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Dendrimers-delivered short hairpin RNA targeting hTERT inhibits oral cancer cell growth *in vitro* and *in vivo*

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ABSTRACT

Promising therapeutic application of RNA interference (RNAi) depends on the availability of safe and efficient intracellular delivery systems. Human telomerase reverse transcriptase (hTERT), the catalytic subunit of telomerase complex, is an attractive therapeutic target for oral cancer. Here we investigated the characteristics and anticancer effect of polyamidoamine (PAMAM) dendrimer-mediated short hairpin RNA (shRNA) against hTERT in oral cancer. Dendrimer-mediated shRNA efficiently silenced the hTERT gene *in vitro*, resulted in cell growth inhibition and apoptosis. Treatment with the shRNA dendriplex attenuated tumor growth in a xenograft model. These studies suggest that RNAi-mediated hTERT gene silencing, coupled with dendrimer delivery, may provide a promising approach for the treatment of oral cancer, in which hTERT is abundantly expressed.

1. Introduction

Oral cancer is a prevalent cancer worldwide [1]. Despite improved understanding of the pathogenesis of this malignancy, the treatment outcome for oral cancer remains poor. Only modest improvements in survival have been reported and these are attributed mainly to earlier diagnosis [2]. Thus, novel therapies for oral cancer are greatly needed.

Manipulating only a handful of cell pathways can transform a normal human cell into a cancer cell [3]. One of these key pathways involves telomerase, a ribonucleoprotein complex which maintains the ends of chromosomes and may have other oncogenic roles. Several normal human cell types can be transformed into cancer cells by expressing only hTERT, an oncogenic variant of H-ras and the simian virus 40 (SV40) early region, which inactivates both the pRb and p53 pathways [4]. More recently, oral epithelial cells could be immortalized by overexpressing cyclin D1 and dominant negative p53, but required over-expression of EGFR and c-myc to acquire the ability to grow in an anchorage-independent manner and form tumors in nude mice [5]. Oral epithelial cells with these 4 genetic alterations that are common in oral squamous cell carcinoma (OSCC) also over-express hTERT.

Telomerase activity is predominantly regulated at the level of telomerase reverse transcriptase (TERT) gene transcription. In the oral mucosa, hTERT was previously found to be expressed in most oral cancers, but to be undetectable in normal oral epithelium [6–8]. Recently, studies also showed that hTERT was up-regulated in the surrounding OSCC tissues/cells, including the normal epithelia and the phlogistic cells [9]. Strikingly, ectopic expression of hTERT restores telomerase activity in telomerase-negative cell lines, including normal human oral keratinocytes (NHOK) [10], normal human oral fibroblasts (NHOF) [11], and cells with atypical dysplasia (D17) [12], suggesting an essential role of hTERT in oral carcinogenesis and malignant transformation. Inhibition of hTERT expression in various human cancers has been reported previously [13–16]. Recent results also suggested an anticancer role of hTERT, which might be independent of the telomere-elongating activity of the enzyme [17,18]. In oral cancers, however, the effects of silencing hTERT on tumor growth, and the cellular mechanisms, by which it induces apoptosis, remain unclear.

Polyamidoamine (PAMAM) dendrimers have unique physicochemical properties such as well-defined shape, highly branched architecture, monodispersity and multivalency [19]. Because of their molecular structure, PAMAM dendrimers have become attractive tools for the development of nanomedicine. Indeed, PAMAM dendrimers are widely used as drug delivery carriers and

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gene transfection agents (see reviews [19–21]). The ability of PAMAM dendrimers to deliver siRNAs, especially *in vitro* cell culture systems, has been documented [22–25]. On the other hand, recent studies showed that the dendrimer-mediated siRNA delivery and gene silencing depends on the stoichiometry, concentration of siRNA and the dendrimer generation [22].

Herein, we aim to explore the physicochemical characteristics and potential of PAMAM dendrimers for the delivery of short hairpin RNA (shRNA) targeting hTERT in oral cancer. Our results show that PAMAM dendrimer is an effective vector for siRNA delivery. Using dendrimer-delivered shRNA, we examined the role of hTERT in oral cancer and its effectiveness as a potential target for gene silencing in oral cancer *in vitro* and in mouse xenografts. We found that depletion of hTERT results in cellular apoptosis, at least partially, via caspase-9 activation and Bcl-2 down-regulation, and inhibits tumor outgrowth in xenotransplanted mice.

2. Materials and methods

2.1. Reagents

PAMAM dendrimers, generations (G) 2 and 5, were purchased from Sigma–Aldrich (St. Louis, MO). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA). Plasmid vector pRNA-U6.1/neo was obtained from Genscript (Piscataway, NJ). Primary antibody against hTERT was obtained from Novus Biologicals (Littleton, CO), Bcl-2 and cleaved caspase-9 antibodies were purchased from Santa Cruz (San Diego, CA), PCNA and beta-actin was purchased from Neomarker (Fremont, CA). *TeloTAGGG* Telomerase PCR ELISA kit was obtained from Roche Applied Science (Mannheim, Germany).

