*Original Research*

# Lanthanum chloride enhances cisplatin-induced apoptosis in ovarian cancer cells

F. Wang[#], Y. Zhu[#], S. Fang, S. Li, S. Liu[*]

Department of Obstetrics and Gynecology, The First Affiliated Hospital of Nanchang University, Nanchang, China

**Abstract:** Ovarian cancer begins in an ovary. It is the leading cause of mortality from gynecologic cancer. Cisplatin is an anti-ovarian tumor drug. However, repeated use of cisplatin causes serious resistance. Recently, Lanthanum chloride (LaCl3) was found to inhibit the proliferation and promote the apoptosis in some cancer cells. In this study, we investigated the influence of LaCl3 on cisplatin-induced apoptosis in the ovarian cell line (COC1). Cell-based assays and analysis of cellular ultra structure were used. Result showed that LaCl3 enhanced cisplatin-induced apoptosis in COC1. Furthermore, the application of Cisplatin also altered the expression level of tumor related proteins, such as ERCC1, Ki67, and CDK6. Overall, we determined the inhibitory effect of LaCl3 on the COC1 growth when treated with Cisplatin, which may serve as a basis for the clinical application of LaCl3 in the treatment of ovarian cancer.

*Key words:* Lanthanum chloride, Cisplatin, COC1 cell line, apoptosis.

## Introduction

Ovarian cancer is the leading cause of mortality from gynecologic cancer and the fifth overall for cancer in women (1-4). It is estimated that more than 21,000 new cases will be diagnosed in the United States in 2015 and that more than 14,000 women will die of the disease (5). Since 1978, cis-[Pt(II)(NH(3))(2)Cl))(PtCl2(NH3)2], known as cisplatin or CDDP, has been used as a ovarian cancer therapeutic, binding to DNA, forming adducts, and activating apoptosis (6). More than 70 % of patients initially respond to cisplatin, but the long-term outcome remains poor with the 5-year survival rate of only about 30 % (7, 8). The main reason for the poor survival rate is that repeated use of cisplatin promotes drug resistance or tolerance (9). The mechanisms responsible for cisplatin resistance are still not fully understood up to date. The reported mechanisms result in the development of cisplatin resistance including, increased DNA repair, decreased accumulation of the drug within the cells, and post-translational modification (3, 10).

Rare earth elements such as lanthanum compound have the capacity to promote anti-tumor and immune regulation (11, 12). It was reported that a lanthanum compound (KP772) could inhibit growth and induce apoptosis even in notoriously drug-resistant tumor types (13). Lanthanum chloride (LaCl3) inhibited the proliferation and induced the apoptosis of cervical cancer cells both in vivo and in vitro (14). LaCl3 also induces telomerase-mediated apoptosis of chicken lymphoblastoid tumor cell line (15). Lanthanum chloride significantly inhibits the growth and induced the apoptosis of leukemia cell line HL-60 and NB4 (16). Furthermore, LaCl3 has little toxic effect on normal cells, indicating its potentials for clinical application (16, 17).

However, little is known about the role of the biological effects of lanthanum in ovarian cancer. Therefore, we used the ovarian cancer cell line COC1 as a model to demonstrate the biological effects of LaCl3 as an anti-ovarian tumor agent. We used cell-based assays to examine effect of LaCl3 on the cisplatin-induced growth inhibition and apoptosis. We also detected the alteration in expression level of some tumor related proteins. This study provides an experimental basis for the possible clinical application of LaCl3 in the treatment of the ovarian cancer.

## Materials and Methods

### Cell culture

A cisplatin-sensitive ovarian cancer cell line was cultured in RPMI 1640 medium (Hyclone, Beijing, China) enriched with 10% fetal bovine serum (Hyclone) at 37°C and 5% CO2. Cisplatin-resistant human ovarian cancer subline COC1/DDP derived from COC1, culturing in 0.5 μg/ml DDP. Cells were transferred to DDP-free medium for 48 h before experiments.

### Treatment

Cells were pretreated with LaCl3 at different concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0μmol/L). Then, cells were cultured for 8 hours before adding 23.08μg/ml cisplatin. The incubation with cisplatin lasted for 48 hours.

### TUNEL Assay

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay was performed by using an in situ cell death detection kit (Santa Cruz Biotechnology). Briefly, cells were fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. Fragmented DNA was labeled with fluorescein-

12-dUTP at 37uC for 1 hour. The reaction was terminated by the addition of SSC, followed by washing twice with PBS. TUNEL-positive nuclei were detected with a fluorescent microscope (Nikon).

