研究报告

A LIF Mutation at The 29th Amino Acid Totally Abolished The Biological Functions*

NING Hong-Xiu[1,2)**], CHEN Yue[1)**], ZHANG Yuan-Jiang[1,2)], RONG Yu[1,2)], WU Xiao-Jun[3)], ZHANG Xiu-Fang[1)], CHANG Zhi-Jie[1,2)***]

([1)]*Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing* 100084, China;
[2)]*The Institute of Biomedicine, Tsinghua University, Beijing* 100084, China;
[3)]*The Third Hospital, School of Medicine, Peking University, Beijing* 100083, China)

Abstract Leukemia inhibitory factor (LIF) plays important roles in varieties of biological processes. This factor is highly conserved in mammalian animals and only one heterozygous *LIF* mutation was reported to cause the infertility of women. A LIF mutation was generated and the evidences were provided that the mutation of mature LIF at the 29th amino acid totally abolished its functions, including stimulation of STAT activation assayed by Luciferase reporter gene expression and EMSA experiments. In addition, the mutated LIF failed to inhibit the proliferation of M1 cells. The data indicated that the mutation of LIF did not have a dominant negative effect but lost the biological functions, suggesting that the 29th amino acid is critical for maintaining the activities of LIF.

Key words leukemia inhibitory factor(LIF), mutation, functions, proliferation

LIF (leukemia inhibitory factor), identified as a glycoprotein[1], suppresses the proliferation of myeloid leukemic cells (murine M1 cell line)[2, 3] and inhibits the differentiation of murine embryonic stem cells to maintain the pluripotency feature [4]. LIF has been reported to play very important roles in varieties of biological processes including blastocyst implantation [5,6], platelet formation, proliferation of some hematopoietic cells, kidney formation [7], bone formation, adipocyte lipid transport, adrenocorticotropic hormone production, neuronal survival and formation [8], muscle satellite cell proliferation, and acute phase production by hepatocytes [9]. LIF still remains functions in tissue regeneration in the adults[10~12].

LIF is a highly conserved protein in most of the mammalian animals[13]. It has been rarely reported *LIF* gene was mutated in human diseases except the case that *LIF* mutation leaded to the women infertile[14]. In the infertile women, three heterozygous point mutations were identified: one in close proximity to the start codon of exon 1 and two in exon 3. The first point mutation occurred in 6 bp before ATG but the other two remained LIF protein expressed with one amino acid changed as V64M and A72Y [14]. These results were consistent with *LIF* knockout phenotype in mice, which developed normally except the adult female homozygous null mice could not be pregnant[15].

LIF functions on cells by binding to the gp190 receptor and then forming a heterodimer with the common receptor gp130. The amino acids crucial for LIF binding to its receptors gp190 and gp130 were characterized by authors[16, 17]. Recently, Fairlie *et al*.[18] reported that incorporation of two mutations (Q29A and G124R) led to the generation of highly potent antagonists of LIF-induced bioactivity, indicating the importance of those two residues in LIF. However, it remains unclear whether the 29th amino acid single mutation in LIF has any dominant negative effect to the function because this residue is highly conserved in all mammalian animals. In this study, we report a point mutation of Q29R in LIF generated via a random PCR. We observed that this mutation abolished the normal functions in the activation of STAT signaling and growth arrest of M1 cells but had no dominant negative effect.

1 Materials and methods

1.1 RNA isolation, RT-PCR and subcloning

The total RNA from human stromal cells isolated from bone marrow was isolated using Trizol (Invitrogen, USA). RT-PCR was performed using one-step RT-PCR kit (Qiagen, Germany). The Taq enzyme was added in the RT-PCR reaction to increase the possibility of random mutation. The primers used are: forward, 5′ gctagaattccccataatgaaggtcttg 3′; reverse, 5′ gatactcgagtcctgctagaaggcctg 3′. The reaction was performed for 30 cycles with conditions of denaturation (94℃, 30 s), annealing (55℃, 50 s)

* This work was supported by grants from Funds for 973 Program (2001CB510006, 2002CB51300), The National Natural Science Foundation of China (39970369, 30070703, 30470888 and 30030050).
**Equally contributed authors.
***Corresponding author.
Tel: 86-10-62785076, Fax: 86-10-62773624
E-mail: zhijiec@mail.tsinghua.edu.cn
Received: October 14, 2004 Accepted: December 8, 2004

and extension (72℃, 1 min). The expected LIF cDNA was directly cloned into pcDNA6 with *Eco*R Ⅰ and *Xho* Ⅰ and subcloned into pEFBOS with *Kpn* Ⅰ and *Xho* Ⅰ for expression. The positive clones with insert were sequenced.