2.2. Plasmid construction

hTERT (GenBank accession number: AH007699.1) target sequences: 5′-AAGAAGCCACCTCTTTGGAGG-3′ (shRNA1) and 5′-AACATCTACAAGATCCTCCTG-3′ (shRNA2) were used. The shRNA expression vectors were constructed to encode a green fluorescent protein reporter to monitor transfection efficiency and a neomycin resistance gene cassette for cell selection. A survivin-oriented shRNA expressing vector (target sequence [26]: 5′-GGACCACCGCATCTC-TACA-3′) was constructed for use as a positive control, and a scramble shRNA plasmid consisting of a negative control #1 siRNA (P/N: 4404021) obtained from Ambion (Austin, TX) was constructed.

2.3. shRNA dendriplexes preparation and characterization

Purification and resuspension of dendrimers were performed as previously described [27]. shRNA dendriplexes were prepared by the addition of 50 nmol of shRNAs to varying proportions of PAMAM dendrimers (G2 and 5), in opti-MEM I medium (Gibco BRL, Rockville, CA) and incubated at room temperature for 30 min to allow complex formation. The samples were allowed to equilibrate for 30 min and then dropped on a carbon-coated copper TEM grid. A transmission electron microscope (Philips, Netherlands) was used to examine particle morphology according to the standard protocol. Hydrodynamic diameter and zeta potential of the dendriplex were performed on a dynamic laser scattering BIC-Zeta PALAS instrument (Holtsville, NY), and the polydispersity index was defined by the normalized *z*-average variance of the distribution of the diffusion coefficient.

2.4. Agarose gel electrophoresis

Dendriplexes were prepared at various N/P ([total terminal amines in dendrimers]/[phosphates in DNA]) ratios in Tris–HCl buffer. Stability of the dendriplexes was tested by gel electrophoresis with 1% agarose in standard TBE buffer. The DNA retardation was visualized under ultraviolet illumination at 375 nm.

2.5. Cell culture and transfection

Human oral cancer cell lines (Tca8113 and SCC-9) were maintained in Dulbecco's minimum essential medium (DMEM, Invitrogen) supplemented with 10% fetal calf serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). The cells were sequentially transfected three times with 80 nM shRNA dendriplexes (N/P = 5). Transfection efficiency was calculated 48 h after the final transfection by counting the percentage of green fluorescent protein expressing cells under a LSM 510 META laser scanning confocal microscope (LSCM) (Carl Zeiss), or by flow cytometry (FACScan, Becton Dickson). G418 (200 μg/mL) was used to select for stably transfected cells.

2.6. Real-time RT-PCR analysis

Primer sets and probes (5′ to 3′) for hTERT were: gctgctcaggtctttcttttatg, ctcttcaagtgctgtctgattcc, and (FAM)caacttgctccagacactcttccg(TAMRA); For actin were: gctcaccatggatgatgatatc, gccagattttctccatgtcgt, and (FAM)caacggctccggcatgtgc (TAMRA). Total RNA was prepared with a Trizol protocol and reverse-transcribed to cDNA with the ThermoScript™ RT-PCR Systems (Invitrogen) according to standard protocols. Real-time PCR was performed using the standard Taqman Gene Assays protocol on ABI 7900 HT real-time PCR detection system. The relative expression level was computed using the $2^{-\text{delta delta Ct}}$ analysis method, where beta-actin was used as an internal reference.

2.7. Western blot analysis

Cells were harvested from the plates and aliquots of cell extracts were separated on a 10% SDS–polyacrylamide gel. The proteins were transferred to polyvinylidendifluoride (PVDF) membrane and incubated overnight at 4 °C with the primary antibodies anti-hTERT (1:500) and beta-actin (1:2000), respectively. The protein bands were visualized using Immun-Star™ HRP Chemiluminescence Kits (Bio-Rad, Hercules, CA). The percentage reduction in band intensity was calculated relative to the untreated samples and normalized to beta-actin.

2.8. Immunocytochemistry

Treated cells were fixed, permeabilized, and blocked with 1% BSA. The chamber slides were immunostained with primary antibody against Bcl-2 (1:100) and cleaved caspase-9 (1:50), and then visualized with 3,3-diaminobenzidine (DAB). Hematoxylin was used to counterstain the cell nuclei.

2.9. Telomerase activity assay

Telomerase activity from cell extracts was analyzed using a PCR-based telomeric repeat amplification protocol (TRAP) assay following the manufacturer's instructions. Each condition was performed three times independently and the mean values were calculated.