## MTT Assay

MTT was used to detect cytotoxicity of LaCl3 at different concentrations. Briefly, MTT was incubated with cells for at least 4 hours to produce formazan. When formazan was completely dissolved by SDS-HCl, the absorbance at 570 nm was measured with a Universal Microplate Reader (Bio-Tek instruments).

## Annexin V-FITC assay

5μl Annexin V-FITC was added to each well and incubated in the dark for 5 min. After centrifuge for 5 min at 1000r/min, supernatant was discarded, and added 190 μl Annexin V-FITC binding solution. Then, cells were stained with propidium iodide (PI). The fluorescence were detected by flow cytometry.

## Western blot

2 μg cell lysates were loaded on each lane of 10% polyacrylamide gel, and then blotted onto a polyvinylidene difluoride (PVDF) membrane. After blocking with a PBST containing 5% nonfat dry milk, the blots were incubated with antibodies against EB1, ERCC1, Ki-67, CDK6, c-CBL and GAPDH (Cell Signaling Technologies, Beverly, MA). Peroxidase-linked anti rabbit IgG (Invitrogen) were used as secondary antibodies. These proteins were visualized by using an ECL western blotting detection kit (Amersham Biosciences).

## Immunohistochemistry

The sections were blocked for 60 min in 0.3% Triton X-100 in phosphate-buffered saline with 5% bovine serum albumin, and then incubated at 4°C with the anti-BCL2,Ki67, and ERCC1 (purchased from Cell Signaling Technology) overnight. HRP-conjugated secondary antibody was applied to the slides, and then were  for 1 hour at room temperature. Procedurally, DAB/H2O2 was added to the surface of the slide to develop the color.  The slides were visualized by light microscopy.

## Trypan staining

Cells were resuspended and stained with 0.4% Trypan blue for 3 min. Then, the cells were counted by hemocytometer. The survival rate was calculated as (Total cell number-blue cell number)/ total cell number.

## Transmission Electron Microscope

The cells were fixed in 2% glutaraldehyde and 2% paraformaldehyde for 2 hours, rinsed with PBS three times, conventionally dehydrated, embedded in paraffin and cut into 0.1 mm sections. The sections were stained with uranyl acetate and lead citrate, and examined with a transmission electron microscopy (TEM; JEM-1230 electron microscope, JEOL Ltd., Tokyo, Japan).

## Statistical analysis

Data were presented as mean ± SEM. Paired Student's t-tests were used to determine significant differences. A p value less than 0.05 is considered as different and highly different if p was less than 0.01. Relative density of protein expression level was analyzed by Image J.

## Results

### Lanthanum chloride enhanced the inhibitory effect of cisplatin

In the cisplatin-sensitive COC1 cell line, the percentage of TUNEL positive cells in the presence of cisplatin was significantly higher than the control group (p<0.01, Fig.1A). Besides, the co-application of lanthanum chloride and cisplatin significantly enhanced the inhibitory effect on the COC1 cell growth, since the ratio of TUNEL positive cells in the co-application group was higher than the cisplatin-treated group (p<0.01, Fig.1A). However, the application of lanthanum chloride alone did not show any effect (p>0.1, Fig.1A). Similarly, the same tendency was observed in the COC1/DDP cells (Fig.1B). We also confirmed the effect of cisplatin and LaCl3 through Annexin V-FITC assay. The results indicated that Annexin V-FITC labeled COC1 cells were increased with the treatment of cisplatin, while the application of lanthanum chloride subtly enhanced the inhibitory effect of cisplatin in the growth of COC1 cells (Fig.2). By contrast, the effect of lanthanum chloride on the cisplatin-induced apoptosis in the cisplatin-resistant COC1/DDP cells was stronger. The co-application of cisplatin and lanthanum chloride induced more significant apoptosis than the cisplatin-treated group (p<0.01, Fig.2). We also detected the effect of lanthanum chloride on the cell proliferation. As shown in the Table.1 and Figure.3, the MTT assay indicated that inhibition rate was increased with the increasing concentration of
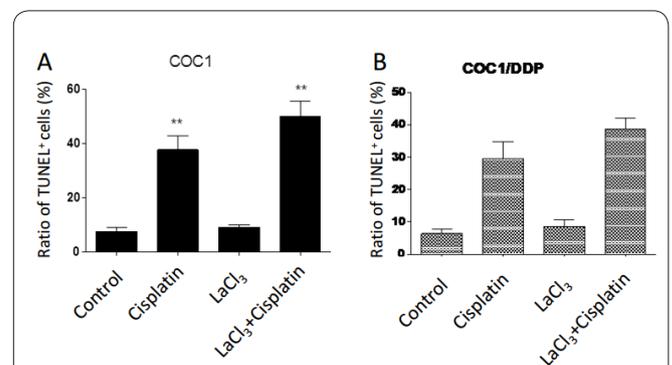


