Biometals (2010) 23:1179–1189
DOI 10.1007/s10534-010-9368-3

Proteomic analysis of lanthanum citrate-induced apoptosis in human cervical carcinoma SiHa cells

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Received: 18 May 2010 / Accepted: 20 July 2010 / Published online: 1 August 2010

Abstract Lanthanides possess diverse biological effect and have been shown to promote cell proliferation and induce apoptosis. Our previous studies showing that lanthanide citrate complex has significant antitumor activity in human cervical cancer HeLa cells. This study aims at determining if $[LaCit_2]^{3-}$ have the activity against another type of human cervical cancer cell line SiHa and the changes in protein expression that contribute to the mechanism(s) of $[LaCit_2]^{3-}$-mediated apoptosis in SiHa cells. Cell growth inhibition was measured by MTT method, and apoptosis was detected by means of Hoechst 33258 staining and flow cytometry analysis. After $[LaCit_2]^{3-}$-treatment the results show that the growth of SiHa cells was inhibited, the cells displayed typical apoptosis morphological changes, and increase in the rates of apoptosis. Using proteomics approaches, a variety of differentially expressed proteins were identified in SiHa cells before and after treatment with $[LaCit_2]^{3-}$. There were profound changes in 10 proteins relating to mitochondrial function and oxidative stress, suggesting that mitochondrial dysfunction plays a key role in $[LaCit_2]^{3-}$-induced apoptosis. This was confirmed by a decrease in the mitochondrial transmembrane potential ($\Delta\psi_m$), and increases in H_2O_2 generation in $[LaCit_2]^{3-}$-treated cells. Among them the alerted proteins, Prx I, ANXA1 and TRAF5 were validated by western blotting analyses. These results suggest that there is an intrinsic molecular pathway of cell apoptosis in $[LaCit_2]^{3-}$-treated SiHa cells. This observation is in accordance with our previous reports about the effects of $[LaCit_2]^{3-}$ and $[YbCit_2]^{3-}$ on HeLa cells and it provide a molecular mechanism underlying lanthanide citrate complex-mediated cell apoptosis.

Keywords Lanthanum citrate complex · SiHa cells · Proteomics · Apoptosis

Abbreviations

$[LaCit_2]^{3-}$	Lanthanum citrate complex
$[YbCit_2]^{3-}$	Ytterbium citrate complex
2-DE	2-Dimensional polyacrylamide gel electrophoresis
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide
JC-1	5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanineiodide
DCFH-DA	2′7′-dichlorofluorescein
ROS	Reactive oxygen species

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$\Delta\psi_m$	Mitochondrial transmembrane potential
ND2	ND2 protein
PrxI	Peroxiredoxin-1
ANXA1	AnnexinI
TRAF5	TNF receptor-associated factor 5

Introduction

Novel therapeutic and diagnostic metals and metal complexes are having an impact on medical practice. However, the clinical success of cisplatin and other platinum complexes is limited by the significant side effects or intrinsic resistance of those compounds. Therefore, much attention has focused on developing new compounds with improved pharmacological properties and a broader range of antitumor activity (Kostova 2005). Lanthanide complexes have been found to play a role in cancer treatment. Up to the present, Phase I, II and III studies have shown that motexafin gadolinium (MGd), a redox modulator, is selectively taken up by tumor cells and may increase the therapeutic index of radiotherapy in the management of brain metastases and other brain tumors (Khuntia and Mehta 2004). Lanthanides (Ln) have been shown to promote cell proliferation and induce apoptosis depending on Ln species, concentrations and cell types. Most studies have shown that the proapoptosis effect appeared at the concentration higher up to millimolar levels, while proliferative effect often occured at micromolar concentration (Zhang et al. 2009). However, recently some lanthanum (III) complexes have been synthesized with diverse ligands including chrysin (Zeng et al. 2003), phenanthroline derivatives (Wang et al. 2000, 2002; Heffeter et al. 2006) and coumarin derivatives (Kostova et al. 2005, 2006). Their anti-proliferative activity on various cancer cell lines has been demonstrated and the IC_{50} values are generally in the low μM range (Zeng et al. 2003; Wang et al. 2000, 2002; Heffeter et al. 2006; Kostova et al. 2005, 2006).