2.10. Xenotransplant experiments

BALB/c nude mice (SPF grade, 6–8 weeks of age), purchased from the Animal Experimental Center of Sun Yat-sen University, were used for experiments. For carcinogenesis experiments, hTERT shRNA1 stably transfected Tca8113 cells (4×10^6) were injected

subcutaneously in 100 μL PBS into the right flank of each mouse. Scramble shRNA transfected or untreated Tca8113 cells were injected as the same procedures. For *in vivo* treatment experiments, mice were injected with untreated Tca8113 cells and when the tumors reached 3–5 mm in diameter, scramble shRNA or hTERT shRNA1 (20 μg) dendriplexes in 100 μL PBS or PBS alone was injected intratumorally. This treatment was performed every other day for three times. Tumor growth was monitored at regular intervals, and tumor volume was calculated according to the following equation: $V\ (mm^3) = width^2 \times length/2$.

2.11. Immunohistochemistry

Three weeks after the final transfection, the mice were euthanized, and the grafts were fixed and paraffin embedded. Four-micron sections were incubated with primary antibody against hTERT (1:100) and PCNA (1:200) overnight at 4 °C. All stained slides were analyzed for immunoreactivity using a standard 0 to 3+ semi-quantitation scoring system.

2.12. Cell apoptosis and proliferation assay

The apoptotic level was performed as previously described [28]. For proliferation assay, cells were seeded in 96-well plates and transfected with dendriplexes as described above. Cells were washed with PBS 24 h post-transfection and continue to grow in fresh culture medium for additional 2 days. MTT assays were carried out and OD_{495nm} value was determined.

2.13. Statistical analysis

Statistical analysis was using one-way ANOVA followed by two-tailed Student *t*-test (Statview 4.01, Abacus Concepts). hTERT and PCNA expression was analyzed by Mann–Whitney *U*-test. *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. Characterization of hTERT shRNA dendriplexes

PAMAM dendrimers are able to form compacted complexes ('dendriplexes') with plasmid DNA via electrostatic interaction. As shown in Fig. 1A, the dendrimer G5 could compact shRNA into nanoscale spherical complexes, with aggregation of particles occurred. At the same N/P ratio, the particle size of G5/shRNA complexes are about 110 nm in diameter while the particle size of G2/shRNA complexes are bigger than 300 nm (Table 1). The higher generation dendrimer, with geometric higher density of peripheral functional groups, displays an increased surface charge. In accordance with this phenomenon, the zeta potential for G5/shRNA was higher than that for G2/shRNA.

Next, we tested the binding ability of dendrimers using agarose electrophoresis. Fig. 1B shows the gel retardation results of dendriplexes at different N/P ratios. Complete retardation of shRNA was achieved at N/P ratio of 10 and 5 for G2 and G5, respectively. Additionally, we also evaluated the cytotoxicity of dendrimers in cultured oral cancer cells. Our results showed that both dendrimer G2 and G5 were nontoxic at the indicated shRNA concentrations and N/P ratios (data not shown). These results highlight the excellent physicochemical properties of dendrimer G5 for shRNA delivery and hence, it was used for further *in vitro* and *in vivo* experiments.

3.2. Inhibition of hTERT expression and telomerase activity

Tca8113 cells were transfected with hTERT shRNAs under optimal conditions. A high percentage of cells (58.6%) expressed

