CD59 is overexpressed in human lung cancer and regulates apoptosis of human lung cancer cells

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Abstract. CD59, belonging to membrane complement regulatory proteins (mCRPs), inhibits the cytolytic activity of complement and is overexpressed in many types of solid cancers. The aim of the present study was to detect the expression of CD59 in non-small cell lung cancer (NSCLC) and to investigate the relationship between decreased CD59 expression and tumorigenesis of NSCLC by transfecting recombinant retrovirus encoding shRNA targeting human CD59 into the human NSCLC cell line NCI-H157. CD59 expression in NSCLC was detected by immunocytochemistry (IHC). In the human NSCLC cell line NCI-H157, CD59 mRNA and protein expression suppressed with lentivirus-mediated RNAi was confirmed by using RT-PCR and western blotting, respectively. The proliferation and apoptosis of NCI-H157 cells was measured by using MTT assay and FACS. The resistance to complement cracking ability was detected by LDH assay. Caspase-3 expression in cells was assessed by IHC. Bcl-2 and Fas protein was determined by western blotting both *in vitro* and *in vivo*. CD59 is overexpressed in human NLCLC cancer. In NCI-H157 cells, lentivirus-mediated RNAi significantly reduced both CD59 mRNA and protein expression, which resulted in suppressing cell proliferation and increasing cell apoptosis. When incubated with fresh normal human serum (8%, v/v) for 1 h at 37°C, the cell viability was decreased and cell apoptosis was increased in siCD59-infected NCI-H157 cells compared to siCD59-C-infected cells. Reduced CD59 expression led to increased expression of caspase-3 and Fas and decreased expression of Bcl-2. Furthermore, the nude mouse tumor graft weight was significantly decreased and survival rate was significantly increased in the siCD59 group. CD59 is overexpressed in human NLCLC. CD59 silencing in NSCLC cancer cells via retrovirus-mediated RNAi can enhance complement-mediated cell apoptosis, inhibiting the growth of NSCLC. CD59 may serve as a potential target for gene therapy in NSCLC.

Introduction

Lung cancer is the most common cancer worldwide and the leading cause of cancer-related mortality (1). Non-small cell lung cancer (NSCLC) accounts for ~80% of all lung cancer cases and has a 5-year overall survival rate of <15% (2,3). Approximately 40% of patients diagnosed with NSCLC have unresectable stage III disease or inoperable disease (4). Therefore, additional efforts are needed to understand and identify molecular targets for gene therapy (5,6).

The cluster of differentiation 59 (CD59), also called protectin, is a type of complement regulated proteins (CRPs). CD59 inhibits the complement cytolytic activity by binding to C8 and C9, which blocks the assembly of the membrane attack complex (MAC) (7). CD59 is overexpressed in most solid malignancies and presents at low levels in normal tissues. CD59 overexpression may assist malignant cells to escape immunologic surveillance and complement-mediated cytolysis, limiting the effect of complement-fixing monoclonal antibodies (7-10). Immune escape of tumor cells is a primary cause of failed immunotherapy. Blocking of CD59 function on the surface of tumor cells might allow effective complement-mediated clearance of tumor cells that improve the effect of complement-activating antitumor antibodies (11,12). Therefore, CD59 is a promising therapeutic target for antitumor gene therapy.

RNA interference (RNAi) is an economical, fast and highly efficient technique for silencing gene expression (13-15). Recently, siRNA-encoding plasmids delivered by virus has been rapidly developed and widely applied in mammalian cells (16,17). In this study, we examined the expression of CD59 in NSCLC and constructed a CD59 small interfering RNA (siRNA) lentiviral vector. We assessed its effect on the proliferation and apoptosis of lung cancer cells and further characterized the functional role of CD59 during lung cancer tumorigenesis.

Materials and methods

Expression of CD59 in tissue specimens by immunohistochemistry (IHC). Twenty primary NSCLC specimen and

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corresponding surgical margin specimens were obtained from the People's Hospital of Guangxi Zhuang Autonomous Region. A mouse polyclonal antibody for CD59 was obtained from Santa Cruz Biotechnology (SC-133171) to assess the expression of the CD59 protein. Briefly, paraffin-embedded tissue sections (5-μm thick) were deparaffinized with xylene and then dehydrated in sequential diluted ethanol before rinsing in PBS. Sections were heated for 10 min in 0.01 M citrate buffer (pH 6.0) twice to unmask the antigens. Endogenous peroxidase activity was then blocked with 3% hydrogen peroxide for 20 min at room temperature. Before being incubated with CD59 antibody (dilution, 1/100 in 0.01 M PBS) at 4°C overnight, sections were incubated with 5% normal goat serum in 1% BSA in PBS for 30 min to block non-specific IgG binding. A biotinylated goat antimouse IgG was used for further incubation and a strepavidin-biotin complex system (SABC) with diaminobenzidene as chromogen was used for color development. The sections were weakly counterstained with hematoxylin before mounting and then examined under a light microscope. PBS (0.01 M) was used to replace primary antibody to serve as negative staining controls. Immunohistochemical staining was evaluated by two independent pathologists.