Cervical cancer is the second most common type of tumor in women. More than 500,000 new cases are reported worldwide each year and about 250,000 women die of it (Longworth 2008). In recent years, the link between human papilloma virus (HPV) and cervical cancer has been proven conclusively (Walboomers et al. 1999; Steben and Duarte-Franco 2007). It is of interest that a short treatment of affected cells with La^{3+} changes the cellular chemistry into a state in which RNA replication of flaviviruses is blocked specifically without interfering with host cell multiplication. These findings could open the way for the development of a general mode of chemotherapy against flaviviral diseases. It will also lead to studies on the inhibitory activity of lanthanides on the replication of other viruses (Wengler et al. 2007). In our previous work, a dose-dependent effect of $[LaCit_2]^{3-}$ was studied on the growth and viability of different cancer cell lines. The most potent cytotoxicity of $[LaCit_2]^{3-}$ was observed in HeLa cells (Shen et al. 2009a). The mechanism of $[LaCit_2]^{3-}$ or $[YbCit_2]^{3-}$ on HeLa cells was also investigated by comparative proteomics and the results showed that they induced apoptosis of HeLa cells *via* MT pathways and ROS were involved in the mechanism (Shen et al. 2009b, c). About 80 to 85% of cervical cancers are squamous cell carcinoma; most of the rest are adenocarcinomas. Sarcomas and small cell neuroendocrine tumors are rare. SiHa and HeLa cell lines are HPV positive cell lines, which are derived from squamous and adenocarcinoma of the cervix, respectively (Shen et al. 2009d). Therefore, the effects of $[LaCit_2]^{3-}$ on SiHa was investigated in this study.

Proteomics offers a good chance of identifying the proteins that mediate the apoptotic pathways involved when cells are treated with chemotherapeutic agents (Wang and Chiu 2008). The potential value of proteomics in metal-based drug development, especially in mapping the mechanisms of drug action, has been demonstrated in many successful examples (Wang et al. 2006a, b; Wong et al. 2008). With the aids of proteomic approaches, differentially expressed proteins were observed and identified from the control and $[LaCit_2]^{3-}$-treated SiHa cells. Confirmed by biochemical studies, the results suggest that the mitochondrial apoptosis pathway was involved in $[LaCit_2]^{3-}$-induced SiHa cell apoptosis.

Materials and methods

Chemical reagents

$[LaCit_2]^{3-}$ solution was prepared as described previously from lanthanum oxide (purity >99.9%, Yuelong New Materials Co., Ltd., Shanghai, PR

China) and citric acid (BBI, Canada) (Shen et al. 2009b).All other reagents were obtained from GE Healthcare (Yian Plaza, Guangzhou, China), except where otherwise noted.

Cell culture

The human cervical cancer cell line SiHa was purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Cells were cultured in RPMI-1640 medium plus 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA), 100 U/ml penicillin and 100 μg/ml streptomycin (Merck & Co., Inc, Whitehouse Station, NJ, USA). The cells were maintained in a humidified incubator in 95% air and 5% CO_2 at 37°C. When the cells reached 80% confluence, they were harvested and plated for either subsequent passages or drug treatments. For the treated samples, the medium was removed and fresh FBS-free RPMI-1640 medium containing $[LaCit_2]^{3-}$ were added.

Cell viability assay

Cell viability was determined by MTT assay as described previously (Shen et al. 2009b). SiHa cells were plated in 96-well microplates. After 16 h, the medium was replaced with fresh medium at various concentrations of $[LaCit_2]^{3-}$ in a final volume of 200 μl. After 24 h treatment, 20 μl of 5 mg/ml MTT (Sigma-Aldrich, St Louis, MO, USA) was added to each well for an additional 4 h, then the medium was removed and 100 μl DMSO (Amresco, Solon, OH, USA) was added. The absorbance of samples was measured at 570 nm with a SpecTRA MAX 190 microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Hoechst 33258 staining

To detect morphological changes during apoptosis, after treatment with 0.06 mM $[LaCit_2]^{3-}$ for 12 and 24 h, nuclear staining was performed with 5 μg/ml Hoechst 33258 (Beyotime Institute of Biotechnology, Jiangsu, PR China) and cells were analyzed using a fluorescence microscope (Olympus, Japan).

Annexin V/PI assay

After treatment with 0.06 mM $[LaCit_2]^{3-}$ for 12 and 24 h, respectively, quantitative apoptotic cell death was measured by Annexin V/PI assay. The Annexin V-FITC kit was purchased from Nanjing Keygen Biotech. Co. LTD (PR China). Cells were processed as described in the manufacturer's protocol and analyzed using flow cytometry.

Two-dimensional gel electrophoresis

After treatment with 0.06 mM $[LaCit_2]^{3-}$ for 24 h, the cells were harvested and protein were extracted as described previously (Shen et al. 2009b). Briefly, the cells were harvested, washed, spun down and the cell pellet was lysed in lysis buffer, sonicated and centrifuged. The supernatant was used for 2-DE analysis. Whole cell protein lysates, 90 μg for analytical gels and 500 μg for preparative gels, were mixed with rehydration solution to a final volume of 240 μl. Protein separation on 2-DE was performed using IPGphor isoelectric focusing (IEF) and electrophoresis units (GE Healthcare). Precast 13 cm immobilized pH gradient (IPG) strips were rehydrated for 12 h at 30 V. IEF was performed in the following conditions: a step-and-hold pattern was used initially at 100 V for 2 h, 200 V for 1 h and 500 V for 1 h. A gradient pattern was performed successively at 1000 V for 1 h, 8000 V for 3 h, followed by a step-and-hold pattern of 8000 V for a total of 48 kVh for analytical gels and 68 kVh for preparative gels. After the first dimensional run, proteins were reduced and alkylated in the equilibration buffer. Proteins were then separated in the second dimensional gel made from 12.5% SDS-polyacrylamide using the SE 600 Ruby system (GE Healthcare). The second dimensional gels for analysis were stained with silver nitrate and the gels for mass spectrometry were stained with Coomassie brilliant blue R-250. The gels were scanned with ProXPRESS 2D imaging system (PerkinElmer Inc., Waltham, MA, USA) and analyzed with ImageMaster 2D Elite software (GE Healthcare). Data were normalized and expressed as percentages of all valid spots to account for differences in protein loading and staining. Only those spots that changed consistently in three replicates and significantly (more than 2.0-fold) were selected for the analysis with MS.