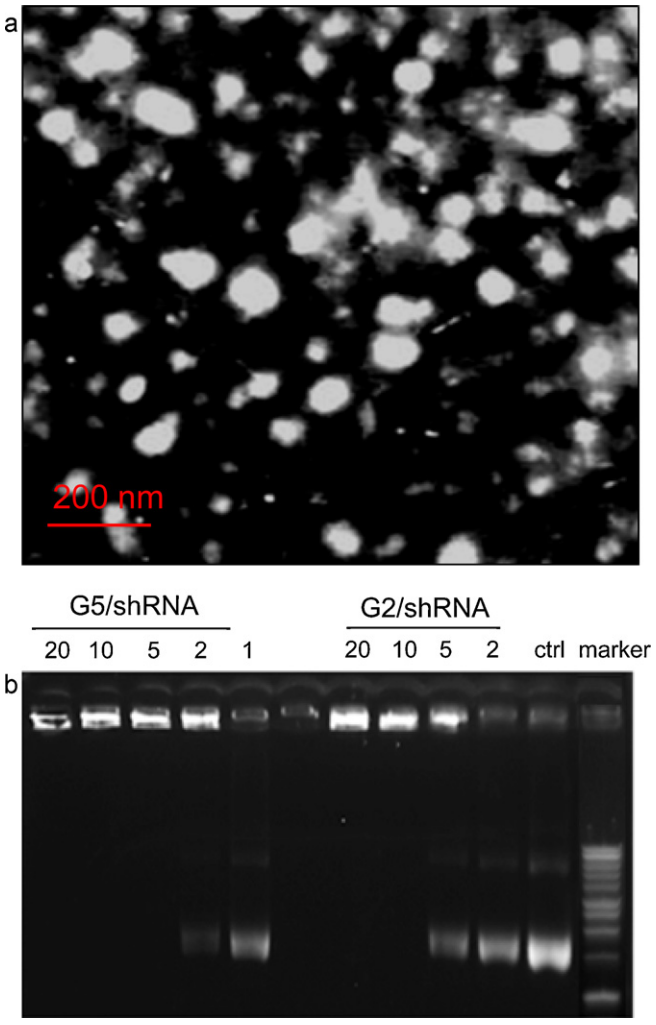

Fig. 1. Characterization of hTERT shRNA dendriplexes. (A) TEM image of hTERT shRNA/PAMAM dendrimer G5 complexes ('dendriplexes') showed that the dendriplexes possessed nanoscale spherical complexes. (B) The binding ability of dendrimers with shRNA at different N/P ratios was tested using agarose gel electrophoresis assay. Naked hTERT shRNA was used for internal control.

GFP 48 h after the final transfection (Fig. 2A, *left*), and cells became uniformly GFP+ after further selection in G418 (Fig. 2A, *right*). hTERT mRNA was markedly reduced in a dose-dependent manner (Fig. 2B). hTERT protein was reduced by more than 80% in Tca8113 cells after shRNA1 transfection (Fig. 2C). Another sequence (shRNA2) also silenced expression, but less effectively. Telomerase activity was significantly decreased in parallel with hTERT knockdown (Fig. 2D). Neither hTERT expression nor telomerase activity were affected by a scramble shRNA or survivin shRNA (Fig. 2B–D). Moreover, we also observed that hTERT expression and telomerase activities in SCC9 cells treated with shRNA1 and shRNA2 were significantly decreased compared that untreated or treated with control shRNA (Fig. 2B–D).

Table 1
Particle size and zeta potential of shRNA dendriplexes.

Samples	N/P ratio	Particle size (nm)	Zeta potential (mV)
G2-shRNA	5	309.2 ± 15.0	8.8 ± 1.3
G2-shRNA	10	315.9 ± 18.4	10.1 ± 0.8
G5-shRNA	5	107.5 ± 8.2	23.3 ± 2.5
G5-shRNA	10	112.6 ± 3.1	29.7 ± 0.7.