**Figure 1.** Lanthanum chloride enhanced the inhibitory effect of cisplatin on COC1 (A) and COC1/DDP cells (B), tested by the TUNEL assay.
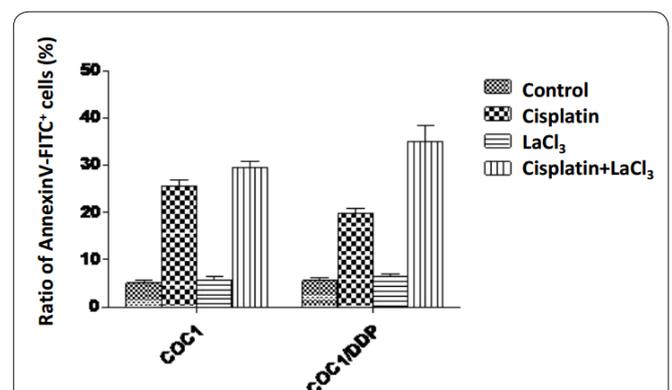


**Figure 2.** Lanthanum chloride induced apoptosis in the COC1 and COC1/DDP cells, showed by AnnexinV-FITC assay.
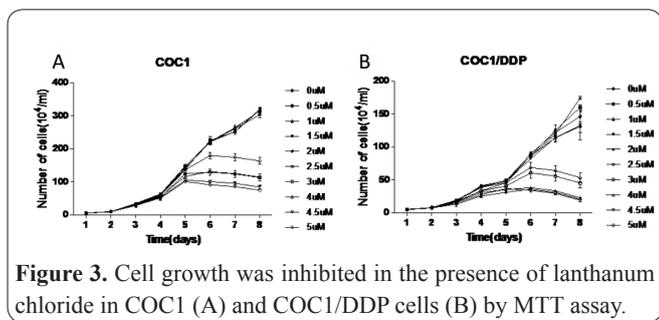
**Figure 3.** Cell growth was inhibited in the presence of lanthanum chloride in COC1 (A) and COC1/DDP cells (B) by MTT assay.

cisplatin, while the application of lanthanum chloride (>1.5μM) further increased the inhibitory effect of cisplatin in COC1 cells, and it showed the similar effect in COC1/DPP cells when the concentration of LaCl3 was more than 2μM. Similarly, Trypan blue staining showed the effective concentration of LaCl3 in the COC1 cells was more than 1.5 μM (Fig. 4).

## Application of lanthanum chloride affects the expression of tumor related proteins

Through the western blot analysis, we found that the expression level of EB1 and c-CBL was higher in the cells treated with Cisplatin compared to the control group (Fig.5). Furthermore, the expression level was even higher in the cells treated with both LaCl3 and Cisplatin, indicating that LaCl3 enhanced the effect of Cisplatin. On the other hand,  the expression level of ERCC1, Ki67, and CDK6 was opposite to the EB1 and c-CBL. In the presence of Cisplatin, the expression of these three proteins decreased, while the coapplication of Cisplatin and LaCl3 enhanced this inhibitory effect (Fig.5). The application the LaCl3 alone did not alter the expression of these proteins, excluding the direct effect of LaCl3 on the tumor related proteins.  The similar results were obtained by immunohistochemistry. The number of BCl2 and Ki67 positive cells was decreased in the presence of Cisplatin, while the ratio of positive
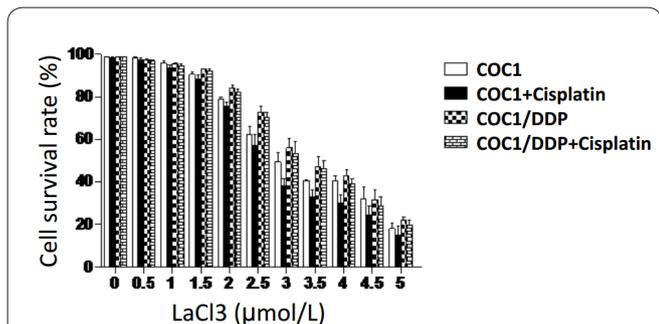
cells continues to decrease when treated with both Cisplatin and LaCl3 (Fig. 6A,B). The alteration in ratio of ERCC1, BRCA1 and BRCA2 positive cells showed the same tendency as Ki67 and BCl2 (Fig.6C-E). Collectively, lanthanum chloride altered the expression of tumor related proteins in the ovarian cancer cells.