MALDI-TOF-MS

In-gel digestion with trypsin and protein identification using MALDI-TOF-MS was performed as described previously (Shen et al. 2009b). Mass spectra were recorded on an Applied Biosystems 4700 Proteomics Analyzer (Framingham, MA, USA) and the data were processed using 4700 Explorer software. MASCOT was used in database searching for protein identification by MS data in the NCBI database (http://www.ncbi.nlm.nih.gov/).

Measurement of mitochondrial transmembrane potential

Flow cytometry was used for the analysis of $\Delta\psi_m$. After 0.06 mM $[LaCit_2]^{3-}$ treatment for 12 and 24 h, the cells were incubated at 37°C for 1 h with 5 mg/l JC-1 (Beyotime Biotech, Jiangsu, PR China), then washed twice with PBS and placed in fresh medium without serum. Flow cytometry was performed using a BD FACSCalibur system (BD Biosciences, USA) equipped with a single 488 nm argon laser.

Measurement of oxidative stress

DCFH-DA was used to evaluate the intracellular ROS level in the form of cellular peroxides, mainly hydroperoxide (H_2O_2). After 0.06 mM $[LaCit_2]^{3-}$ treatment for 12 and 24 h, cells were harvested and then incubated with 10 μM DCFH-DA (Beyotime Biotech) at room temperature for 30 min in the dark and then measured using a BD FACSCalibur system.

Western blotting analysis

The method of western blotting has been described previously (Shen et al. 2009b).Western blotting was performed using primary antibodies (Abs) against peroxiredoxin-1, TNF receptor-associated factor 5 (from Santa Cruz Biotechnology, Santa Cruz, CA, USA), Annexin I (from Boster, Wuhan, PR China) at optimized dilutions. β-acting (Boster) was used for the normalization of each protein to ensure equal protein loading.

Statistical analysis

Data were expressed as the mean ± SD of triplicate samples. Comparisons between multiple groups were performed using the one-way ANOVA followed by LSD test. Differences were considered to be significant at $P < 0.05$.

Results

$[LaCit_2]^{3-}$-induced cell growth inhibition and apoptosis

To determine the growth inhibitory activity of $[LaCit_2]^{3-}$, MTT assay was performed on SiHa cells. As shown in Fig. 1a, after treatment cell viability decreased significantly in a dose-dependent manner compared with the untreated control ($P < 0.05$). The IC_{50} value for $[LaCit_2]^{3-}$ on SiHa cells was 0.14 ± 0.01 mM. After treatment with 0.06 mM $[LaCit_2]^{3-}$ for 12 and 24 h, the morphology of apoptosis were observed by Hoechst 33258 staining under fluorescence microscope. Typical morphology of apoptosis, such as cell shrinkage, nuclear condensation and nuclear fragmentation, was detected in $[LaCit_2]^{3-}$-treated SiHa cells (Fig. 1b). In addition, $[LaCit_2]^{3-}$ mediated SiHa cell apoptosis was detected by flow cytometry (FACS) assay using Annexin V-FITC/PI staining. There was a significant increase in the percentage of apoptotic cells after $[LaCit_2]^{3-}$ treatment ($P < 0.05$) with 2.83 ± 1.01% for the controls and 14.00 ± 0.82% and 67.43 ± 3.27% for the treated cells after 12 and 24 h treatment, respectively (Fig. 1c).

Comparison of the protein expression patterns between $[LaCit_2]^{3-}$-treated and control cells

The proteomes of control and $[LaCit_2]^{3-}$-treated cells were analyzed by 2-DE. Representative gel images are shown in Fig. 2a and detailed alternations in Fig. 2b. 10 proteins showed significant changes and were identified by peptide mass fingerprinting (PMF) as shown in Table 1. The identified up-regulated proteins were as follows; ND2 protein (ND2), 3-oxoacid CoA transferase (Scot-S), TNF receptor-associated factor 5(TRAF5). The down-regulated proteins were CD209 antigen(DC-SIGN1), chain A, cyclophilin A complexed with dipeptide Gly-Pro(PPIA), eukaryotic

