BioMetals **18:** 89–95, 2005.

Proteomic detection of changes in protein synthesis induced by lanthanum in BGC-823 human gastric cancer cells

Ping Shi[1,*] & Zhiwei Huang[2]
[1]*State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, PR China;* [2]*Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Shanghai 200032, PR China; *Author for correspondence (Tel.: +86-021-64252920; Fax: +86-021-64252250; E-mail: ship723@yahoo.com)*

Received 2 June 2004; Accepted 4 August 2004; Published online December 2004

Key words: BGC-823 human gastric cancer cells; differential protein identification; lanthanum; mass spectrometry analysis; two-dimensional electrophoresis.

Abstract

There is increasing interest in the use of rare earth elements in medicine. However, the biological mechanism of action of this metal ion remains unclear. In the present study, changes in protein synthesis induced by lanthanum in BGC-823 human gastric cancer cells were investigated. The proteins were separated using two-dimensional polyacrylamide gel electrophoresis and four proteins were significantly affected by lanthanum treatment when compared to an untreated control. Among them, one was down-regulated and three were up-regulated. Of these, three were successfully identified as RHOJ, CSP6 and MPI2 with peptide mass fingerprinting using matrix-assisted laser desorption/ionization time of flight mass spectrometer (MALDI-TOF-MS) after in-gel trypsin digestion. Among them, RHOJ was down-regulated and CSP6 and MPI2 were up-regulated. The three proteins are involved in various cellular functions, which are correlated with the regulation of cell morphology, gene transcription and cell cycle, respectively. It is suggested that the possible involvement of rare earth elements in the growth arrest of tumor cells is significantly associated with the differential protein expression induced by rare earth ions.

Introduction

The rare earth elements of the periodic table include lanthanum and actinium series elements. There is an increasing interest in their medical uses. The therapeutic use of lanthanides as an anticancer agent has been proposed. Daily i.p. injection of 2.5 mg $LaCl_3$ retarded the growth of sarcoma in rats (Angilheri 1979). Administration of Sm-EDTMP (ethylenediaminetetra (methylenephosphonic) acid) to dogs with bone carcinoma resulted in regression of tumor growth (Ketring 1987). The influences of lanthanoid ions (Ln^{3+}) on cancer cell proliferation have been investigated for several cell lines. For example, in the presence of Ln^{3+}, growth rates of B16 melanoma cells were significantly lower than that of the control cells (Sato *et al.* 1998). By examining the colony-forming ability in soft agar, microtubule structure, calmodulin levels and regulation of some gene expression by Northern blot analysis, Xiao *et al.* (1997) and Ji *et al.* (2000) found that Ln^{3+} have certain suppression effects on the proliferation of the human gastric cancer cell PAMC82 and leukemic cell K562. Work on the probable mechanism suggest that it may be concerned with some proto-oncogenes relating to proliferation, protein expression and the regulation of the cell cycle (Xiao *et al.* 1997; Li *et al.* 1999, 2000).

To further understand the intracellular events leading to lanthanides stimulation, we have

investigated changes in protein synthesis induced by lanthanum ion in BGC-823 cells using two-dimensional electrophoresis and matrix-assisted laser desorption ionization-time of flight mass spectrometer (MALDI-TOF-MS). This methodology has been considerably improved over the last few years to allow sensitive detection of individual protein changes studied from a total cell extract. The main finding of this paper is that lanthanum ion induced changes of four proteins in BGC-823 cells. Furthermore, three identified of these proteins were correlated with the regulation of cell morphology, gene transcription and the cell cycle, respectively.

Materials and methods

Cell culture

The human gastric cancer cell line BGC-823 was supplied by the Institute of Cell Biology, Shanghai, China. They were cultured at 37 °C under a humidified 5% CO_2 atmosphere in RPMI 1640 medium (Sigma Chemical Company, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) and 2% penicillin–streptomycin (10,1000 U/ml penicillin and 10 mg/ml streptomycin).