Construction of small interfering RNA targeted CD59 expression vector, production of lentivirus and transfection into NCI-H 157 cells. The pSUPER vector was digested by *Bgl*II and *Hin*dIII restriction enzyme and annealed oligos, siCD59: 5'-GATCCCCGCGTGTCTCATTACCAAAGttcaagagaCTT TGGTAATGAGACACGTTTTA-3. siCD59-C: 5'-AGCTTA AAAAGCGTGTCTCATTACCAAagtctcttgAACTTTGGTA ATGAGACACGCGGG-3' were ligated with this vector. The recombinants were identified by PCR, restriction endonuclease analyses and DNA sequencing, respectively.

A packaging cell line Phoenix A and human non-small lung cancer cell line NCI-H157 cells (18) were cultured in DMEM and RPMI-1640 supplemented with 10% FBS (Gibco, Carlsbad, CA, USA) and penicillin/streptomycin, incubating in a humidified incubator (37°C, 5% CO_2). For retroviral production, Phoenix A cells were transfected with this recombinant using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Culture supernatants were collected after 48 h. NCI-H157 cells were infected with siCD59 and siCD59-C in the presence of 8 g/ml polybrene, follow by clonal selection with G418 (400 mg/l) to generate stable clones.

Detection of the expression of CD59 mRNA by real-time quantitative PCR (RT-qPCR) test. Total RNA from different groups of NCI-H157 cells was isolated using TRIzol reagent (Invitrogen). Two microgram of total RNA was reverse-transcribed in a 20-μl reaction using reverse-transcription system (Promega, Madison, WI, USA). Primers were designed based on sequences of human CD59 and β-actin. The forward primer of CD59 was 3'-ACACCATTGCTGGGGACCTC-5' and the reverse primer was 3'-GCTGAATCTTAAAGTCAGGCAA AGG-5'. The forward primer of β-actin was 3'-CACACCCGC CACCAGTTCGC-5' and the reverse primer was 3'-AGCACAG GGTGCTCCTCAGGG-5'. The amplified sequence was 356 and 332 bp, respectively. Thermo cycling was carried out as follows: CD59; 94°C for 5 min, then 40 cycles of 94°C for

45 sec, 52°C for 45 sec and 72°C for 45 sec, followed by 72°C for 7 min or β-actin 94°C for 5 min, then 40 cycles of 94°C for 40 sec, 58°C for 35 sec and 72°C for 45 sec, followed by 72°C for 7 min. PCR products were quantified by using Quantity One 6.4.0 (Bio-Rad, Hercules, CA, USA) Software. CD59 levels were normalized to β-actin.

Determination of CD59 protein expression by western blotting. Different groups of NCI-H157 cells were harvested at the indicated time-points, washed twice with cold phosphate-buffered saline (PBS), lysed in fresh cell lysis buffer for 2 h on ice and centrifuged at 12,000 g for 15 min at 4°C to remove insoluble materials. Protein concentrations were determined by BCA assay. Protein (20 μg) was separated by using 8 and 5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to PVDF membranes. The membrane was incubated with anti-CD59 (1:500) and anti-β-actin (1:200) antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), respectively, followed by incubation with horseradish peroxidase-conjugated goat anti-mouse secondary antibody. Western blots were developed by chemiluminescence and quantified using Quantity One 6.4.0 (Bio-Rad) software. CD59 levels were normalized to β-actin.

Determination of the NSCLC cell proliferation by MTT assay. Different groups of NCI-H157 cells were cultured for 3 days in 96-well plates and then incubated in 5% MTT at 37°C for 4 h. DMSO (100 μl/well) was added and the light absorption value at 490 nm was measured.

Determination of the NSCLC cell apoptosis by flow cytometry. Different groups of NCI-H157 cells were seeded in 6-well culture plates. Each group contained five culture flasks. After 24 h, the cells were harvested and washed in cold PBS. Annexin V and PI staining were carried out using the Annexin V-FITC Apoptosis Detection kit (BD Biosciences, USA), according to the manufacturer's protocol. After a 20-min incubation in the dark at room temperature, the cells were immediately analyzed by FACSCalibur Flow Cytometry (BD Biosciences).

Determination of the NSCLC cell resistance to complement cracking ability by LDH release test. Different groups of NCI-H157 cells were treated with complement inactivated 8% fresh normal human serum (NHS) for 60 min at 37°C. Triton X-100 (0.1%) in RPMI-1640 was used as the 100% lysis control and RPMI-1640 alone was used for the 0% lysis control. Following incubation, 40 μl of sample supernatant was taken for LDH assay. To each well 100 μl solution C was added. Just before analysis, 10 μl solution B was added. The absorbance at 440 nm was calculated, reflecting the activity of LDH present and the following equation applied: LDH leakage rate.