## Application of LaCl3 and Cisplatin leads to the ultrastructural change

Then, we observed the possible ultrastructural alteration in the ovarian cancer cell caused by the treatment of LaCl3 and Cisplatin. The result from transmission electron microscope (TEM) observation showed the clear organelle and nuclear structure, integral nuclear membrane, and no vacuole in the cytoplasm of COC1 and COC1/DDP cells (Fig. 7A, 7E). No apparent change was observed when treated with 0.5μM LaCl3 (Fig.7B, 7F). In the presence of 1.5μM LaCl3, cells were shrunk subtly, and several vacuoles appeared in the cytoplasm (Fig. 7C, 7G). When the concentration of LaCl3 was increased to 4μM, the chromatin was condensed and aggregated around the nuclear membrane. The endoplasmic reticulum became loose and fused with the cell membrane to form the vacuoles. The nuclei was split, and apoptotic body was formed (Fig.7D, 7H). Therefore, application of LaCl3 and Cisplatin led to the ul-



**Figure 6.** The number of BCl2 (A), Ki67 (B), ERCC1 (C), BRCA1 (D) and BRCA2 (E) positive cells was decreased in the presence of Cisplatin, while the ratio of positive cells continues to decrease when treated with both Cisplatin and LaCl3.



**Figure 4.** Trypan blue staining showed the cell growth was affected in the presence of lanthanum chloride.



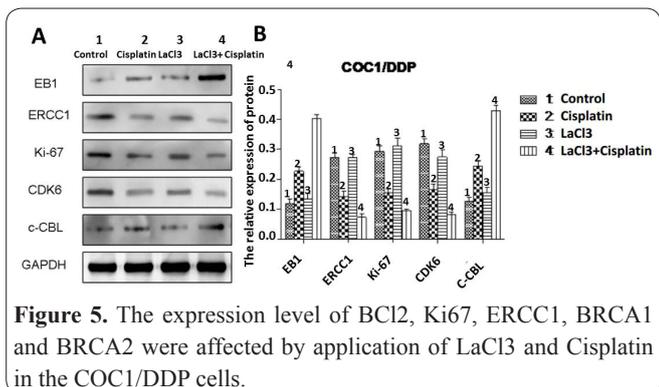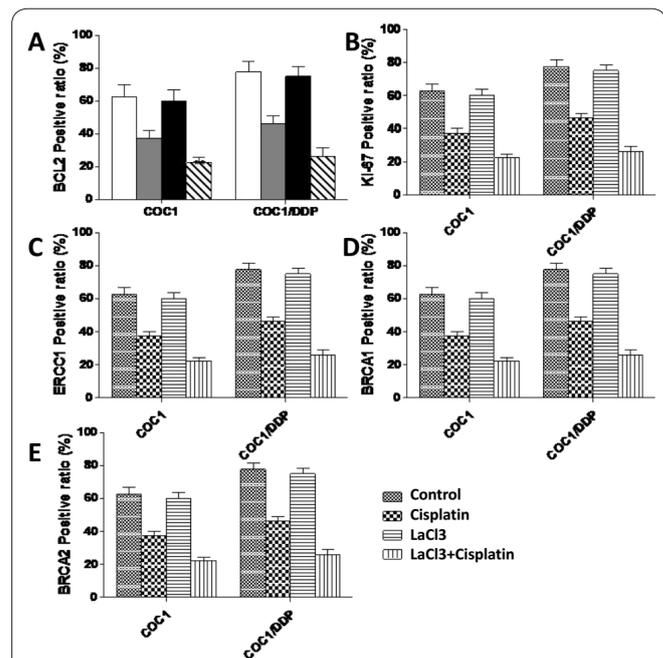**Figure 5.** The expression level of BCl2, Ki67, ERCC1, BRCA1 and BRCA2 were affected by application of LaCl3 and Cisplatin in the COC1/DDP cells.
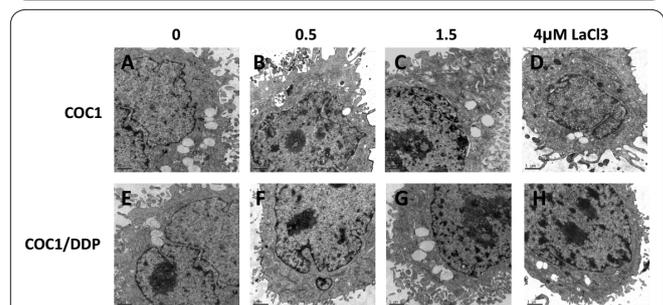