Protein sample preparation

BGC-823 cells were plated at 0.5×10^5 cells in six-well plates in 2 ml of RPMI 1640 medium supplemented with 10% FBS. After overnight adherence, $LaCl_3$ was added to cells at a final concentration of 1.0 and 2.0 mM, respectively for 5 days. The medium was replaced at 2-day intervals. The cells were collected by gently scraping with a rubber scraper, then centrifuged. Cell pellets were snap frozen and thawed in PBS repeatedly. Insoluble materials were removed by centrifugation, and the sample was subjected to two-dimensional electrophoresis.

Two-dimensional polyacrylamide gel electrophoresis (2-DE)

Two-dimensional polyacrylamide gel electrophoresis was performed as follows: proteins were homogenized in lysis solution (9.8 M Urea (P.A), 2% (W/V) NP-40, 2% carrier ampholytes, pH 3–9.5, 100 mM DTT) at room temperature for at least 40 min, then separated by nonequilibrium pH gradient electrophoresis in gels containing 4% acrylamide using pH 3–9.5 ampholytes at 400 V for 18 h. After equilibration for 15 min in equilibration buffer (2% SDS, 100 mM DTT, 10% glycerol, 0.06 M Tris-HCl, pH 6.8), the tube gel was sealed to the top of the stacking gel (0.75 mm thick), which was placed on top of a 12% acrylamide slab gel. The standard Tris/glycine buffer system was used as described by Laemmli (1970). Per gel for 30 min followed by a maximum of 30 mA per gel for 3–4 h, until the tracking dye reached the bottom of the gel. Then, gels were stained with Coomassie brilliant blue R-250. The isoelectric focusing (IEF) and the 2-DE experiments were repeated three to four times.

Image analysis and spot identification

Image analysis was performed using the PDQuest system according to the protocol provided by the manufacturer in order to search the differential spots of different treatments. To account for experimental variations, three gels were prepared for control cells and those treated with $LaCl_3$.

In-gel protein digestion

The differential proteins were excised from two-dimensional gel and digested with trypsin as previously described (Bergman *et al.* 2000). Briefly, the spots were washed several times with 50% acetonitrile, which was then removed. Gel pieces were dried in a vacuum centrifuge. The cysteine reduction and alkylation steps consisted of incubation first in 10 mM DTT, followed by a 45 min dark incubation with 55 mM iodoacetamide at room temperature. The gel pieces were then dried again and rehydrated in a minimal volume of 50 mM NH_4HCO_3, pH 8.0 containing 5 mM $CaCl_2$ and 12.5 ng/μl sequencing grade modified trypsin for 45 min in ice. The excess liquid was removed and the pieces of gel were immersed overnight in the same buffer (without enzyme) at 37 °C. The gel pieces were first extracted in 20 μl 20 mM NH_4HCO_3, pH 8.0 followed by three extractions in 20 μl 5% formic acid in 50% acetonitrile. The resulting pooled eluates were dried prior to analysis by mass spectrometry.

Mass spectrometry analysis and protein identification

Mass spectra of the spot was acquired with a MALDI-TOF-MS (ReFlex^{TM}III, Bruker Co., USA) operating in the delayed-extraction reflection mode. Peptide mass fingerprints (PMF) of the tryptic peptides from MALDI-TOF-MS data on differential spots, together with the isoelectric points and molecular weights, were used to search the SWISS-PROT protein database with the Peptident program (http://expasy.org/tools/peptident.html) or National Center for Biotechnology Information (NCBI) with MS-Fit program (http://prospector.usef.edu/uesfhtm4.0/msfit.him).