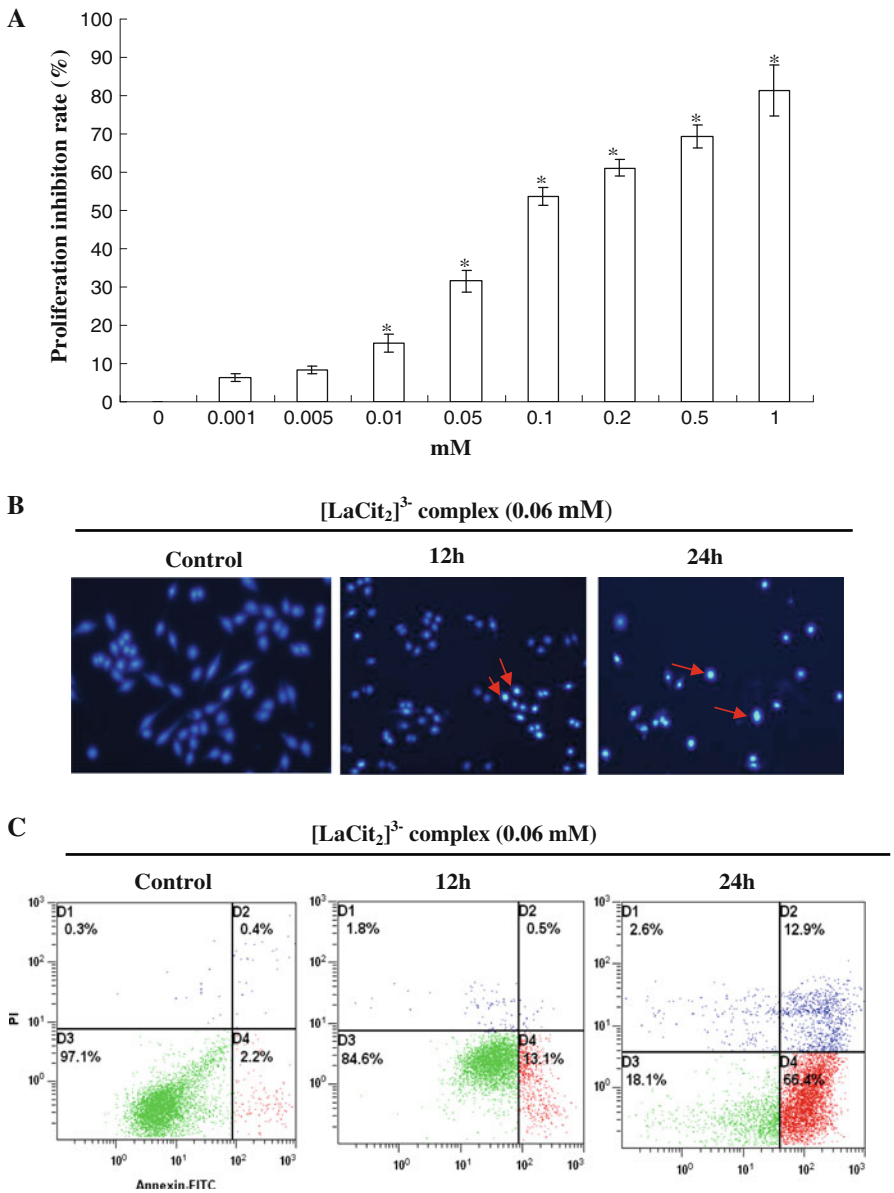

Fig. 1 Effects of $[LaCit_2]^{3-}$ on the proliferation and apoptosis of SiHa cells. **a** Effects of $[LaCit_2]^{3-}$ on cell proliferation by MTT test. **b** Hoechst 33258-staining of SiHa cells treated with $[LaCit_2]^{3-}$ (* $P < 0.05$). *Red arrows* indicate several apoptotic cells with typical condensation of chromatin. **c** Annexin V-FITC/PI staining flow cytometry analysis of the rate of apoptosis rate in SiHa cells treated with $[LaCit_2]^{3-}$. This is a representative data of triplicate experiments

translation initiation factor 5A-1 (eIF-5A-1), protein furry homolog (FRY), OTTHUMP00000016934 (ANAPC5), annexin I(ANXA1), and peroxiredoxin-1 (PRXI).

These proteins could be classified into several groups based on their functions. The first group was proteins involved in apoptosis and cell proliferation, including ANXA1, DC-SIGN1, and TRAF5. The second group of proteins was related to stress response and redox activity, ND2 and PRXI. Another group of proteins was related to translation and protein synthesis, including eIF-5A-1, FRY, chain A, cyclophilin A complexed with dipeptide Gly-Pro, and ANAPC5.