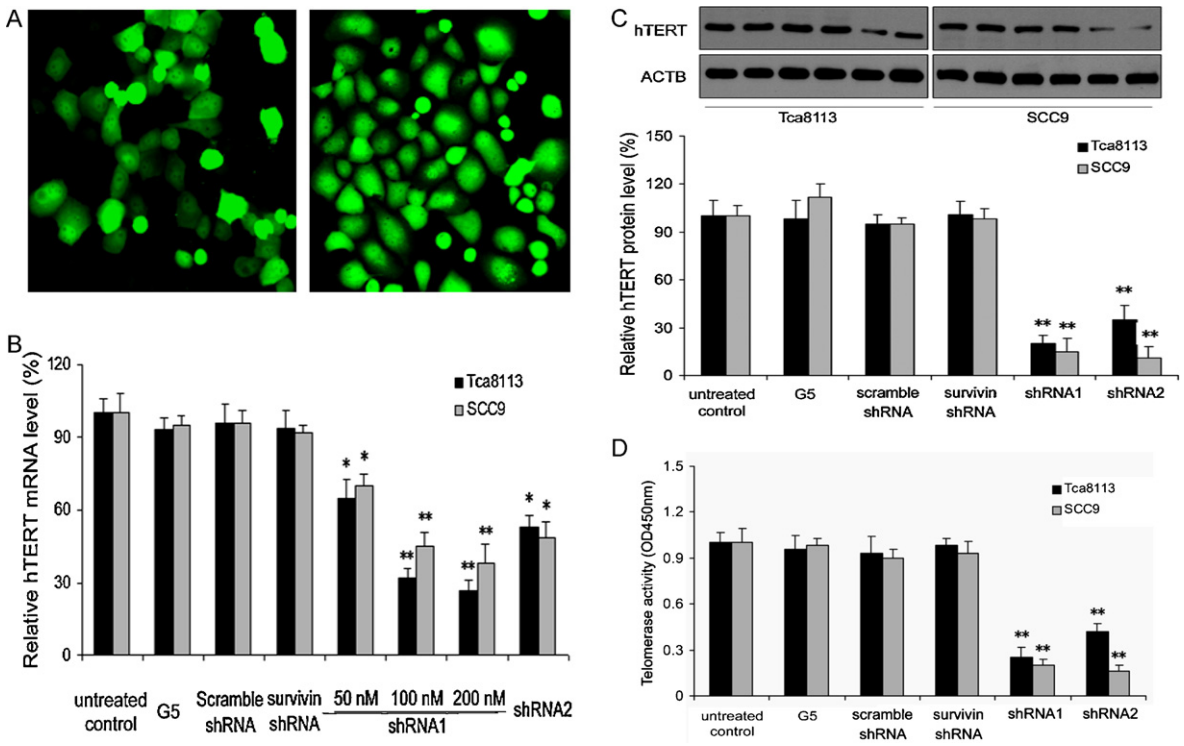

Fig. 2. Specific inhibition of hTERT expression and telomerase activity by hTERT shRNA. Tca8113 and SCC9 cells were transfected with hTERT shRNAs by PAMAM dendrimer G5 under optimal conditions. (A) Fluorescence images for GFP protein expression 48 hr after the final transfection (*left*) and 2 weeks after G418 selection (*right*) were taken. Cells transfected with the indicated shRNAs were analyzed for hTERT or β-actin expression level by quantitative RT-PCR (B) or immunoblot (C). (D) Telomerase activity was substantially reduced in tumor cells transfected with hTERT shRNA1 or shRNA2. *, $P < 0.05$; **, $P < 0.001$, compared with untreated cell control. Data are the mean ± SD of three independent experiments performed on duplicate samples.

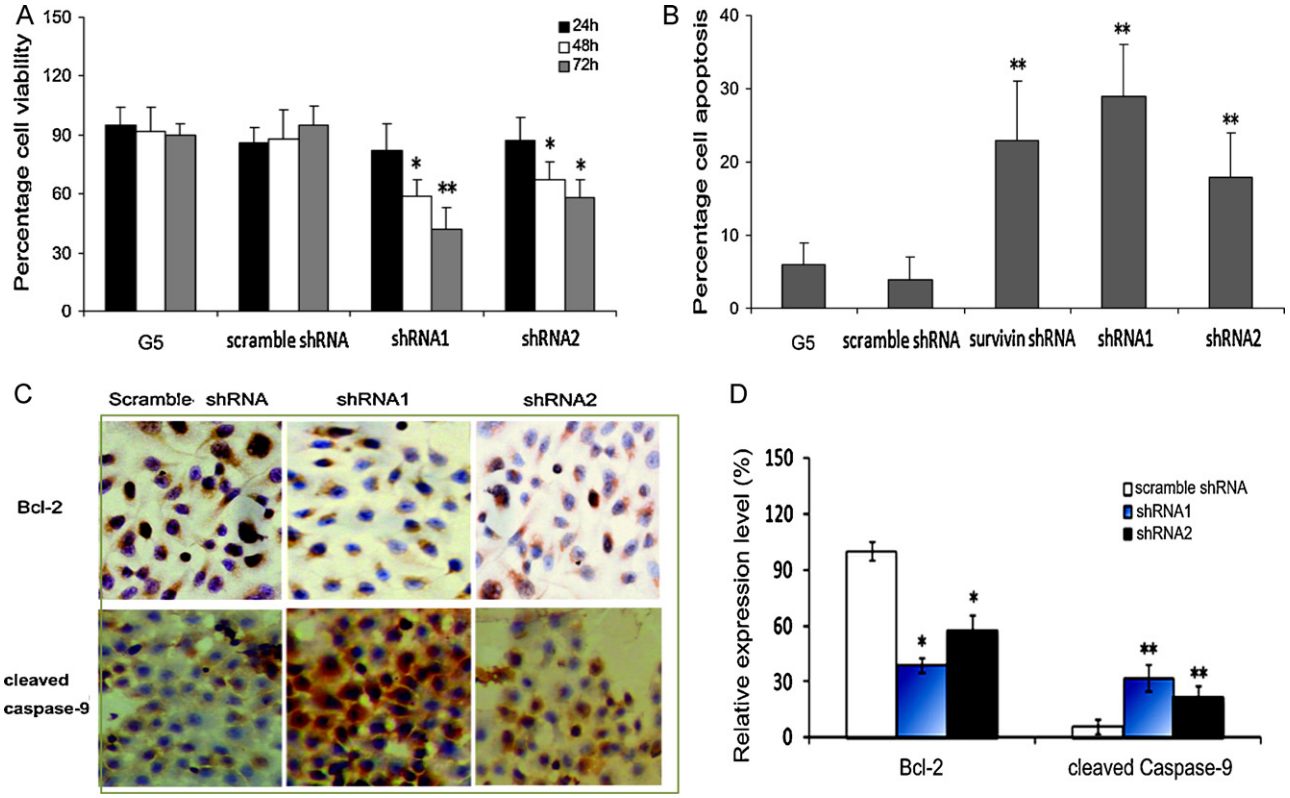

Fig. 3. Cell growth inhibition induced by dendrimer delivered hTERT shRNAs. (A) hTERT shRNAs reduce the numbers of viable Tca8113 cells assessed by MTT assay. Results are normalized to the untreated cell control (100%, control). (B) Apoptosis, assessed by annexin V-FITC/PI staining. (C) Representative immunostaining for Bcl-2 and cleaved caspase-9 in hTERT shRNA-treated Tca8113 cells compared to scramble control. (D) Quantification of Bcl-2 and cleaved caspase-9 protein expression by immunocytochemical assays, and scored as described in Section 2. *, $P < 0.05$; **, $P < 0.001$, as compared with control.