Results

Two-dimensional map and differential proteins search

Proteins of BGC-823 cells were separated using two-dimensional electrophoresis and the protein spots were visualized following Coomassie R-250 staining. Three pairs of gels from different batches

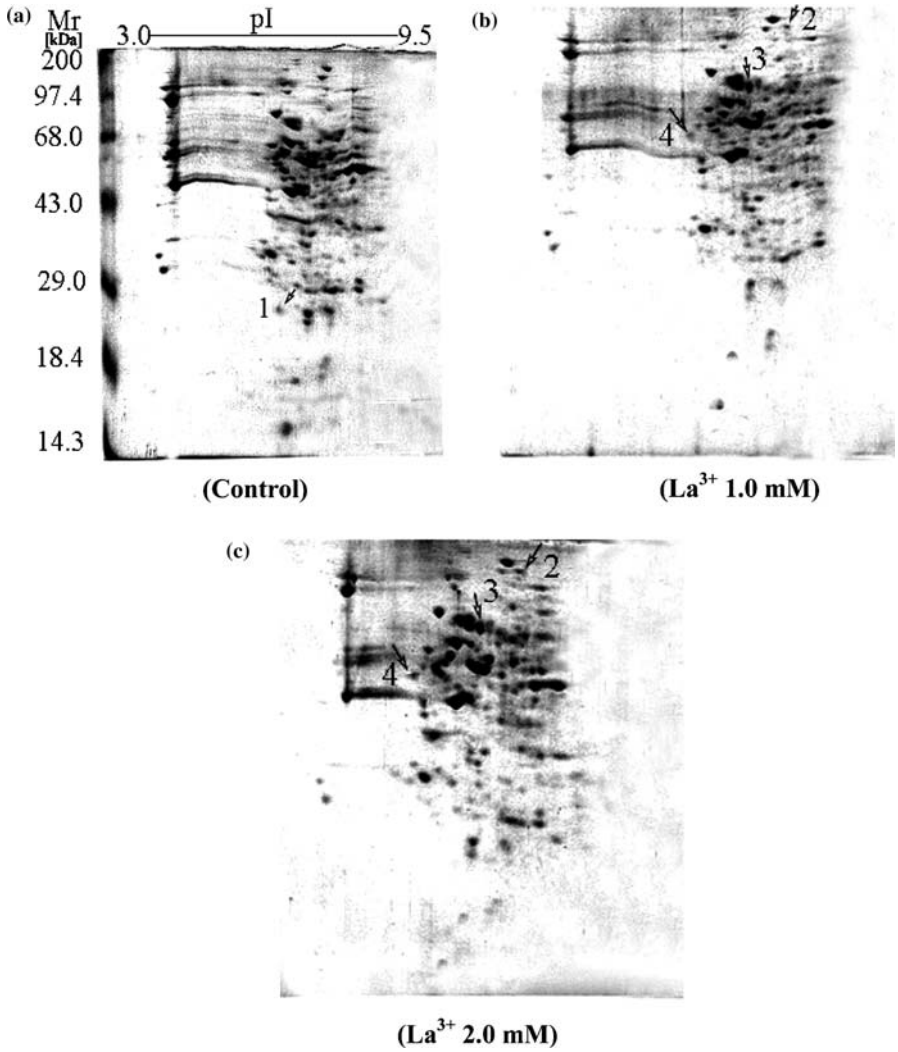

Figure 1. Two-dimensional electrophoresis map of total proteins in BGC-823 cells. (a) Control; (b) treatment with 1.0 mM $LaCl_3$; (c) treatment with 2.0 mM $LaCl_3$. Digit 1 shows the differential spot which is down-regulated in cells treated with $LaCl_3$. Digits 2–4 show the three differential spots which appear up-regulated in cells treated with $LaCl_3$.