**Figure 7.** Cellular ultrastructure of COC1 and COC1/DDP cells was altered in 1.5 and 4 μM LaCl3 (C, D, G, H) compared to the 0 and 0.5 μM LaCl3 (A, B, E, F). Scale bar, 1μm.

trastructural cellular alteration, indicating the process of apoptosis.

## Discussion

As a representative of rare earth elements, lanthanum has been shown to induce apoptosis in several kinds of cells (18-20). The safety of clinical usage of lanthanum was assessed by many tests as well (21), which makes it possible to develop lanthanum as a promising clinical drug for humans in the future. In this study, we found that lanthanum chloride can enhance the cisplatin-induced inhibitory effect on the growth of ovarian cancer cell line, and lead to the increasing number of apoptotic cells. This process may be related to the altered expression level of tumor related protein. This work provides a new piece of evidence to support the clinical application of lanthanum chloride in the treatment of cancer.

Since lanthanum chloride's precise biological role remains largely elusive, further investigations of the underlying mechanisms are required. Recently, the anticancer properties of a new La complex (KP772) were clarified to be related with p53-independent G0/G1 arrest and apoptosis induction. Apoptosis is closely associated with the cell cycle (22). Apoptosis can maintain the balance between proliferation and cell death. In this study, we showed that the coapplication of lanthanum chloride and cisplatin increase the apoptosis in the ovarian cancer cell. It is possible that the increasing ratio of apoptotic cells was due to the percentage of cells in S-phase and G2/M-phase decreased while percentage of cells in G0/G1-phase increased. The cell cycle status need to be investigated in the future work.

In this study, cisplatin in combination with lanthanum chloride induced apoptosis in COC1 cells. This coapplication led to the downregulation of Ki67, CDK6 and ERCC1, and the upregulation of EB1 and c-CBL. Ki67 is a proliferative cell marker. CDK6 is important for the control of G1 to S phase transition, essential to the cell proliferation (23). ERCC1 participates in DNA repair after lesion. Downregulation of these genes indicated that cell proliferation was impaired after the treatment of cisplatin and lanthanum chloride. By contrast, EB1 binds to the APC, and functions as a tumor suppressor (24). CBL is an anti-cancer gene. Mutations to this gene have been implicated in a number of human cancers, particularly acute myeloid leukaemia (25). Thus, upregulation of EB1 and c-CBL indicated the suppression of cancer, which is a direct evidence to support the anti-tumor application of lanthanum chloride.

In summary, our results suggested lanthanum chloride was capable of inhibiting proliferation and inducing apoptosis in the ovarian cancer. This study provides novel evidence to establish an experimental basis for the clinical application of lanthanum chloride as an anti-cancer drug. Further studies will be needed to reveal the mechanism of the effect of lanthanum chloride.