3.3. hTERT knockdown reduces proliferation and induces apoptosis

We evaluated the cellular effects of hTERT knockdown in Tca8113 cells. Overexpression of scramble shRNA did not affect cell growth on day 7 after transfection. In contrast, inhibition of cell growth was evident by day 2 after transfection of cells with hTERT shRNA (Fig. 3A). Apoptosis was also assessed by flow cytometry after transfection (Fig. 3B). Cellular toxicity induced by dendrimer transfection on its own or scramble shRNA transfection was negligible (Fig. 3A and B). Survivin shRNA, as expected for a positive control, increased cell apoptosis. hTERT shRNA-treated cells had significantly higher apoptotic level than untreated, G5 alone or scramble shRNA-treated cells ($P < 0.001$). Moreover, the onset of apoptosis was rapid, being evident within 2 days after transfection, reaching a plateau at day 3 in Tca8113 cells. Similar findings were observed in SCC9 cells treated with hTERT shRNA1 or shRNA2 (Supplementary Fig. 1).

3.4. hTERT silencing leads to down-regulation of Bcl-2 and caspase-9 activation

To explore potential mechanisms responsible for the pro-apoptotic effect of hTERT shRNAs, we examined an upstream and downstream mediator of cellular apoptosis in Tca8113 cells after hTERT knockdown. Expression of the anti-apoptotic Bcl-2 protein was attenuated, whereas caspase-9 activation was significantly increased in the shRNA-treated cells, compared to scramble control cells (Fig. 3C and D). Moreover, Bcl-2 expression and caspase-9 activation correlated with hTERT knockdown (Bcl-2, $r = 0.79$, $P < 0.01$; caspase-9, $r = -0.6$, $P < 0.001$).

3.5. Reduced tumorigenicity of Tca8113 cells after hTERT knockdown

To determine whether hTERT silencing could inhibit tumor formation in a xenotransplant setting, Tca8113 cells that were stably transfected with hTERT shRNA or controls were injected subcutaneously into nude mice. Both untransfected Tca8113 cells and cells transfected with scramble shRNA (4×10^6 cells/mouse) rapidly and uniformly formed tumors in BALB/c nude mice. As illustrated in Fig. 4A, tumor formation in mice injected with hTERT-silenced Tca8113 cells was markedly suppressed. Only 10 of 14 mice treated with hTERT shRNA1 transfected cells developed tumors, compared to 100% of control mice. More importantly, tumor growth was delayed. While control tumors were palpable within 7 days of injection, hTERT silenced tumors did not become detectable until 15 days or later. The size of tumors in mice that formed tumors was also significantly smaller when the mice were sacrificed at day 21.Therefore, silencing hTERT significantly inhibited tumor growth *in vivo*.

3.6. Intratumoral injection of hTERT shRNAs complexed with dendrimers inhibits subsequent tumor outgrowth

To investigate whether hTERT shRNA could be administered *in vivo* to already established tumors (a setting more relevant to cancer treatment) Tca8113 subcutaneous tumors were allowed to grow to ~3–5 mm in diameter before mice were treated with intratumoral injection of PBS or dendrimer-delivered hTERT shRNA1 or scramble shRNA. Tumor outgrowth was significantly slower in mice treated with shRNA1 dendriplexes (Fig. 4B). When mice were sacrificed 15 days after treatment, tumors of the treated mice were approximately half the size of the control tumors. Moreover, the hTERT shRNA-treated xenografts had signs of necrosis and involution (Fig. 5A). In addition, expression of hTERT and PCNA protein was also reduced in xenografts of tumors injected with the hTERT shRNA1 dendriplexes ($P < 0.01$) (Fig. 5B and C). Therefore, dendrimer efficiently delivered shRNA1 into tumor xenografts and had a substantial antitumor effect *in vivo*.