1.2 Cell culture and transfection

HepG2 cells were cultured in DMEM, supplied with 10% fetal bovine serum (Hyclone, USA), 100 U/ml penicillin, and 100 mg/L streptomycin at 37℃ in 5% CO_2. 293T cells were cultured under the same conditions except for replacement of FBS with NCS (new-born calf serum). M1 cells were fed with RPMI1640 medium supplied with 10% FBS. 293T cells were plated onto six-well plates at a density of 4×10^5 cells per well and cultured at 37℃ overnight for transfection. 5 μg DNA per well was used for transcient transfection with Tfx™-20 (Promega) according to the manufacturer's protocol. The expression vectors for different genes were balanced with pcDNA6 or pEFBOS in the specific experiments. After 48 h transfection, the cells were harvested for different experiments.

1.3 Preparation of conditioned medium

293T cells, transfected with pcDNA6, pcDNA6/LIFw or pcDNA6/LIFmu for 12 h in the complete medium, were cultured in serum free medium for 48 h. The conditioned medium was collected and stored at 4℃ or directly used for experiments.

1.4 Western blot

Cells were harvested with lysis buffer (20 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, 1 mmol/L $NaVO_3$, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerol-phosphate, 1 mmol/L PMSF, 5 mg/L aprotinin, and 5 mg/L leupeptin, pH 7.5). Equal amounts of proteins were subjected to 12% SDS-PAGE. The proteins were blotted onto NC membrane (Pharmacia) and detected with anti-LIF antibody (Santa Cruz). The image was captured using Molecular Dynamics (Pharmacia).

1.5 Luciferase assay

HepG2 cells stably transfected with hSIE/m67 (kindly provided by Dr. Fu at Yale University, Medical School.) were seeded into 24-well cell culture plates with a density of 3×10^5 cells/well. After 24 h culturing, the conditioned medium (with wide type LIF, LIFw, or mutated LIF, LIFmu, secreted by transfected 293T cells) was added. The cells, stimulated for 6 h, were harvested with the lysis buffer provided by Luciferase1000 Assay System (Promega) and assayed for luciferase activity according to the manufacturer's protocol. In another experiment, 293T cells were seeded in a 12 well plate with the density of 2×10^5 cells/well. The luciferase reporter with STAT3 binding element (pGL2-APRE-Luc, kindly provided by Dr.Hirano, Division of Immunology, Osaka University, Japan) was cotransfected with mutated or wild type LIF expression plasmid. The luciferase activity was measured by a Dual Luciferase Reporter Assay KIT and normalized with the internal control (pRL-TK) (Promega). The results were presented as $(\bar{x}\pm s)$ from transfections in triplicate.

1.6 Electrophoresis mobility shift assay

293T cells and HepG2 cells were seeded with a density of 1×10^6 cells/well in 60mm dishes. The cells were stimulated with LIF (purchased from Santa Cruz) and the conditioned medium (wild type LIF or mutated LIF) for 30 min and harvested for preparation of nuclear extract. In brief, the cells were collected and lysed with 500 μl of the cell pellet lysis buffer (10 mmol/L Tris-HCl pH 7.5, 1.5 mmol/L $MgCl_2$, 10 mmol/L KCl, 0.5% Nonidet P-40) by pipetting several times. The pellet was collected through centrifugation of the lysates at 4℃ for 10 min at 7 000 r/min and dissolved in 50 μl of nuclear protein extraction buffer (20 mmol/L Tris-HCl pH 7.5, 10% glycerol, 1.5 mmol/L $MgC1_2$, and 420 mmol/L NaCl, 0.2 mmol/L EDTA). The nuclear protein suspension was cleared by microcentrifugation at 13 000 r/min for 15 min. The annealed and purified DNA probe (5′ TCGACATTTCCCGTAAATCGTC GA 3′) labeled with [γ-^{32}P] dATP was incubated with the nuclear extracts in 15 μl binding buffer (including 10 mmol/L Tris-HCl pH 7.5, 10% glycerol, and 0.2% Nonidet P-40, additionally, 2 μg of poly dI-dC) at room temperature for 30 min. The DNA-protein complexes were resolved on 5% nondenaturing polyacrylamide gels in 0.5 × TBE buffer. After electrophoresis, the gels were dried and subjected to autoradiography (PhorphorImager, Pharmacia).

