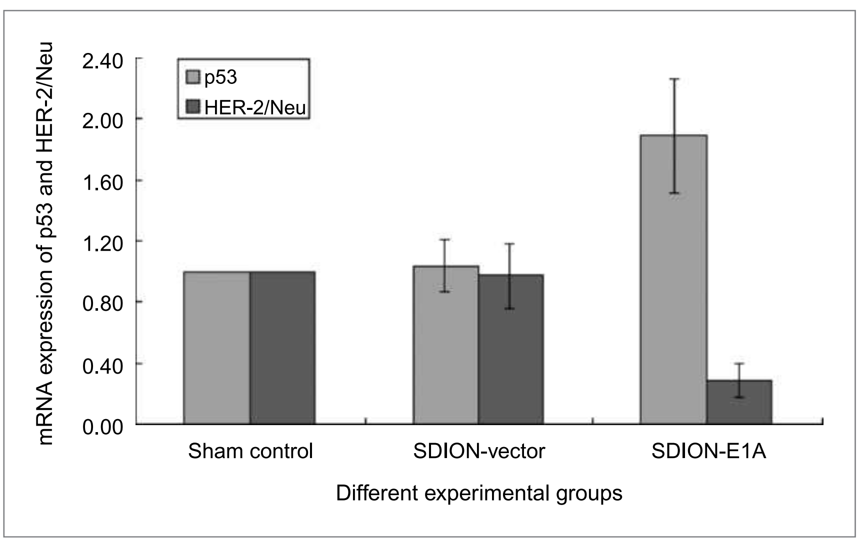

Figure 3. mRNA expression of *p53* and *HER-2/Neu* in xenograft tumor tissue ($n = 10$) identified by real-time RT-PCR. Applying β-actin as the internal loading control in three independent experiments, the mRNA expression levels of target genes in Sham group were set as 1. *, the significantly different expression levels of *p53* and *HER-2/Neu* between Sham control and SDION-*E1A* ($P < 0.05$).

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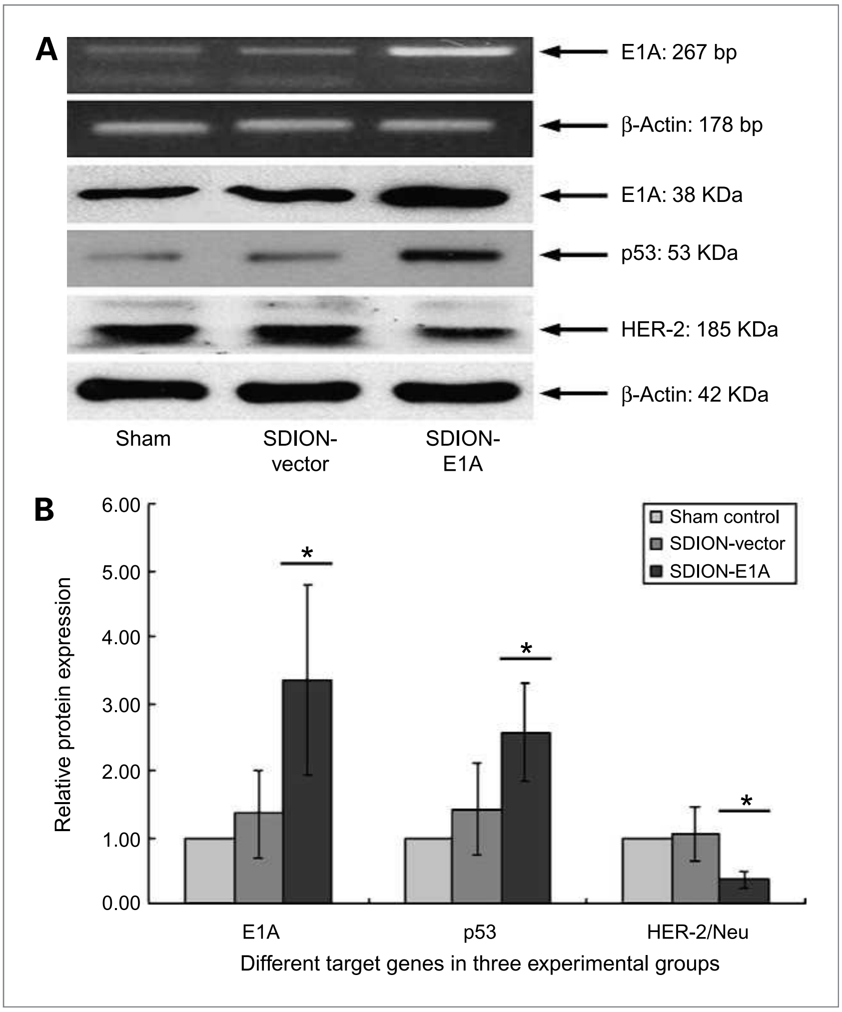

Figure 4. Expression of *E1A*, *p53*, and *HER-2/Neu* in different experimental groups. E1A mRNA expression in tumor tissue was identified by regular RT-PCR, and protein expression of *p53* and *HER-2/Neu* was shown by Western blot. A, representative electrophoretic gels; B, statistical analysis results of gray scale scanning with the gray scales in Sham group as the evaluation standard value of 1. β-Actin was used as the internal loading control in three independent experiments. *, the significantly different protein expression levels of *p53* and *HER-2/Neu* between experimental control and SDION-*E1A* treatment groups.

nanoparticles (16). As compared with the vectors of rival and liposome, another advantage of SDION is its nanodiameter, which is small enough to avoid macrophage phagocytosis and to achieve a prolonged circulating and functioning period (23). In the present project, *E1A* carried by SDION was efficiently and stably expressed by intratumoral injection weekly, even 30 days after the first procedure. In general, nonviral gene delivery vectors, including nanoparticles, have a potential deficiency of lower efficiency compared with viral vector. However, the important characteristic of superparamagnetic of SDION could potentiate the efficacy of magnetic nanoparticle up to several hundred-fold under a magnetic-guided field (24). Moreover, the application of SDION for specific targeting in systemic delivery guided by magnetic field and the magnetic hyperthermia cancer therapy is of considerable clinical significance and worthy of more attention in further investigations.