Detection of the expression of caspase-3 in cells by immunohistochemistry (IHC). Different groups of NCI-H157 cells ($2x10^4$ ml) were seeded onto glass coverslips for 48 h. After incubation with fresh NHS for 6 h, the cover slips were washed thrice with PBS, 2% PBS-paraformaldehyde solution was added for 15 min at room temperature and 0.4% Triton X-100 was added at room temperature for 15 min. After washing thrice with PBS, the cells were treated with 30% H_2O_2. The
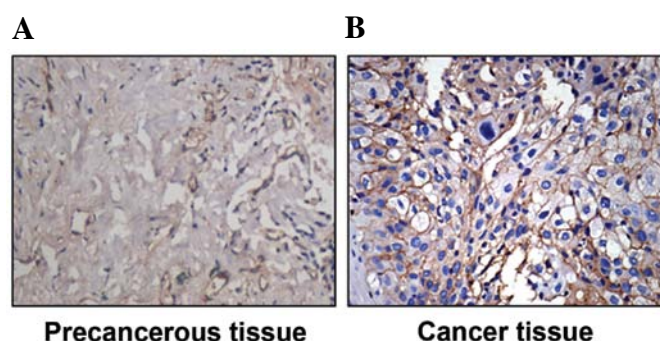
Figure 1. Expression of CD59 in non-small cell lung cancer. (A) Expression of CD59 in the surrounding tissue. (B) Expression of CD59 in the non-small cell lung cancer.

caspase-3 protein expression levels were measured by IHC staining. In brief, cells were incubated in blocking buffer at room temperature for 20 min, followed by the rabbit anti-human caspase-3 antibody (1:500 Abcam, Cambridge, MA, USA) at 4°C overnight followed by an additional washing step (3X) with PBS. Secondary antibody labeled with HRP (mouse anti-rabbit) was added and incubated at 37°C for 1 h followed by washing (3X) with PBS. SABC was added at 37°C for 20 min and DAB at room temperature for 5-30 min and the results were observed under a light microscope. Positive cells were stained brownish yellow. Caspase-3 protien levels were determined by positive index = positive percentage (%) x mean optical density x 100.

Determination of Bcl-2 and Fas protein by western blotting. Total protein extraction of different groups of NCI-H157 was performed, as described above. Twenty microgram (20 μg) was separated using 8 and 5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to PVDF membranes. The membrane was incubated with anti-bcl-2 (1:500), anti-Fas (1:10,000) and anti-β-actin (1:200) antibodies (Santa Cruz), followed by incubation with horseradish peroxidase-conjugated goat anti-mouse secondary antibody. Western blots were developed by chemiluminescence and bands were quantified by using Quantity One 6.4.0 (Bio-Rad) software. CD59 levels were normalized to β-actin.

Xenograft studies. Athymic male nude mice (5- to 6-week-old) were purchased from Laboratory Animal Centre of Guangxi

Medical University and housed under pathogen-free conditions. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the Guangxi Medical University. Thirty mice were randomly divided into three groups of 10 each: control group, siCD59 groups and siCD59-ctr groups. Each mouse of every group was subcutaneously respectively injected with $2x10^5$ luciferase-expressing Luc-NCI-H157 cell, siCD59 and siCD59-ctr cells (Invitrogen) into the right flank. Tumor growth/regression was monitored every 5 day by *in vivo* imaging after intraperitoneal injection of firefly luciferin (150 mg/kg) to the mice using an non-invasive imaging system (Roper, USA). Each mouse cohort was also monitored for 60 days to determine the tumor volume and the survival rate.

Determination of CD59 mRNA and CD59, Bcl-2 and Fas protein expression in vivo. Mice were sacrificed at the end of the experiments by CO_2 asphyxiation. Tumors were excised and immediately snap-frozen in liquid nitrogen. Total RNA and protein extraction of tumor tissue were frozen in liquid nitrogen. CD59 mRNA expression was determined by RT-PCR and CD59, Bcl-2, Fas protein was determined by western blotting.

Statistical analysis. Data were analyzed using GraphPad Prism 5.0 statistical software. Quantitative data were expressed as the means ± standard deviation. Significant difference was determined by P-values <0.05.

Results

Expression of CD59 in lung cancer. CD59 expression was significantly higher in tissues from non-small cell lung carcinoma (NSCLC) than in preneoplastic tissue (67.6 vs. 4.3%, P<0.05) (Fig. 1).