1.7 ^{3}H-thymidine incorporation

M1 cells were seeded in 24 well plates with a density of 1×10^5 cells/well. The cells were starved for 24 h before addition of 1 μCi of ^{3}H-thymidine per well. The ^{3}H-thymidine was allowed to incorporate for 4 h and then the free ^{3}H-thymidine was washed off with PBS and 5% TCA. The labeled cells were lysed with buffer (0.5% SDS, 0.5 mol/L NaOH) and subjected to scintillation assay. The CPM was measured and results were presented as $(\bar{x}\pm s)$ from three independent repeats for each treatment.

2 Results

2.1 Generation of mutated LIF from RT-PCR

In an attempt to search for the mutation of LIF

cDNA, we performed RT-PCR using Taq enzyme mixed in the One-step RT-PCR kit from Qiagen. Via subcloning the PCR products and sequencing more than 20 independent clones, we found three clones with a mutation at 29th amino acid in the LIF cDNA (Figure 1b and 1c). We turned to be interested in this mutation because the amino acid at this position in LIF from all of the species was kept identical as

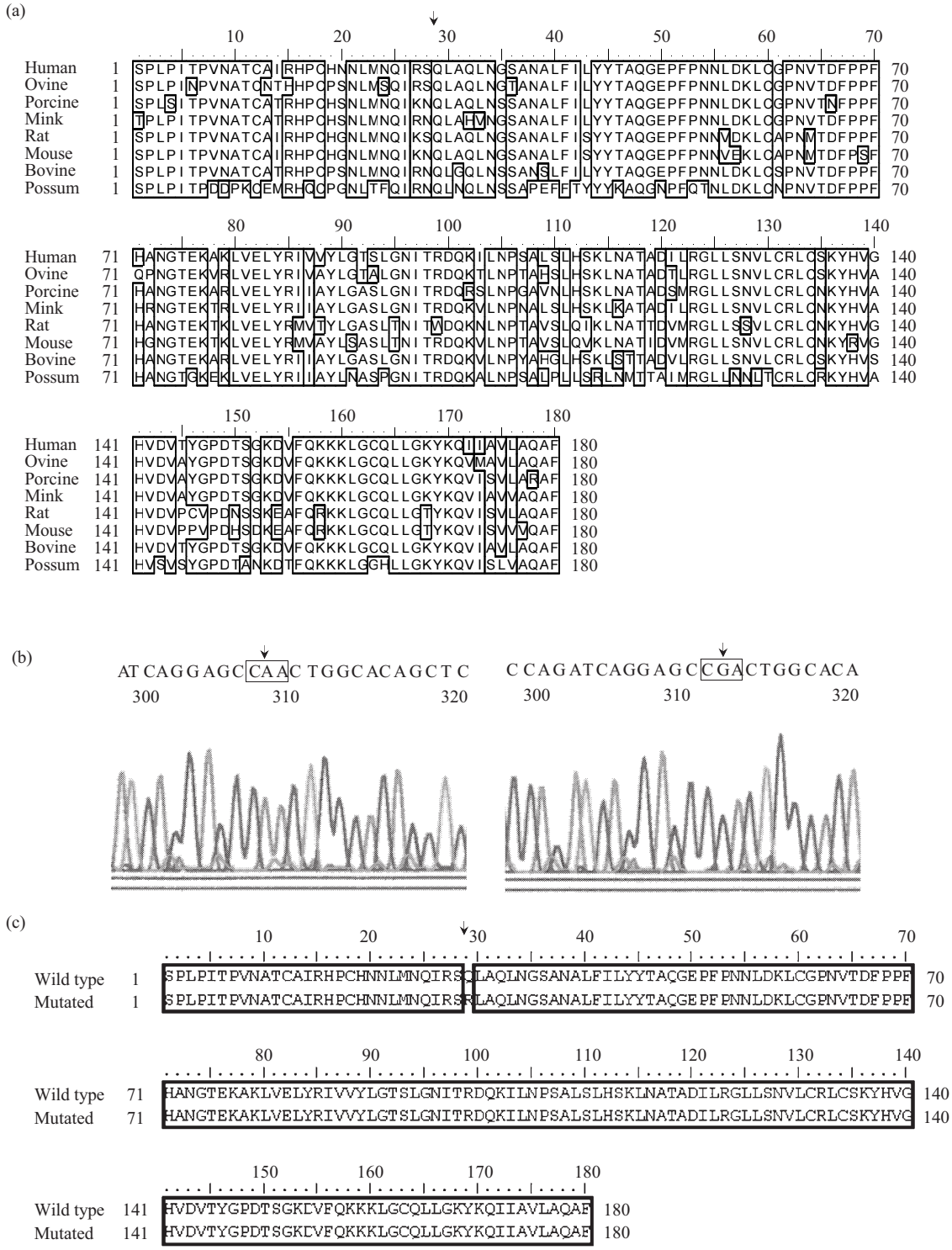

Fig.1 Diagrams of LIF sequence information

(a) Amino acid sequence alignment of LIF from indicated mammals including human. The identical amino acids were boxed. The 29th residue with identical Q between the species is indicated with arrow. (b) Partial cDNA sequence information from the cloned wild type and mutated LIF. The original sequencing graphs were shown under the sequence reading. The boxed triplets indicated mutation of nucleotide A (left panel) to G (right panel). (c) Alignment of amino acid sequences of wild type and mutated LIF. The position where Q (acidic) was mutated to R (basic) is indicated with arrow.