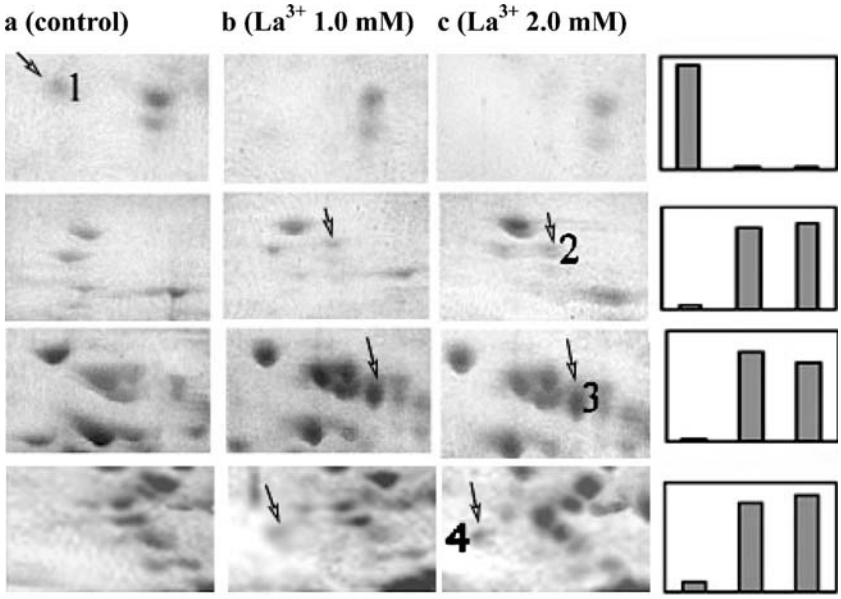

Figure 2. Two-dimensional electrophoresis partial maps of total proteins in BGC-823 cells. (a) Control; (b) treatment with 1.0 mM $LaCl_3$. (c) treatment with 2.0 mM $LaCl_3$. Digits represent the differential spots between the three treatments. Spot 1 is down-regulated and those of 2–4 are up-regulated on the (b) and (c). The histogram plots show the relative quantifications of the four differential proteins according to the intensity of Coomassie R-250 staining in different gels.

of control, 1.0 and 2.0 mM $LaCl_3$ treated cells for 5 days were analyzed for the purpose of differential spot comparisons with the PDQuest analysis software. Figure 1 shows representative example of the proteins separated on two-dimensional gel, where 0.2 mg of total protein was applied. Approximately 200 protein spots were detected on the Coomassie stained gel. The majority of spots distributed on the map had p*I* values ranging from 4.0 to 8.0 and molecular weight from 20 to 70 kDa. After matching analysis, four protein spots were found to be significantly different from these three batches of gels. These spots are marked at the corresponding site in Figures 1 and 2.

MALDI-TOF-MS analysis and protein identification

These four proteins were identified by MALDI-TOF-MS on the basis of peptide mass matching (Henzed *et al.* 1993), following in-gel digestion with trypsin. The peptide masses were matched with the theoretical peptide masses of all proteins from the human species of the SWISS-PROT database, together with the isoelectric points and molecular weights. Figure 3 shows the spectrum of the trypsin digest of protein spots 1–4. Three proteins were successfully identified by MALDI-TOF-MS. The list identified proteins is shown in Table 1. It is to be noted that one protein RHOJ was down-regulated in BGC-823 cells treated with $LaCl_3$. While three others were up-regulated, of these, two were identified as CSP6 and MPI2, respectively.

Discussion

The cytotoxic effects of lanthanum ion on cancer cells have been well documented (Figure 4). For instance, $La(NO_3)_3$ inhibits the proliferation of human neuroglioma SWO cells dose-dependently (Wu *et al.* 2003). Using MTT assay and TUNEL method, Dai *et al.* (2002) reported that the rare earth compounds ($LaCl_3$ and $CeCl_3$) might inhibit the growth of leukemic cells HL-60 and induce them to apoptosis at certain concentrations. Hong *et al.* (2003) studied the effect of rare earths on the proliferation toxicity of K562 and BEL-7402 cells by means of MTT. These previous studies have shown that 0.05–1.5 mM lanthanide ions present inhibitory effects on cancer cells. It is obvious that the inhibition rates of cancer cell growth are different on same concentrations (Figure 4).