Monitoring the mitochondrial transmembrane potential

Figure 3a showed a series of representative JC-1 fluorescence results in both FL-1 and FL-2 channels. The control cells displayed high red fluorescence and weak green fluorescence, indicating hyperpolarized

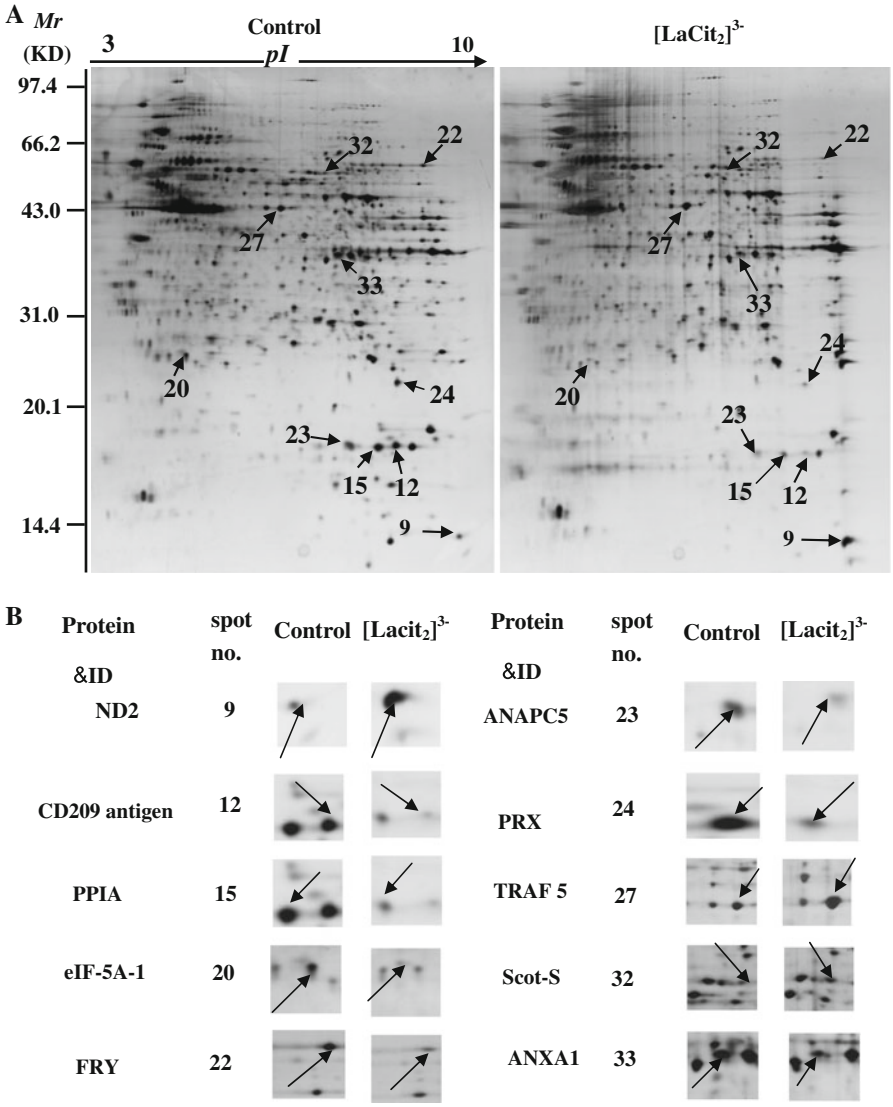

Fig. 2 Representative 2D gel images of SiHa cells. **a** SiHa cells treated with or without 0.06 mM $[LaCit_2]^{3-}$ in FBS-free culture medium for 24 h followed by 2D analysis. Arrows indicate proteins whose expressions were altered and identified by peptide mass fingerprinting. Spot numbers correspond to those listed in the first column of Table 1. **b** Detailed alternation of identified protein spots

MT. However, cells treated with $[LaCit_2]^{3-}$ exhibited increased green and decreased red fluorescence, suggesting the depolarization of MT and reduced $\Delta\psi_m$. The results also showed reduction of $\Delta\psi_m$ upon incubation with $[LaCit_2]^{3-}$ (Fig. 3b). The fluorescence intensity ratio between with green and red increased from 0.14 ± 0.01 to 0.75 ± 0.09 and 7.04 ± 0.98 ($P < 0.05$) after the SiHa cells exposed to $[LaCit_2]^{3-}$ 12 h and 24 h, respectively.

Measurements of reactive oxygen species (ROS)

As shown in Fig. 4, the intracellular H_2O_2 generation was significantly increased ($P < 0.05$), the fluorescence intensity increased from 8.94 ± 1.92 for the control cells to 18.17 ± 0.91 and 29.28 ± 2.82 for the cells under 12 and 24 h treatment with 0.06 Mm $[LaCit2]^{3-}$, respectively.