glutamine (Q, Figure 1a)[13], implying that this residue is important for the activity of LIF. This reminded us of investigating whether the mutation could affect the functions of LIF. Thereafter, we purposely cloned this mutation into expression vector and expressed the protein in mammalian cells (293T). As a control, we expressed the wild type LIF using the same vector.

2.2 Mutated LIF lost the activity to stimulate the downstream signaling

LIF belongs to IL-6 cytokine family, which, by binding to receptor gp130 and gp190 (LIFR), can stimulate STAT3 signal pathway. To examine whether the mutated LIF has any activity, the STAT3 luciferase reporter system was employed. The wild type and mutated LIF cDNA were subcloned to the expression vector respectively (referred to as pEFBOS/LIFw and pEFBOS/LIFmu) and were co-transfected with the STAT3 luciferase reporter into 293T cells. The data showed that the wild type LIF stimulated the reporter activity significantly but the mutated LIF had no effect on the reporter activity (Figure 2b) although both the cells transfected with the constructs showed the same levels of protein expression (Figure 2a). Similarly, in a HepG2 cell line stably transfected with STAT3 binding elements linking to luciferase reporter gene, we obtained same results by addition of the conditioned medium produced by 293T cells transfected with wild type or mutated LIF expression vector (Figure 2c). Taken together, these results showed that the wild type LIF had strong ability to activate STAT3 signal pathway, but the mutated LIF failed to, indicating that the mutation of LIF at 29th amino acid lost the normal function in stimulation of STAT3 signal pathway.

To further confirm the mutated LIF failed to activate STAT3, we performed an EMSA experiment using 293T and HepG2 cells stimulated by the purchased LIF and the conditioned mediums (from 293T cells expressed wild type or mutated LIF). The