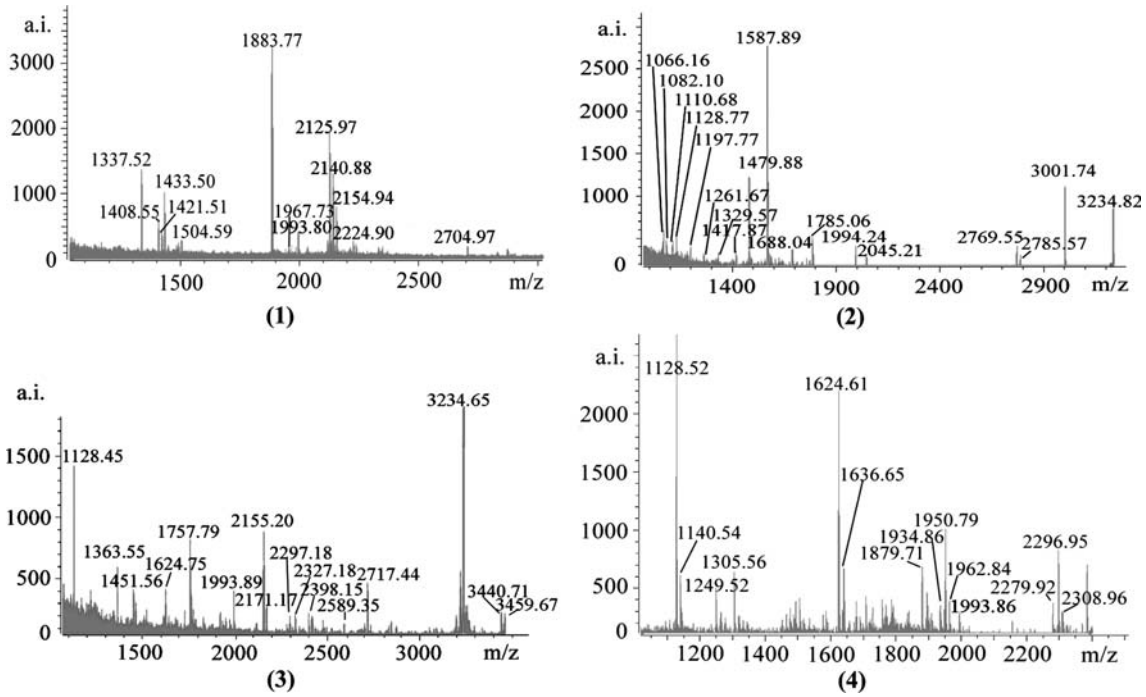

Figure 3. Identification of the differential proteins using MALDI-TOF and peptide mass fingerprint of the four differential protein spots excised from the two-dimensional electrophoresis gel. (1) Spot 1, which is down-regulated in cells treated with $LaCl_3$. (2–4) Spot 2–4, which are up-regulated in cells treated with $LaCl_3$.

Table 1. Identification of the four differential proteins in BGC-823 human gastric cancer cells using peptide mass fingerprint data from matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) to search protein databases.

Spot	Score	Number of Peptide matches	AC	ID	p*I*	M_w
1	0.45	5	Q9H4E5	RHOJ_HUMAN	6.37	23820.52
2[a]	0.39	7	P23497	SP10_HUMAN	8.49	100416.62
	0.39	7	Q9BZJO	CRN1_HUMAN	8.26	100579.00
3	0.40	6	Q9NVCE	CSP6_HUMAN	7.05	72876.13
4	0.54	7	P30305	MPI2_HUMAN	6.00	64987.49

[a]Shows that the protein spot was identified uncertainly by MALDI-TOF-MS and may be two probabilities.

We investigated the growth rate and morphological changes induced by $LaCl_3$ on BGC-823 gastric cancer cells (experiment not shown). Experiments were carried out at 0.1, 0.5, 1, 1.5 and 2 mM of lanthanum ions for 5 days. The results showed that the morphologies and growth rate of the cultured cells in the presence of 0.1 mM of metal ions were not different from those of control. Though the cells cultured in the presence of 0.5 mM of lanthanum ions showed slight inhibition, the morphological changes were small. In the presence of 1.0, 1.5 and 2.0 mM La^{3+}, the morphological transformations and significantly decreased growth rates were induced (Figure 4). In this study, we used proteomic analysis to investigate changes in protein synthesis induced by 1.0 and 2.0 mM La^{3+} in order to further explore its mechanism of tumor inhibition. In the case of Ln^{3+} as medication, Angilheri (1979) reported that daily i.p. injection of 2.5 mg $LaCl_3$ retarded the growth of sarcoma in rats. Some recent studies indicated that 40 mM cerium nitrate has a protective effect on postburn immunosuppression in patients (Scheidegger *et al*. 1992; Deveci *et al*. 2000). Thus, it can be seen that potent effects of