## References

1. Sun C, Li N, Yang Z, Zhou B, He Y, Weng D. et al., miR-9 regulation of BRCA1 and ovarian cancer sensitivity to cisplatin and PARP inhibition. J. Natl. Cancer Inst., 105 (2013), 1750–1758.
2. Xiang Y, Ma N, Wang D, Zhang Y, Zhou J, Wu G. et al., MiR-152 and miR-185 co-contribute to ovarian cancer cells cisplatin sensitivity by targeting DNMT1 directly: a novel epigenetic therapy independent of decitabine. Oncogene, 33 (2014), 378–386.
3. Sorrentino A, Liu CG, Addario A, Peschle C, Scambia G, Ferlini C. Role of microRNAs in drug-resistant ovarian cancer cells. Gynecol. Oncol., 111 (2008), 478–486.
4. Boren T, Xiong Y, Hakam A, Wenham R, Apte S, Chan G. et al., MicroRNAs and their target messenger RNAs associated with ovarian cancer response to chemotherapy. Gynecol. Oncol., 113 (2009), 249–255.
5. Siegel RL, Miller KD, Jemal A. Cancer statistics 2015. CA Cancer J. Clin., 65 (2015), 5–29.
6. Chan JK, Blansit K, Kiet T, Sherman A, Wong G, Earle C. et al., The inhibition of miR-21 promotes apoptosis and chemosensitivity in ovarian cancer. Gynecol. Oncol., 132 (2014), 739–744.
7. Thigpen T, DuBois A, McAlpine J, et al., First-line therapy in ovarian cancer trials. Int J Gynecol Cancer. 21 (2011), 756–762.
8. Ye Q, Zhai X, Wang W, et al., Overexpression of growth-related oncogene-beta is associated with tumorigenesis, metastasis, and poor prognosis in ovarian cancer. Dis Markers. 2015; 2015:387382.
9. Ding YH, Zhou ZW, Ha CF, et al., Alisertib, an Aurora kinase A inhibitor, induces apoptosis and autophagy but inhibits epithelial to mesenchymal transition in human epithelial ovarian cancer cells. Drug Des Devel Ther. 9 (2015), 425–64.
10. D.W. Shen, L.M. Pouliot, M.D. Hall, M.M. Gottesman, Cisplatin resistance: a cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. Pharmacol. Rev., 64 (2012), 706–721.
11. Zhang J, Li Y, Hao X et al, Recent progress in therapeutic and diagnostic applications of lanthanides. Mini Rev Med Chem 11 (2011), 678–694.
12. Heffeter P, Jakupec MA, Korner W et al (2006) Anticancer activity of the lanthanum compound (tris(1,10-phenan-throline) lanthanum(III))trithiocyanate (KP772; FFC24). Biochem Pharmacol 71 (2006), 426–440.
13. Heffeter P, Jakupec MA, Korner W et al Multidrug-resistant cancer cells are preferential targets of the new antineoplastic lanthanum compound KP772 (FFC24). Biochem Pharmacol 73(2007),1873–1886.
14. Yu L, Xiong J, Guo L, et al., The effects of lanthanum chloride on proliferation and apoptosis of cervical cancer cells: involvement of let-7a and miR-34a microRNAs. Biometals. 28 (2015), 879-890.
15. Zhang Z, Wang J, Li J, Xu S. Telomerase-mediated apoptosis of chicken lymphoblastoid tumor cell line by lanthanum chloride. Biol Trace Elem Res. 144 (2011), 657-667.
16. Dai Y, Li J, Li J, et al., Effects of rare earth compounds on growth and apoptosis of leukemic cell lines. In Vitro Cell Dev Biol Anim. 38 (2002), 373-375.
17. Heffetera P, Jakupecb MA, Körnerc W, et al., Multidrug-resistant cancer cells are preferential targets of the new antineoplastic lanthanum compound KP772 (FFC24). Biochem Pharmacol 73 (2007),1873–1886.
18. Dai Y, Li J, Li J et al, Effects of rare earth compounds on growth and apoptosis of leukemic cell lines. In vitro cellular & developmental biology. Animal 38(2002), 373–375.
19. Shen L, Lan Z, Sun X et al, Proteomic analysis of lanthanum citrate-induced apoptosis in human cervical carcinoma SiHa cells. Biometals 23(2010), 1179–1189.
20. Wu J, Yang J, Liu Q et al, Lanthanum induced primary neuronal apoptosis through mitochondrial dysfunction modulated by Ca(2) and Bcl-2 family. Biol Trace Elem Res 152 (2013), 125–134.
21. Xu J, Zhang YX, Yu XQ et al, Lanthanum carbonate for the treatment of hyperphosphatemia in CKD 5D: multi-center, double blind, randomized, controlled trial in mainland China. BMC Nephrol

14(2013), 29.

22. Lapenna S, Giordano A Cell cycle kinases as therapeutic targets for cancer. Nat Rev Drug Discov 8 (2009), 547–566.

23. Meyerson, M and Harlow, E. Identification of G1 kinase activity for cdk6, a novel Cyclin D partner. Molecular and Cellular Biology 14 (1994), 2077–2086.

24. Jennifer S. Tirnauera and Barbara E. Biererb. Eb1 proteins regulate microtubule dynamics, cell polarity, and chromosome stability. J Cell Biol. 149 (2000), 761–766.

25. Naramura M, Nadeau S, Mohapatra B, et al., Mutant Cbl proteins as oncogenic drivers in myeloproliferative disorders. Oncotarget 2 (2011), 245–250.