CD59-siRNA efficiently suppressed CD59 expression. Recombinant retroviruses siCD59 and siCD59-control (siCD59-C) both contain a green fluorescent protein (GFP) reporter gene, which allowed for measuring infection efficiency in NCI-H157 cells. Forty-eight hours post-infection, the infection efficiency was ~70% in both siCD59 and siCD59-C infected cells (Fig. 2). CD59 mRNA and protein levels were decreased significantly in siCD59 infected cells compared to siCD59-C infected cells (Fig. 3).
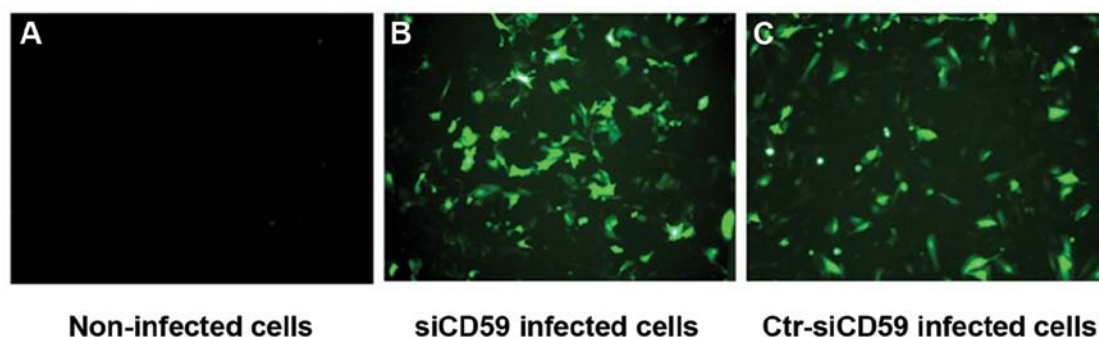

Figure 2. Fluorescence microscope images of NCI-H157 cells 48 h after infection. (A) Luc-NCI-H157 cells without infection. (B) Luc-NCI-H157 cells infected by siCD59. (C) Luc-NCI-H157 cells infected by siCD59-C.
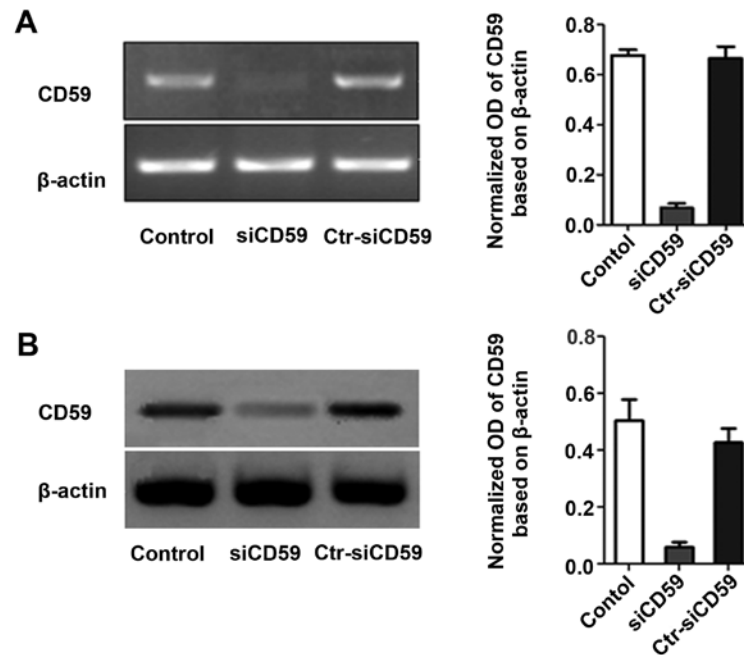
Figure 3. (A) Expression of CD59 mRNA in Luc-NCI-H157 cells, Luc-NCI-H157 (siCD59) and Luc-NCI-H157 (siCD59-ctr) cells. siCD59 significantly decrease the CD59 mRNA expression and the control siRNA did not affect the CD59 expression. (B) Expression of CD59 protein in Luc-NCI-H157 cells, Luc-NCI-H157 (siCD59) and Luc-NCI-H157 (siCD59-ctr) cells. siCD59 significantly decrease the CD59 protein expression, and the control siRNA did not affect the CD59 expression.
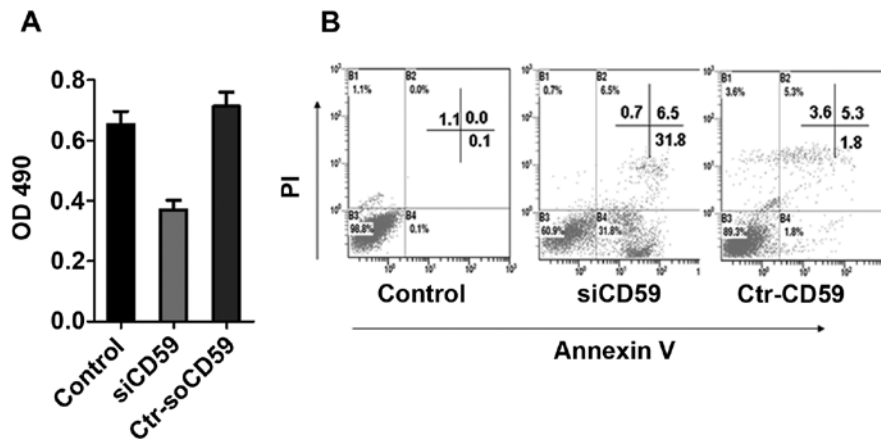

Figure 4. (A) Effect of CD59-siRNA on lung cancer cell proliferation. The proliferation of siCD59 transfected cells decreased significantly compared with the Luc-NCI-H157 and siCD59-ctr transfected cells. (B) Effect of CD59-siRNA on lung cancer cell apoptosis. The apoptosis of siCD59 transfected cells increased significantly compared with the Luc-NCI-H157 and siCD59-ctr transfected cells.