Western blotting analysis

Western blot analysis was used to confirm the differentially expressed proteins in response to $[LaCit_2]^{3-}$ treatment in the proteomics (Fig. 5). The proteins ANXA1 and PRXI were down-regulated and TRAF5 was up-regulated in $[LaCit_2]^{3-}$-treated cells, compared with the control. The ratio of differential expression of these proteins detected by western

Table 1 Proteins and their alterations after $[LaCit_2]^{3-}$ treatment (0.06 mM for 24 h)

Spot no	Protein ID	Accession no(NCBI)	MW (kDa)/pI	Reported function	Expr Level[a]	Peptides matched[b]
9	ND2 protein (ND2)	4261833	1.9/8.26	Oxidative stress	↑ 2.57 ± 0.67	2
12	CD209 antigen (DC-SIGN1)	15281095	34.1/6.87	Cell adhesion	↓ 3.10 ± 0.62	7
15	Chain A, Cyclophilin A Complexed With Dipeptide Gly-Pro (PPIA)	1633054	18.1/7.82	Protein synthesis	↓ 3.38 ± 0.39	10
20	Eukaryotic translation initiation factor 5A-1 (eIF-5A-l)	4503545	17.0/5.08	Translation	↓ 2.54 ± 0.49	9
22	Protein furry homolog (FRY)	117606355	342.1/5.66	Transcription	↓ 2.60 ± 0.46	7
23	OTTHUMP00000016934	55960444	24.86/9.93	Transcription	↓ 2.62 ± 0.53	9
24	Peroxiredoxin-l (PRX I)	55959887	19.1/6.14	Oxidation reduction	↓ 2.12 ± 0.36	8
27	TNF receptor-associated factor 5 (TRAF 5)	2138180	63.7/7.31	Apoptosis	↑ 2.23 ± 0.32	8
32	3-oxoacid CoA transferase (Scot-S)	4557817	56.6/7.14	Metabolism	↑ 2.18 ± 0.26	12
33	Annexin I (ANXA1)	4502101	38.9/6.57	Apoptosis	↓ 2.35 ± 042	18

[a] Expression level: trends of protein expression in $[LaCit_2]^{3-}$-treated SiHa cells when compared with the control. ↑ represents an increase of protein, while ↓ represents a decrease in protein expression

[b] Peptides matched by mass fingerprinting

blotting accorded well with the ratio differences detected by 2-DE.

Discussion

In this study, the effects and potential mechanism of lanthanum citrate complex on human cervical cancer SiHa cell line was investigated. After $[LaCit_2]^{3-}$-treatment, the growth of SiHa cells was inhibited and typical morphological changes were detected relating to apoptosis. Flow cytometry also revealed an increase in the apoptotic cell rate in response to $[LaCit_2]^{3-}$-treatment. These data suggest that $[LaCit_2]^{3-}$ significantly induced the inhibition of cell growth and promoted apoptosis in SiHa cells.

Two of the major pathways of apoptosis are the death receptor (the extrinsic pathway) and mitochondrial pathways (the intrinsic pathway). Reactive oxygen species (ROS) and mitochondria play an important role in apoptosis induction under both physiologic and pathologic conditions. Apoptosis is known to be induced by direct oxidative damage due to oxygen-free radicals or hydrogen peroxide or by their generation in cells by the actions of injurious agents. Interestingly, mitochondria are both the source and target of ROS (Simon et al. 2000). The previous report (Liu et al. 2003; Dong et al. 2009) and our previous studies(Shen et al. 2009b, c) on $[LaCit_2]^{3-}$ and $[YbCit_2]^{3-}$-induced HeLa cells apoptosis have shown that cell apoptosis promoted by rare earth complexes may be related to mitochondrial apoptosis pathway and that ROS were involved in the mechanism. Similar to the result of $[LaCit_2]^{3-}$ and $[YbCit_2]^{3-}$ on HeLa cells, these altered proteins in $[LaCit_2]^{3-}$-treated SiHa cells can be classified into several categories, including the proteins involved in apoptosis and cell proliferation, stress response and redox activity, and translation and protein synthesis. On the other hand, decrease in $\Delta\psi_m$ and increase in H_2O_2 generation were also detected. These results suggest $[LaCit_2]^{3-}$ may induce SiHa cell apoptosis through the mitochondrial apoptosis pathway and the regulation of intracellular H_2O_2 levels.

ROS including superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and highly toxic hydroxyl radical ($OH^{\bullet}$), were generated during aerobic metabolism(Fan et al. 2007). ROS can damage macromolecules essential for the integrity of the cell such as lipids, proteins, and nucleic acids. So cells have evolved a variety of very efficient non-enzymatic and enzymatic antioxidant systems (França et al. 2007). In this study, increase in H_2O_2 generation was detected in $[LaCit_2]^{3-}$-treated cells and alerted