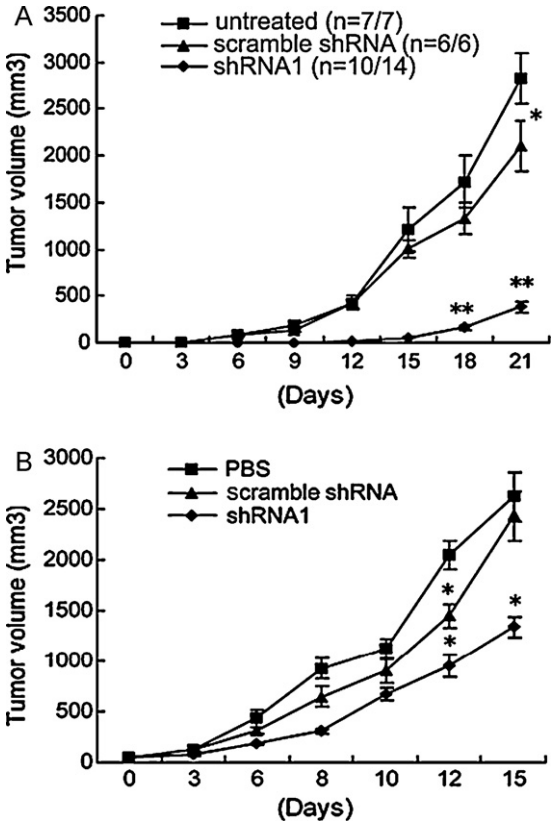

Fig. 4. RNAi-mediated hTERT downregulation inhibits Tca8113 cell growth in nude mice. (A) Tumor cells were either untreated or transfected with dendrimer-delivered scramble shRNA, or stably transfected hTERT shRNA1 before subcutaneous injection. Mean tumor volumes were monitored by calipers. The parentheses indicate the numbers of mice that formed tumors in each group. (B) Tca8113 oral cancer xenografts were treated with intratumoral injection after tumors were established and had reached 3–5 mm diameter. Graphs show mean ± SD. *, $P < 0.05$; **, $P < 0.001$, as compared with mock treated control.

4. Discussion

It has been well documented that cancer cells frequently overexpress hTERT, a limiting factor of telomerase activity, resulting in enhanced proliferation and tumor progression. The up-regulation of hTERT provides an attractive therapeutic target. Indeed, a variety of gene targeting approaches including antisense oligonucleotides [29], hammerhead-like nucleases [30], and dominant-negative hTERT [31] were described to interfere with hTERT function. Although very effective and selective *in vitro*, these gene therapies require pretreatment with cytotoxic transfection reagents and/or enhancers, and therefore could not be readily translated to clinical application. More recently, siRNAs were used to specifically knockdown the targeted hTERT gene [13–16,32]. It is now clear that siRNAs can be potent telomerase inhibitors, but important issues, including incomplete suppression of target genes, *in vivo* delivery systems and nonspecific immune response, hamper the development of this novel therapy for cancer treatment.

In this study, we demonstrated the effect of dendrimers-mediated shRNAs against hTERT on human oral cancer cells and mouse tumor xenografts. Our study showed that shRNAs expressing vectors were efficiently transfected into Tca8113 cells *in vitro* and *in vivo* via dendrimer nanoparticles. The shRNA against hTERT