Effect of CD59-siRNA on lung cancer cell proliferation and apoptosis. To determine the effect of CD59 knockdown on the growth of NCI-H157 cells, an MTT assay was performed on NCI-H157 cells, siCD59 and siCD59-C transfected NCI-H157 cells. SiCD59-transfected NCI-H157 cells displayed a significant decrease in growth rate compared to siCD59-C-transfected cells and NCI-H157 cells (Fig. 4A). To determine the effects of CD59 knockdown on cell apoptosis, flow cytometry were used. Knockdown of CD59 increased apoptosis of NCI-H157 cells compared to NCI-H157 cells and siCD59-ctr cells (31.8% $P>0.1\%$, $P>0.05$; 31.8% $P>0.18\%$, $P>0.05$) (Fig. 4B).

CD59-siRNA reduced cell viability and increased cell damage when treated with complement. The viability and

Table I. LDH test.

Groups	LDH release rate
Cells only	9.6±1.3
Cells + normal serum	17.4±3.7
siCD59 + normal serum	63.5±5.3
Ctr-siCD59 + normal serum	16.3±4.1

cellular DNA damage of siCD59-NCI-H157 transfected cells was also reduced and increased, respectively, compared to the siCD59-C cells and NCI-H157 cells (Table I).
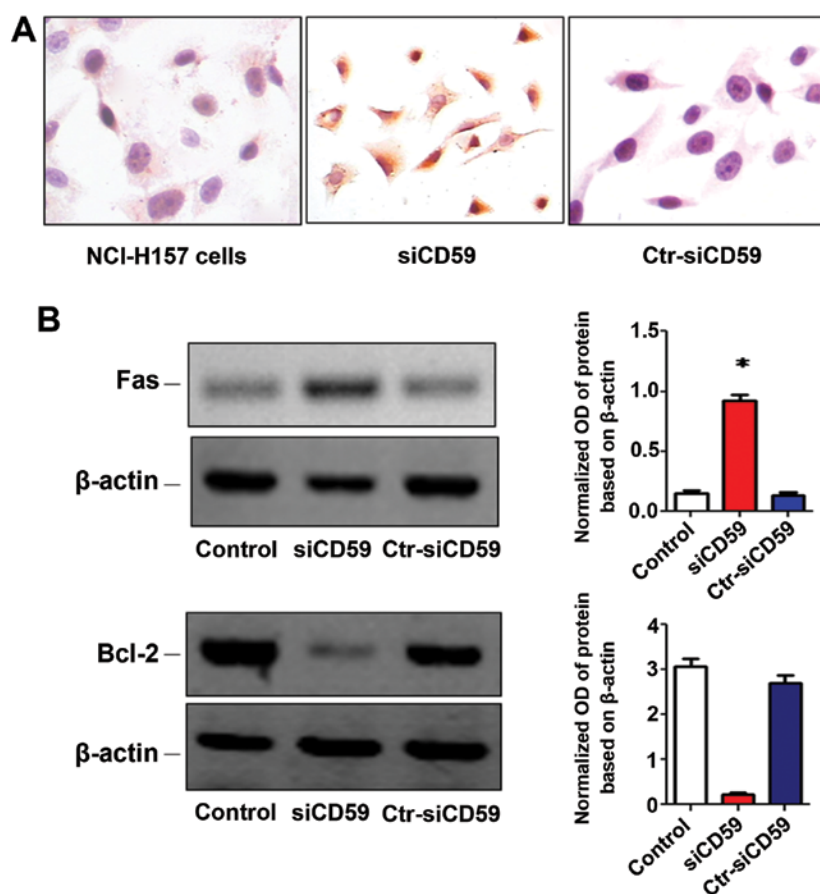
Figure 5. (A) The expression of apoptosis-associated protein caspase-3 in NCI-H157 cells, NCI-H157 (siCD59) and NCI-H157 (siCD59-ctr) cells. siCD59 significantly increased the caspase-3 protein expression, but the control siRNA did not affect the caspase-3 expression. (B) The expression of apoptosis-associated protein Fas and Bcl-2 in NCI-H157 cells, NCI-H157 (siCD59) and NCI-H157 (siCD59-ctr) cells. siCD59 significantly increased the Fas and decreased Bcl-2 protein expression, whereas the control siRNA did not affect the Fas or Bcl-2 expression. These results also confirm the caspase-3 result.