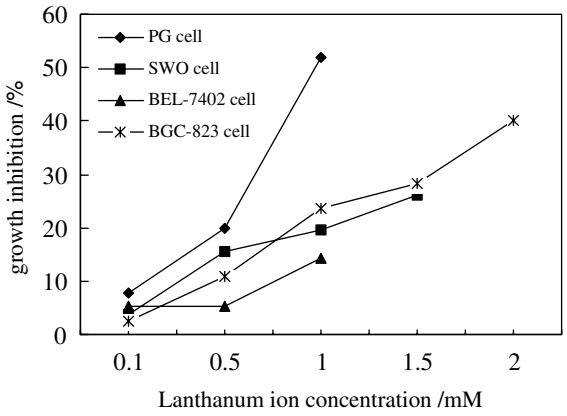

Figure 4. Cytotoxic effects of lanthanum ion (La^{3+}) on various human cancer cells, including neuroglioma cells SWO (Wu *et al*. 2003), lung cancer cells PG (Li *et al*. 2000), liver cancer cells BEL-7402 (Hong *et al*. 2003) and gastric cancer cells BGC-823 (experiment not shown in this paper). Growth inhibition (%) was represented by the rate of cell death compared to control.

Ln^{3+} on either cancer cells or medication *in vivo* attribute to their optimal doses.

Our data indicate that 1.0 and 2.0 mM lanthanum ions induce differential protein synthesis in BGC-823 cells. The differential protein analyses demonstrate that protein RHOJ is down-regulated in the presence of La^{3+}, which belongs to GTP-binding protein with GTPase activity, elicits the formation of F-actin-rich structures in fibroblasts and is involved in the regulation of cell morphology (Vignal *et al*. 2000). In general, cell morphology is known to correlate with the two- or three-dimensional structure of actin filaments. The down-regulation of RHOJ induced by La^{3+} suggests that La^{3+} has a potential to alter the formation F-actin-rich structures in fibroblasts. Changes in synthesis and structures of actin will induce the changes of cell morphology. Therefore, morphological changes in BGC-823 cells are related to the changes of RHOJ. Three other differential proteins are up-regulated when treated with La^{3+}, of these, two were identified as CSP6 and MPI2, respectively. CSP6 is a cofactor required for Sp1 transcriptional activation subunit 6 and plays a role in transcriptional coactivation (Ito *et al*. 1999). The up-regulation of this nuclear protein shows that La^{3+} may affect gene transcription of cancer cells. Another is cell cycle associated protein MPI2, which is a dual specificity phosphatase involved in the control of cyclin-dependent kinases and the progression of cells through the cell cycle (Galaktiono & Beach 1991). It appears to be essential in the G_2/M phase transition in human cells (Lammer *et al*. 1998). It is a suitable target for drug intervention, since it has been shown to be an oncogene when overexpressed, showing up in increased levels in several human breast cancers (Galaktionov *et al*. 1996). It is inconsistent that our study shows that addition of La^{3+} results in the up-regulation of this protein. The mechanism is unknown. However, the result suggests that La^{3+} is involved in mitotic control in cancer cells. In short, proteomic detection of changes in protein synthesis indicated that La^{3+} induced the differential protein synthesis in BGC-823 human gastric cancer cells. Additionally, the present results suggest that Ln^{3+} may be involved in differential protein expression relating to regulation of cell morphology, gene transcription and the cell cycle in cancer cells.