E1A is documented to function as a tumor suppressor gene in an independent manner in the tumor to inhibit the oncogenes of *HER-2/Neu* (25, 26). A series of gene therapy research of *E1A* focuses on breast and ovarian tumor with positive *HER-2/Neu* expression, and some encouraging preclinical trial results have been reported (27, 28). Gene therapy allowing for regional delivery of *E1A* is a clinical challenge and an attractive modality for the treatment of advanced cervical cancer, which expresses *HER-2/Neu* also and has a close correlation with tumor radiosensitivity. Rat embryo cells transfected with *E1A* were relatively radiation sensitive and nonmetastatic *in vitro* (29). *E1A* gene could significantly inhibit the growth rate of lymph node metastasis cell line 686LN-1 of human head and neck squamous cell carcinoma, and enhance the cell sensitivity to irradiation, which is consistent with our findings in an experimental animal model of human cervical cancer. These functions of *E1A* gene were assumed to be associated with its ability to suppress the *HER-2/neu* expression and to arrest the cell at G_2/M phase (30). HER2 has been shown to activate NF-κB; some data suggest a loop-like *HER2*-NF-κB-*HER2* pathway in radiation-induced adaptive resistance in breast cancer cells (31). An anti–*HER-2/neu* antibody trastuzumab at the dose of 10 μg/mL increased the irradiation response for the treatment of esophageal cancers, including adenocarcinoma and squamous cell cancer with *HER-2/neu* expression (32). An anti–*HER2*-antibody is also considered as an irradiation enhancement agent in human head and neck squamous carcinoma cells and breast cancer cells. Moreover, the upregulation of cyclin-dependent kinase inhibitors by *HER2* antibody may occur upstream of irradiation-induced *p53* upregulation (33). In clinical practice, *HER-2/neu* oncoprotein expression detected by immunostaining is useful for the prediction of chemoradioresistance in esophageal squamous cell carcinoma (34). We previously reported that *E1A* inhibited the proliferation of human cervical cancer HeLa cells through activation of the *HER-2/Neu*/caspase-3 pathway (6). The present data support that the downregulated *HER-2/Neu* expression by SDION-*E1A* regional delivery contributes to the radiosensitivity of human cervical cancer *in vivo*.

The tumor suppressor gene of *p53*, which is investigated in the present project, is also an important radiosensitizer and can be regulated by *E1A*. *E1A* was reported to facilitate Mdm4 binding to *p53* and inhibit Mdm2 binding to Mdm4, so as to result in decreased nuclear exportation of *p53* and stabilize the *p53* through targeting Mdm4 in a p14(ARF)-independent manner (4). During the infection of human cells, *E1A* expression may trigger redundant *p53*-independent and *p53*-dependent apoptotic pathways by binding to pRb− and/or p300 (35). Consequently, the intracellular accumulation of *p53* is assumed to be the result of the induction of unscheduled DNA synthesis by *E1A* proteins and that increased levels of *p53* then activate cell death pathways (36). *p53* coding for positive signal transduction factors acts as a central mediator of the cellular response to stressful stimuli and can influence transit through cell cycle checkpoints to further confer radiosensitivity upon tumor cells. *p53* is suggested to have a cooperative role with RAF1 protein in determining cellular radiosensitivity in human cells, which involves control of the G_2-M checkpoint (37). A large amount of evidence over the last two decades have shown that p53 expression is required for the efficacy of radiation and has led to considerable interest in the development of strategies to restore normal *p53* function in tumors with defective p53-dependent signaling (38, 39). *p53* activation has been proven to be an effective radiosensitizer, which is entirely attributable to an increased induction of *p53*-dependent cellular senescence, in some human cancers (40). It is also believed that *p53*-dependent signaling pathways are one of the central molecular mechanisms involved in a cell's response to irradiation and play the key role as a radiation sensitivity biomarker in clinical radiation oncology practice (41). Our experimental results suggest that *p53* expression can be stimulated and accumulated in human cervical cancer tissue in nude mice by SDION-*E1A* regional injection, so as to achieve a radiosensitization of xenograft tumor. On the other hand, there was some evidence also supporting the potent radiosensitivity inducer of *E1A* in a *p53*-dependent and *p53*-independent manner (42). Some murine and human malignant tumors, when expressing adenovirus *E1A*, were very sensitive to irradiation treatment *in vivo*, regardless of the *p53* status of the tumors (43). The most obvious hypothesis for the discrepancy in *E1A* function is that the specific cellular factors involved in the induction of apoptosis by *E1A* may be cell type dependent.

In conclusion, the intratumoral delivery of exogenous *E1A* carried by SDION increases *p53* expression but inhibits *HER-2/neu* expression, and enhances the radiosensitivity of human cervical cancer in xenograft mice.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Molecular Cancer Therapeutics

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