CD59-siRNA affects expression of caspase-3, Fas and Bcl-2. Shi *et al* (6) demonstrated that apoptosis could modulate the expression of CD59. IHC and western blot analysis showed increased caspase-3 (Fig. 5A) and Fas (Fig. 5B) levels while Bcl-2 (Fig. 5B) levels were decreased in siCD59-transfected NCI- H157 cells and NCI-H157 cells. These results indicate that CD59 regulates apoptosis in non-small cell lung cancer cells.

Knockdown of CD59 suppresses lung cancer cell growth in vivo. To test whether loss of CD59 can suppress lung cancer progression, we performed a xenograft study whereby different groups of NCI-H157 cells were subcutaneously injected into mice. Tumors were allowed to reach a size such that $\sim 10^8$ photons/sec/cm^2 were emitted following luciferin processing. Subsequently, luciferase signals in tumors of mice were detected in 10, 15 and 20 days after post-injection. Compared to s-CD59-C tumors and NCI-H157 cells, significant decrease in tumor burden was observed upon CD59 knockdown (Fig. 6A and B). The survival rate of tumor-bearing mice at 60 days was 70, 0 and 0% in siCD59-transfected tumors group, siCD59-C tumors group and NCI- H157 tumors group (Fig. 6C).

In vivo expression of CD59 mRNA and CD59, Bcl-2 and Fas protein. Total RNA and protein were extracted from tumor tissues to determine CD59 levels. Robust decrease in CD59

levels at both the RNA and protein levels were observed in siCD59 cells compared to siCD59-C cells and NCI- H157 cells (Fig. 7A). Furthermore, Fas and Bcl-2 expression was significantly increased and decreased, respectively, in siCD59 cells compared to siCD59-C and NCI- H157 cells (Fig. 7B).

Discussion

Human CD59 is an 18-20 kDa protein anchored through glycanphosphatidylinositol (GPI) to the cell membrane (19). CD59 belongs to the members of Ly6 superfamily (20). The functions of CD59 protein are mainly involved in the following three aspects. First, CD59 functions as an inhibitor of the C5b-9 membrane attack complex (MAC) of human complement (21). Second, CD59 acts as the second signal stimulant, inducing the activation of T lymphocytes and taking part in the regulatory course of immunoreactions (22). Third, CD59 is the ligand of CD2 that can conglutinate with CD59. CD59-CD2 complex activates T cells and then guides adhesion of T and T cells or T and other tissue cells and further regulates the growth of tissue cells (23).

Previous studies have shown that CD59 is highly expressed in many types of tumors, including breast cancer (24-27), colorectal cancer (26,28,29), ovarian cancer (30), malignant gliomas (31), malignant lymphomas (32), prostate cancer (33) and pancreatic cancer (34) and it directly or indirectly
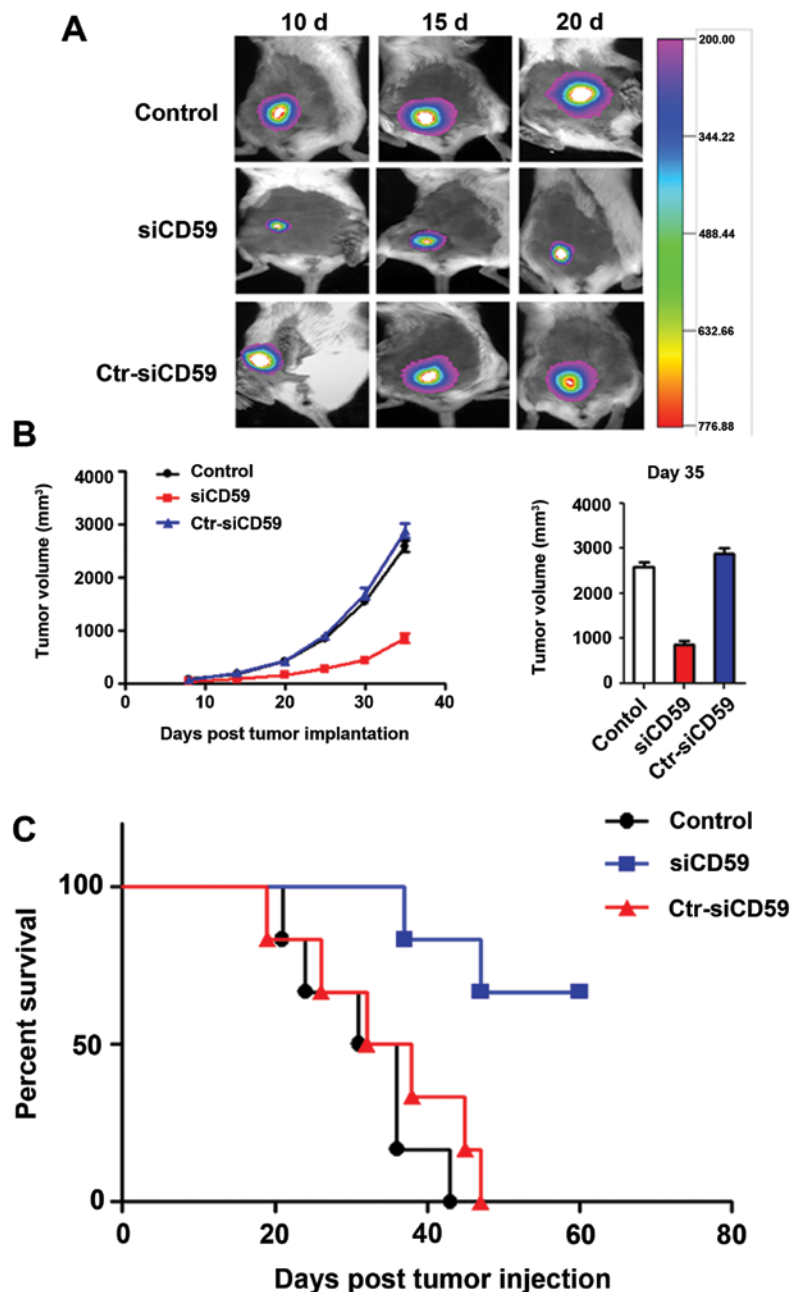
Figure 6. (A) Luciferase activity in tumors. Luciferase signals in tumors was decreased in siCD59 transfected mice in comparison to controls groups and siCD59-ctr treated groups. (B) Tumor size. Seven days after tumor injection, tumor growth and tumor size were observed and results showed that tumor growth was more rapid in the control group and siCD59-ctr transfected group than siCD59 transfected group. (C) Survival rate of tumor-bearing mice. Sixty days after tumor injection, all of the mice had died in Luc-NCI-H157 group and siCD59-ctr transfected group while in siCD59 transfected group only 3 had died.