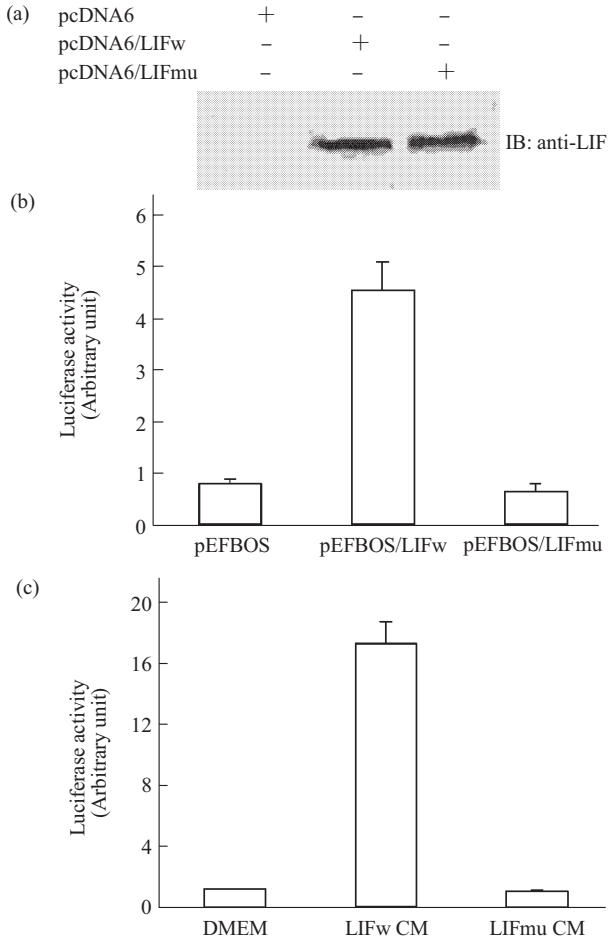

Fig.2 Activation of STAT3 stimulated by LIF

(a) Expression of wild type and mutated LIF. Western blot were recruited to show the protein expression levels of the wild type and mutated LIF in 293T cells transfected with pcDNA6 (1.8 μg), pcDNA6/LIFw (1.8 μg) and pcDNA6/LIFmu (1.8 μg) as indicated respectively. Western blot was performed using anti-LIF antibody (Santa Cruz). (b) Luciferase activity of STAT3 reporter responsible to LIF. 239T cells were co-transfected with STAT3 luciferase reporter plasmid and the indicated wild type or mutated LIF expression vector. The luciferase activities were presented as arbitrary units normalized with the internal control (pRL-TK) for the equal transfection efficiencies. The experiments were repeated 3 times and the results were expressed as $\bar{x} \pm s$ (n=3). (c) Luciferase activity in STAT3-Luc reporter stable cell line. The conditioned medium as indicated was added to the HepG2 cells stably integrated STAT3 response element with luciferase reporter gene. The luciferase assays were performed and the results were presented as (b).

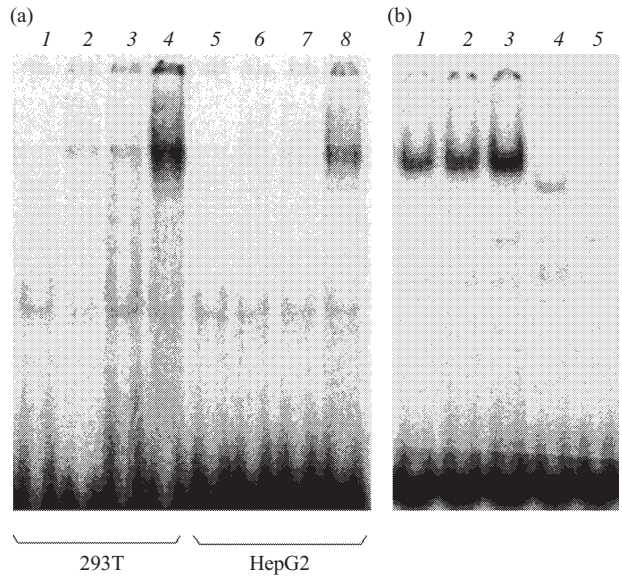

Fig.3 Stimulation of STAT1/3 binding

Electrophoresis mobility shift assays were performed using the cell extracts as indicated. Maximal amounts of conditioned mediums were used to stimulate both 293T and HepG2 cells (a). The different amounts of conditioned mediums (b) were compared with commercial purchased LIF (Santa Cruz). The mediums of wild-type and mutated LIF contained equal amounts of the secreted proteins. The bands were confirmed by super shift assay (data not shown). (a) *1*:control; *2*: pcDNA6 CM; *3*: mutant LIF CM; *4*: wild-type LIF CM; *5*: control; *6*: pcDNA6 CM; *7*: mutant LIF CM; *8*: wild-type LIF CM. (b) *1*: LIF (100 μg/L); *2*: wild-type LIF CM (200 μl); *3*: wild-type LIF CM (400 μl); *4*: mutant LIF CM (200 μl); *5*: mutant LIF CM (400 μl).

data showed that wild type LIF conditioned medium stimulated very strong binding of STAT3 to its binding elements and had the dosage dependent effects on the binding (Figure 3a lane *4* and *8*, Figure 3b lane *2* and *3*), while the mutated LIF had almost no activity on the binding of STAT3 (Figure 3a lane *3* and *7*, Figure 3b lane *4* and *5*). The wild type LIF conditioned medium had the same effect as the purchased LIF (Figure 3b, lane *1* and *2*). These data, consistent with those from the luciferase experiments, suggested that the mutated LIF abolished the activity of stimulation of STAT3 signaling.