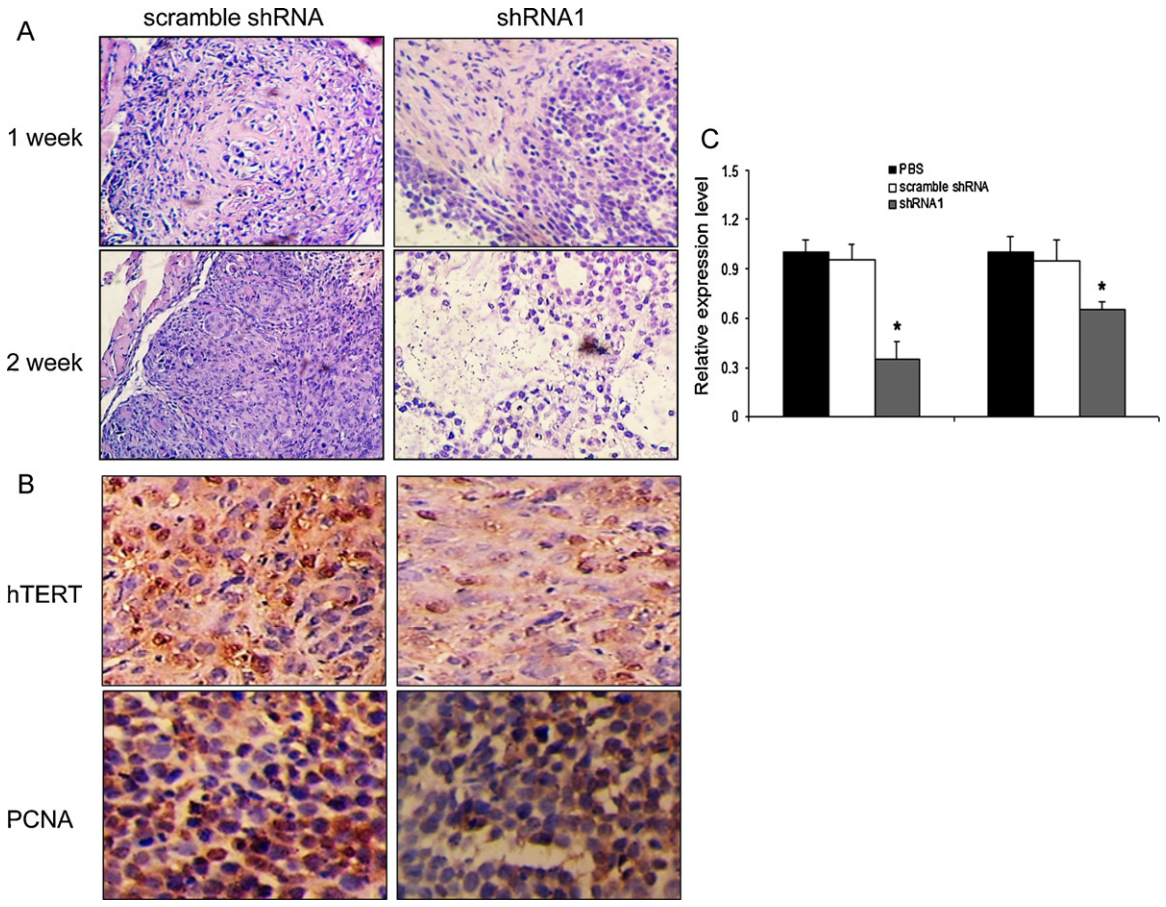

Fig. 5. Intratumoral injection of hTERT shRNA1 dendriplexes leads to tumor necrosis, decreased hTERT and PCNA protein expression in Tca8113 xenografts. (A) Xenografts treated with hTERT shRNA1 showed necrosis and degeneration compared with those receiving scramble shRNA (×40). (B) Representative immunostaining for hTERT and PCNA protein in Tca8113 xenografts which were obtained from the hosts two weeks after the final transfection (×200). (C) Expression levels of hTERT and PCNA protein were determined and scored as described above. *Bars*, ±SD. *, $P < 0.05$, as compared with PBS.

led to a marked reduction of hTERT expression, tumor growth and tumorigenesis. These results may partly be attributed to hTERT gene pathway selectivity via shRNA or by passive tumor accumulation through 'enhanced permeability and retention' effect [33], or both. While there are differences between the two mechanisms, the key issue is whether distribution into the tumor achieves cellular uptake and siRNA activity [34]. Previous studies have demonstrated that dendrimers have favorable characteristics including monodispersity, well-defined structure, low cytotoxicity, and host–guest entrapment properties for delivering nucleic acids for clinical use [19–21]. We have investigated that the average size of the dendriplexes (G5) used in this study was ~110 nm and the zeta potential was ~30 mV (Table 1), which are favorable biophysical parameters for escape from the vasculature and intracellular delivery. We now show that dendrimers are efficient carriers to deliver shRNA plasmids and induce gene silencing *in vitro* and *in vivo*. The high transfection rate and gene silencing was maintained for at least 3 days, without apparent cytotoxicity. These results suggest that shRNA targeting hTERT, coupled with dendrimer-based delivery, may be therapeutically useful for OSCC and potentially for other malignancies. Silencing of hTERT might also be combined with silencing of other genes, such as EGFR or c-myc, implicated in transformation of OSCC. This method may also potentially be used to deliver siRNAs in place of shRNA plasmids. In this study we only investigated intratumoral injection; further work is needed to determine whether this approach could also be used for systemic intravenous delivery, which would be required to treat most types of cancer. It also should be noted that an unexpected growth inhibition in day 21 (Fig. 4A) and day 12 (Fig. 4B) was observed in scramble shRNA transfected cells, which may due to the limited sample size, the *in vivo* 'off-target' effect [35], or other unknown reasons.

In addition to its role as a cellular survival factor, accumulating evidence suggests that hTERT also has an anti-apoptotic effect in tumor cells [17,36]. However, the mechanisms reported by these studies are different. In this study, we demonstrated for the first time that shRNAs against hTERT not only led to a significant decrease in surviving cells, but also triggered apoptosis of OSCC cells (Fig. 3). To explore the possible mechanisms underlying the role of hTERT in regulating apoptosis, we investigated the effect of silencing hTERT on the expression of Bcl-2 and caspase-9, critical regulators of apoptosis. We found that Bcl-2 expression was down-regulated, while caspase-9 activation was increased. Moreover, these changes correlated with the degree of hTERT silencing. Our data suggest that Bcl-2 and caspase-9 may be involved in the regulation of mitochondrial apoptosis induced by treatment with hTERT shRNAs in human OSCCs, similar observations are also reported in cervical cancers, colon cancers and pancreatic cancers [16,37]. However, changes in expression or activation of other molecules may also be important in inducing apoptosis and need to be investigated.

In conclusion, these studies suggest that the ability of dendrimers-based protocol to transfect cells efficiently in oral cancers *in vitro* and *in vivo*, coupled with the effectiveness of shRNA targeted for hTERT demonstrated herein, will extend the application of shRNA to nano-based therapies and provide the rationale

for the development of telomerase-targeted therapies to improve patient outcome in oral cancers. Meanwhile, demonstrated potential of targeted inhibition of hTERT as a putative therapeutic strategy in this study necessitates further study to elucidate the intricacies linking genetic and epigenetic modulations of the gene.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2011.03.017.

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