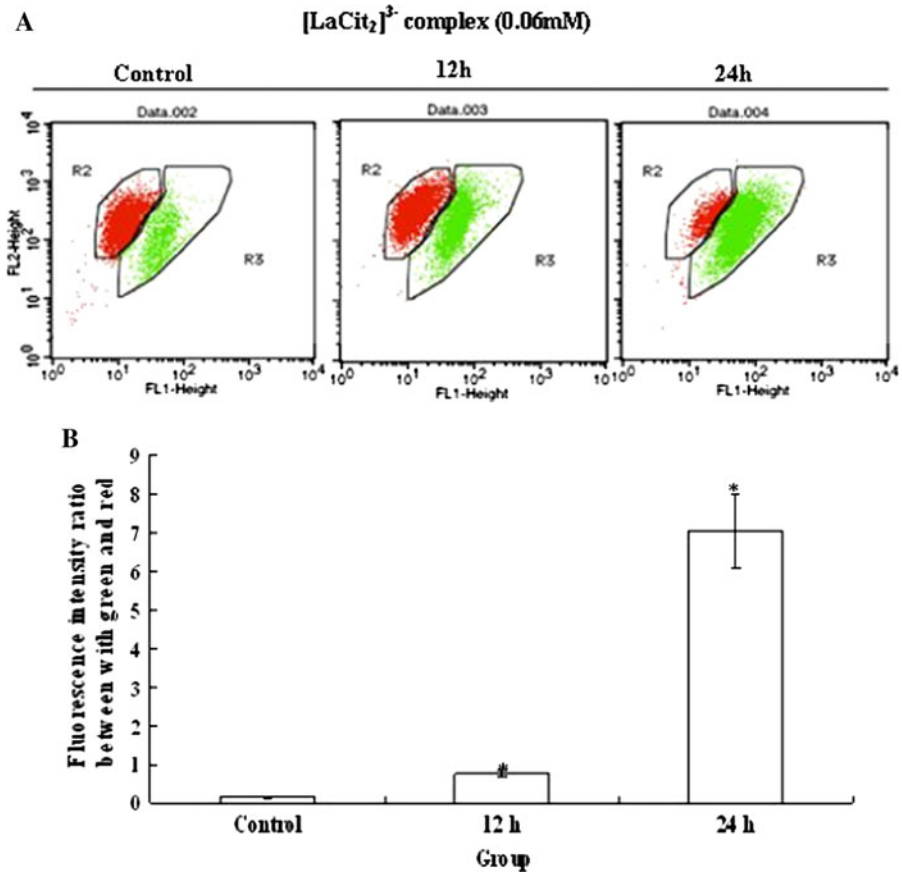

Fig. 3 Cytofluorimetric analysis of $\Delta\psi_m$ by JC-1 staining. SiHa cells exposed to 0.06 mM $[LaCit_2]^{3-}$ for 12 h and 24 h were analyzed and compared with control cells. One representative profile out of three replicates. **a** Cells with high $\Delta\psi_m$ are indicated in *red* and those with low $\Delta\psi_m$ in *green*; numbers refer to the percentage of cells. **b** The change in the *green* to *red* fluorescence intensity ratio (%). Each point represents the mean ± S.D. of three replicates (* $P < 0.05$)

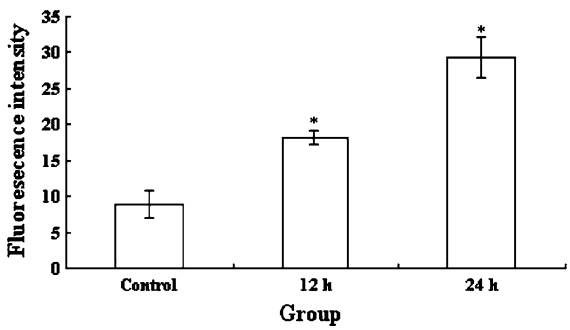

Fig. 4 $[LaCit_2]^{3-}$ induced the generation of H_2O_2 in SiHa cells. SiHa cells were pretreated with 0.06 mM $[LaCit_2]^{3-}$ (FBS free) for 12 h or 24 h and incubated with 10 mM DCFH-DA for 30 min at 37 °C. The fluorescent intensity was measured using flow cytometry (* $P < 0.05$)

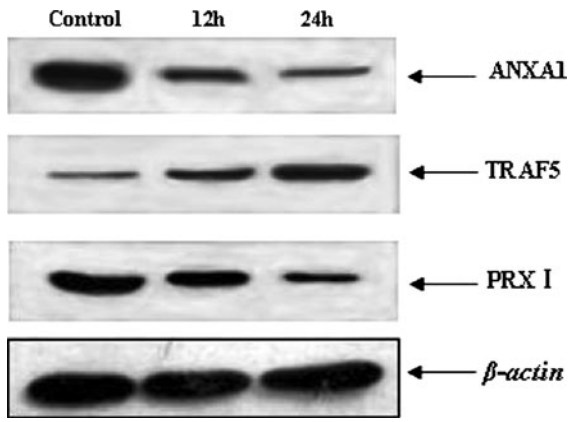

Fig. 5 The $[LaCit_2]^{3-}$-sensitive proteins in SiHa cells identified by western blot analysis. This is a representative result from three independent experiments

protein expression was observed for two redox-related proteins, PrxI and ND2. Hydrogen peroxide (H_2O_2), which is the primary sources of ROS, can be reduced to water by peroxiredoxin (Prx). Prx is a family of novel antioxidant proteins containing two conserved Cys residues, which are essential for the enzymatic scavenging of hydrogen peroxide (Chae et al. 1993). Prx I and II transfection enabled MCF-7 cells to resist H_2O_2-induced cell death and suggest

that they have important functions as inhibitors of cell death during cellular response to oxidative stress (Bae et al. 2007). Here PrxI was down-regulated (Figs. 2, 5 and Table 1) and thus it can be proposed that the down-expression of PrxI is a cause of the increased H_2O_2 generation induced by $[LaCit_2]^{3-}$ in SiHa cells.