participates in carcinogenesis and tumorigenesis. Our studies focused on the role of CD59 during tumorigenesis of NSCLC cells (NCI-H157) upon silencing CD59.

In the present study, we first detected high expression of CD59 in tissues of patients with lung cancer (Fig. 1). CD59 expression in non-small cell lung cancer tissues is much higher than in the surrounding tissue based on IHC. This suggests that CD59 might be a new biomarker for lung cancer progression.

RNA interference has emerged as a genetic tool for silencing gene expression. Because siRNA can be integrated into the host genome, long-term gene silencing can be achieved of siRNAs (17,35). Retroviral infection of siCD59 to NCI-H157 cells

was able to significantly decrease CD59 mRNA and protein levels compared to siCD59-C cells (Fig. 3). Knockdown of CD59 decreased cell viability and increased cell damage and apoptosis of lung cancer cells (Fig. 4). Furthermore, CD59 knockdown also reduced tumor growth *in vivo* (Fig. 6).

Loss of CD59 can induce complement-mediated cytolysis and lead to apoptosis of tumor cells (5). Complement-mediated cytolysis can also assist in apoptosis by inducing phagocytosis (36,37). MAC-triggered cell death can occur through a caspase-dependent pathway, specifically via caspase-3. Animal models of renal disease also implicate that MAC can trigger apoptosis (38-40). Korty *et al* demonstrated that CD59 can also increase calcium flux to increase cytoplasmic
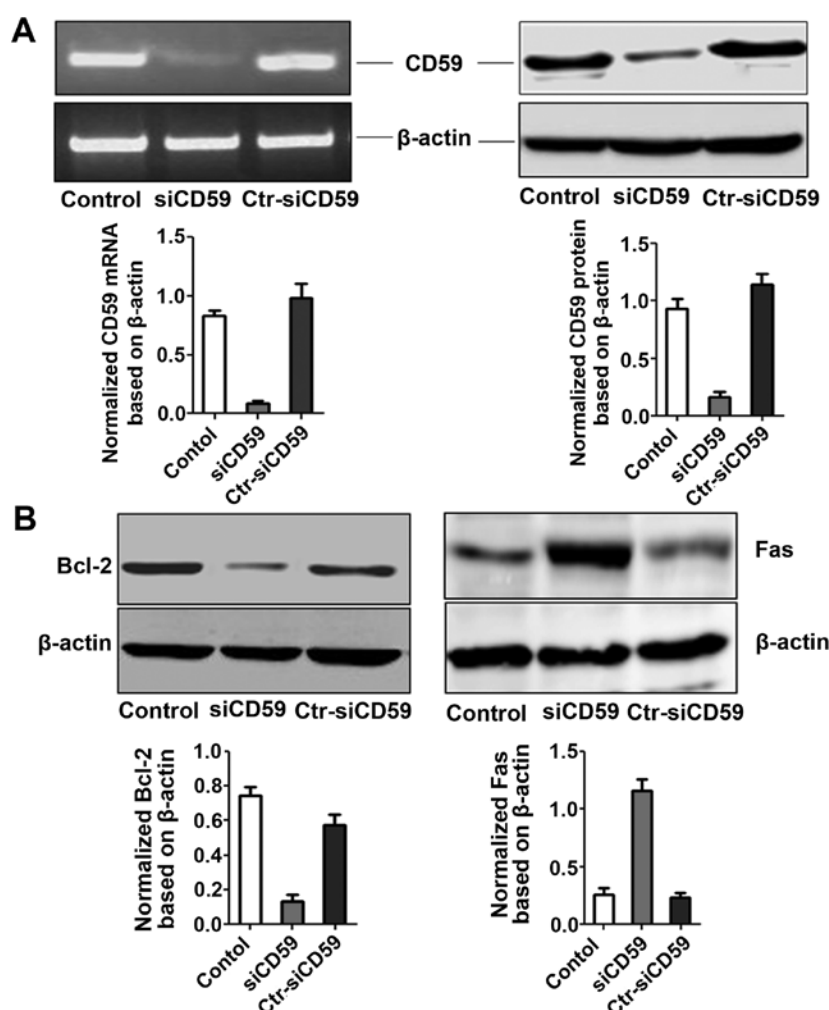
Figure 7. (A) CD59 expression in tumor tissues. The CD59 expression of mRNA and protein is dramatically less in siCD59 transfected group. (B) Fas and Bcl-2 expression in tumor tissues. The Fas is significantly increased and the Bcl-2 is dramatically reduced in siCD59 transfected group.