Acknowledgements

This work was sponsored by grants from China Postdoctoral Science Foundation and the Foundation of Fujian Province for Natural Science (C0210003).

References

Angilheri LJ. 1979 Effects of gallium and lanthanum on experimental tumor growth. *Eur J Cancer* **15**, 1459–1462.

Bergman AC, Benjamin T, Alaiya A. *et al*. 2000 Identification of gel-separated tumor marker proteins by mass spectrometry. *Electrophoresis* **21**, 679–686.

Dai YC, Jian LI, Jie LI *et al*. 2002 Effects of rare earth compounds on growth and apoptosis of leukemic cell lines. *In vitro Cell Dev Biol – An* **38**, 373–375.

Deveci M, Eski M, Sengezer M, Kisa U. 2000 Effects of cerium nitrate bathing and promote burn wound excision on IL-6 and TNF-α levels in burned rats. *Burns* **26**, 41–45.

Galaktionov KI, Beach D. 1991 Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins. *Cell* **67**, 1181–1194.

Galaktionov K, Lee AK, Eckstein J, Draetta G, Meckler J, Loda M, Beach D. 1996 Cdc25 phosphatases as potential human oncogenes. *Science* **269**, 1575–1577.

Henzed WJ, Stults TM, Wong SC, Grimley C, Watanabe C. 1993 Identifying proteins from two-dimensional gels by molecular mass searching of peptide fragments in protein sequence databases. *Proc Natl Acad Sci USA* **90**, 5011–5015.

Hong F, Wang PL, Wang K, Li RC. 2003 Effects of rare earths on proliferation toxicity of K562 and BEL-7402 cells. *J Chin Rare Earths Soc* **21**, 597–599.

Ito M, Yuan C-X, Malik S *et al*. 1999 Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. *Mol Cell* **3**, 361–370.

Ji YJ, Xiao B, Wang ZH, Cui MZ, Lu YY. 2000 The suppression effect of light rare earth elements on proliferation of two cancer cell lines. *Biomed Environ Sci* **13**, 287–292.

Ketring AR. 1987 ^{153}Sm-EDTMP and ^{186}Re-HEDP as bone therapeutic radiopharmaceuticals. *Nucl Med Biol* **14**, 223–232.

Laemmli UK. 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Lammer C, Wagerer S, Saffrcih R, Mertens D, Ansorge W, Hoffmann I. 1998 The Cdc25B phosphatase is essential for the G2/M phase transition in human cell. *J Cell Sci* **111**, 2445–2453.

Li XB, Zhou AR, Yu WH, Chen XA. 1999 The effect of cerium compounds on two cell lines: human lung cancer cells PG and human gastric carcinoma cells BGC-823. *Chin J Biochem Mol Biol* **15**, 651–654.

Li XB, Zhou AR, Yu WH, Chen XA. 2000 Effect of lanthanum citrate on two cell lines: human lung cancer cells PG and human carcinoma cells BGC-823. *J Chin Rare Earths Soc* **18**, 156–159.

Sato T, Hashizume M, Hotta Y, Okahata Y. 1998 Morphology and proliferation of B16 melanoma cells in the presence of lanthanoid and Al^{3+} ions. *Biometals* **11**, 107–112.

Scheidegger D, Sparkes BG, Lüscher N, Schoenenberger GA, Allgöwer M. 1992 Survival in major burn injuries treated by one bathing in cerium nitrate. *Burns* **18**, 296–300.

Vignal E, De Toledo M, Comunale F *et al*. 2000 Characterization of TCL, a new GTPase of the Rho family related to TC10 and Cdc42. *J Biol Chem* **275**, 36457–36464.

Wu CL, Liu JS, Yang WD, Sheng ZG. 2003 Effects of $La(NO_3)_3$ on human neuroglioma cells. *J Chin Rare Earths Soc* **21**, 321–325.

Xiao B, Ji YJ, Wang ZH *et al*. 1997 Effect of lanthanum and cerium on malignant proliferation and expression of tumor-related gene. *Chin J Prev Med* **31**, 228–230.