2.3 Mutated LIF did not show dominant negative effect

To question whether the mutated LIF had a dominant negative effect on the wild type LIF rather than just abolished the activity, we performed luciferase assays using both the wild type and mutated LIF expression vectors as described above. The data showed that the mutated LIF did not affect the luciferase activity stimulated by the wild type LIF (Figure 4a) even though we increased the mutated LIF expression vector amount to enough high level (Figure 4a, the right three columns). To avoid different transfection efficiencies, we used the conditioned medium as prepared in Figure 2 to repeat the luciferase assays. As shown in Figure 4b, the conditioned medium containing the mutated LIF did not affect the luciferase activity induced by the wild type LIF. Therefore, these data suggested that the mutated LIF did not show a dominant negative effect on the wild type LIF.

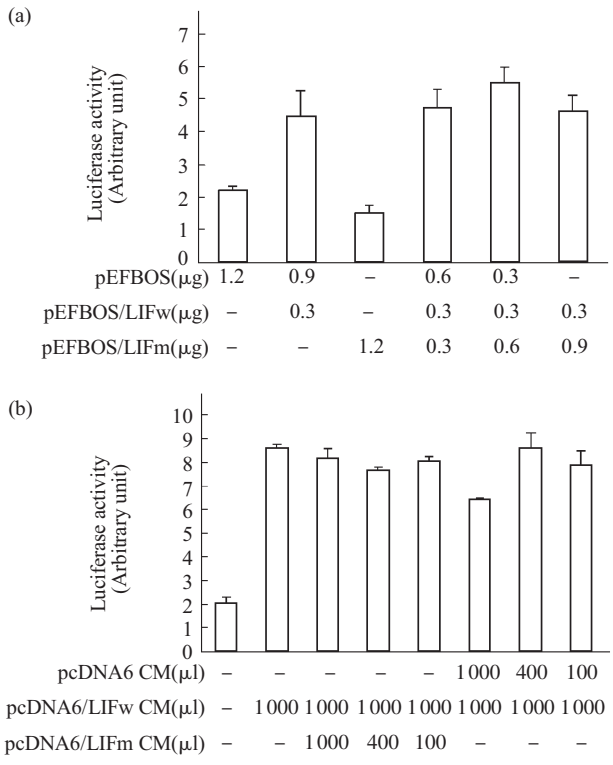

Fig.4 Mutated LIF did not show dominant negative effect

Luciferase assays were performed as described in Figure 2. (a) The indicated LIF expression vectors were co-transfected into the cells with the reporter plasmids. (b) The conditioned medium was added in the cells transfected with only the luciferase reporter plasmid.

2.4 Mutated LIF remained M1 cells growth

The most important feature of LIF is the inhibition role on the growth of M1 cells. To investigate whether the mutated LIF had any effect on the biological function in cells, we performed a proliferation experiment by ^{3}H-thymidine incorporation in M1 cells. The results demonstrated that M1 cells cultured with the wild type LIF