In addition, ND2 protein, encoded by the mtDNA. NADH: ubiquinone oxidoreductase (complex I) and cytochrome c reductase (complex III) of the electron transport chain have been shown to be the major sites of mitochondrial ROS production (Gusdon et al. 2008). NADH dehydrogenase has been observed that Rotenone, an inhibitor of NADH dehydrogenase resulted in inhibition of apoptotic cell death (Matasunaga et al. 1996), and its deficiency may contribute to the resistance to apoptosis in VC-33cells, indicating the probable role of NADH dehydrogenase in apoptosis (Kataoka et al. 1997). Thus the up-regulated expression of NADH dehydrogenase subunit could be another reason for the increase of H_2O_2 in $[LaCit_2]^{3-}$-treated SiHa cells in this study.

During apoptosis there is a considerable and rapid reduction in the global rate of protein synthesis (Morley et al. 1998; Barber 2005). Similar to the result of previous study on HeLa cells(Shen et al. 2009b, c), several proteins related to transcription, translation and protein synthesis displayed significant expression changes in response to $[LaCit_2]^{3-}$ treatment in this work. The proteins FRY and OTTHUMP00000016934, which are related to transcription, are found to be down-regulated by SiHa treatment. Expression of eIF-5A-1 and cyclophilin a complexed with dipeptide Gly-Pro was also significant down-regulated, which are involved in translation and protein synthesis respectively. These results implied that the apoptosis induced by $[LaCit_2]^{3-}$ in SiHa cells was also mediated *via* the translation elongation and protein synthesis-related pathway.

In addition, the protein ANXA1 was down-regulated in this study (Fig. 2, Table 1, and Fig. 5). ANXA1 is a member of the calcium-dependent phospholipid binding protein family. Besides its anti-inflammatory function, ANXA1 has been involved in several mechanisms such as the Erk repression pathway or apoptosis (Debret et al. 2003). The expression of ANXA1 mRNA and protein in cervical cancer were higher than that in normal cervix and CIN, and it may be biological markers in detecting the progression of invasive cervical cancer (Lee et al. 2008). In SiHa and HeLa cell lines, with the treatments of all trans retinoic acid, decreased expressions of ANXA1 were noted with correlated decreased proliferation of cells (Lew et al. 1999). Here the down-regulation of ANXA1 expression in $[LaCit_2]^{3-}$-treated SiHa cells suggested that ANXA1 might play a role in the apoptosis *via* regulating the related growth factors.

Another protein TRAF5 appears to be up-regulated in response to $[LaCit_2]^{3-}$ treatment (Fig. 2, Table 1, and Fig. 5). Tumor necrosis factor receptor-associated factors (TRAFS) were initially discovered as adaptor proteins that couple the tumor necrosis factor receptor family to signaling pathways. To date, six members of the TRAF family have been identified. TRAF proteins are thought to be important regulators of cell death and cellular responses to stress. The major pathways mediated by TRAF2 and TRAF5 are the classical and alternative pathways of NF-kB activation, and MAPK and JNK activation (Au and Yeh 2007). During the past few years it has become clear that NF-kB-mediated inhibition of cell death, prolonged JNK activation contributes to cell death (Wullaert et al. 2006). Here the up-regulate of TRAF5 expression implied its prolonged JNK activation contributes to $[LaCit_2]^{3-}$-induced SiHa cell apoptosis. This clearly needs further study.

In conclusion, this study demonstrated that $[LaCit_2]^{3-}$ induced significant growth inhibition and apoptosis in human cervical cancer cell line SiHa. Using a proteomics strategy, we identified 10 differentially expressed proteins. Most of those proteins are involved in cell apoptosis and proliferation, and redox balance, protein translation, transcription and synthesis. Of those proteins, the altered expression of Prx I, ANXA1 and TRAF5 were further confirmed by western blotting. Mechanism study showed that $[LaCit_2]^{3-}$ treatment induced an increase in MT permeability associated with ROS generation. Similar to $[LaCit_2]^{3-}$ and $[YbCit_2]^{3-}$ on HeLa cells, it could be concluded that $[LaCit_2]^{3-}$ induced the apoptosis of SiHa cells through oxidative stress mediated pathway involving MT participation. Together with the La^{3+} can blocked the ability of the host cell to support the replication of flavivirus RNA and potential inhibitory activity on the replication of other viruses. Further studies will be performed regarding the generality of the potential inhibitory activity of lanthanide ions on the replication of human papillomavirus (HPV).

Acknowledgments This work was supported financially by the National Natural Science Foundation of China (Grant No. 20637010) and Shenzhen Bureau of Science, Technology and Information (FG200805230223A).

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