calcium levels which induces mitochondrial DNA damage and cytochrome *c* (41). Fas expression also initiates apoptosis under certain conditions and Fas antigen and ICAM-1 molecule can have a synergistic effect (42). Bcl-2 has also emerged as a proto-oncogene that blocks programmed cell death independently of promoting cell division (43). Bcl-2 can inhibit the synthesis and activation of caspase-3 to inhibit apoptosis. Moreover, Bcl-2 can also be degraded by caspase-3 a specific enzyme, which activates apoptosis. Apoptosis can inhibit tumorigenesis by removing unwanted and damaged cells (52).

Cell apoptosis typically occurs during tumor development and regression. Therefore, an important mechanism to prevent tumorigenesis is the induction of cell apoptosis that takes place continuously in many tissues to remove unwanted, damaged or aberrant cells. In this study, we investigated the effects of CD59 silencing to understand the biochemical mechanism underlying CD59 inactivation-induced apoptosis. We also studied the role of CD59 in regulating the growth and apoptosis of NCI-H157 cells. We showed that CD59 knockdown significantly decreased the growth and increased apoptosis of NCI-H157 cells compared to siCD59-C cells (Fig. 4), consistent with previous observations (44,45). CD59-induced

apoptosis might be mediated by MAC. Further studies are needed to understand this mechanism.

NCI-H157 cells treated by human complement (NHS, 8%, v/v) were used to assess MAC-mediated cytolysis. We showed that CD59 knockdown significantly inhibited the viability of NCI-H157 cells (Table I), decreased Bcl-2 expression and increased caspase-3 and Fas expression (Fig. 5). Caspases are executioners of apoptosis and regulate Fas/TNF-R1, mitochondria dysfunction and TNF-related apoptosis-inducing ligand (TRAIL). Caspase-3 activation was observed upon Fas/TNF-R1 treatment (Fig. 5A). Overall, the present data suggest that the loss of CD59 induces caspase-dependent apoptosis in cultured cells.

To investigate the inhibitory role of siCD59 *in vivo*, siCD59 infected cells were injected into athymic nude mice. Knockdown of CD59 significantly decreased tumor weight and growth and increased the survival rate of mice compared to siCD59-C cells and NCI-H157 cells (Fig. 6), which suggests that silencing CD59 gene expression could markedly inhibit the growth of cancer *in vivo*. The silencing of CD59 expression in tumor tissue was confirmed by RT-PCR and western blotting (Fig. 7A), which showed significantly less CD59 expression in siCD59 group than in siCD59-C group, or the NCI-H157 cell

group. Decreased tumor growth could be attributed to apoptosis, as indicated by Fas and Bcl-2 expression in these tumor tissues (Fig. 7B). The present data suggest that the CD59 loss induces caspase-dependent apoptosis *in vitro* and *in vivo*.

Overall, our study illustrates that CD59 is increased in human lung cancer and loss or inactivation of CD59 can lead to apoptosis of NCI-H157 lung cancer cells. This may be mediated by inducing Fas expression on the surface of NCI-H157 cells, leading to apoptosis through caspase-3 activation and complement-induced cytolysis. This might provide insight into new treatment for lung cancer patients by inhibiting CD59 expression.

In conclusion, the present study demonstrates that CD59 is overexpressed in human lung cancer and retroviral-mediated RNAi delivery is an efficient system for CD59 gene silencing of human lung cancer. Suppression of CD59 expression enhanced complement-mediated cytolysis of lung cancer cells, which may be mediated by induction of Fas expression on the surface of NCI-H157 cells, leading to apoptosis through caspase-3 activation, CD59 may serve as a candidate targeting gene in gene therapy for human carcinomas such as lung, ovarian, prostate carcinoma and cervical carcinoma.

Acknowledgements

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