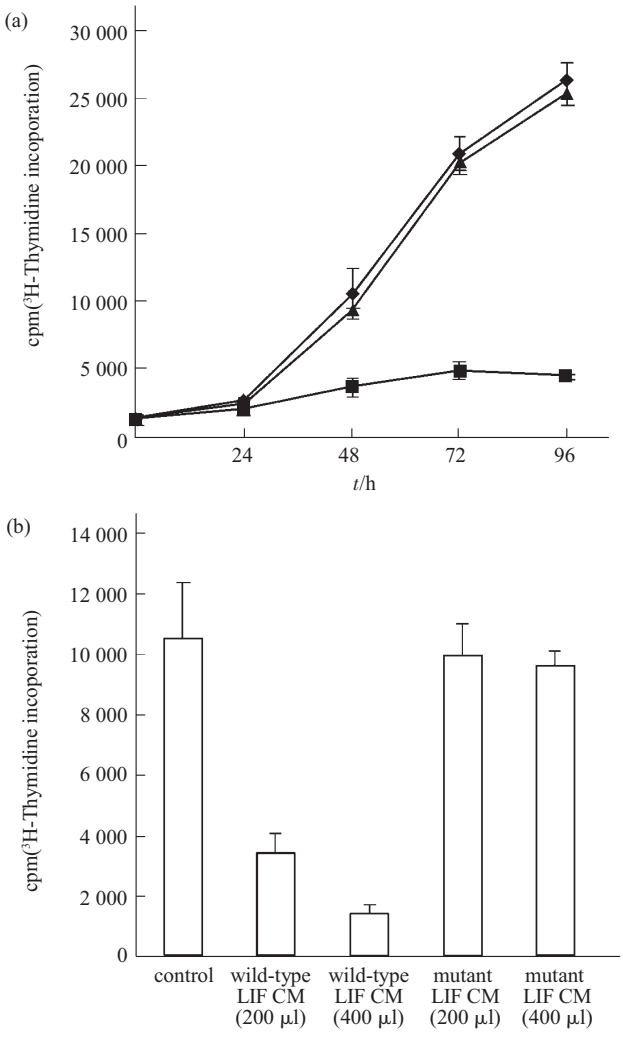

Fig.5 Growth inhibition of LIF

^{3}H-thymidine incorporation assays were performed using M1 cells stimulated by the conditioned mediums as indicated. The different times (a) and dosages (b) of conditioned medium treatments were assigned and the cell proliferation was presented as incorporated ^{3}H-thymidine (cpm). The experiments were repeated three times independently and the results were presented as $\bar{x} \pm s$ (n=3). ◆—◆: control; ■—■: wild-type LIF; ▲—▲: mutant LIF.

conditioned medium had a strong growth arrest, but the cells cultured with mutated LIF conditioned medium remained of rapid growth, as in the case of control cells (Figure 5a and b). Intriguingly, this inhibition of growth arrest showed dosage dependent effects with the increasingly amounts of wild type LIF conditioned medium but remained no effect with the maximal amounts of the mutated LIF conditioned medium (Figure 5b). These experiments indicated that the mutated LIF could not inhibit M1 cells growth, suggesting that the mutation of LIF totally lost the biological functions.

3 Discussion

LIF is a glycoprotein cytokine belonging to IL-6 family, which exerts many important functions through activation of STAT signal pathway. Three mutations in *LIF* gene, occurred in exon 1 and exon 3, were reported in the infertile women [14]. Interestingly, although LIF was reported to function in varieties of biological processes, *LIF* brain deficient mice did not display lethal but abnormal in nervous system development[19]. However, the *LIF* mutation women did not show any abnormal development[14] although it was unclear whether the mutation altered the expression or the activity of LIF. No other mutation of *LIF* was reported in patients. In this report, we, via random selection from RT-PCR, reported LIF mutation in the 29th amino acid in the mature protein. We found this mutation totally abolished LIF activities, including activation of STAT3 signal pathway and inhibition of M1 cell proliferation.

LIF remains high conservation in the amino acid sequences between species [13]. Our data showed that LIF kept totally identical at the 29th amino acid in all mammalian animals, suggesting that this residue is critical to the activity. In this study, we observed that the amino acid glutamine (Q) at the 29th position had been mutated into arginine (R) in the mutated clones from RT-PCR. Although this mutation did not affect the protein expression examined by Western blot (Figure 2), it dramatically changed the activity of LIF measured by STAT3 luciferase reporter, EMSA and M1 cell proliferation assays. It has been noted that the six conserved cysteine residues in LIF molecule could be involved in disulfide bond linkages [20] and 9 conserved glycosylation sites could be important for the glycosylation of this factor. However, no report was proposed to the function of the residue Q at the 29th amino acid in the molecule. Importantly, this residue is located in helix A region, where a marked kink was observed [2,17,20], while V64M and A72Y mutations in the infertile women were located in AB loop. Interestingly, the Q (glutamine) is acidic and R (arginine) is basic. We speculate the change of the amino acid altered the feature of the helix. How this mutated site abolishes LIF function is to be elucidated.

Several reports indicated the different residues critical for the LIF activity by keeping the binding affinity to the receptor[16,17]. Especially, a recent report demonstrated that two mutations (Q29A and G124R) of LIF could generate a highly potent antagonist effect of LIF-induced bioactivity [18]. The report indicated a mutation at the 29th position but different residue replacement from the one we reported here. Interestingly, we observed no dominant negative effect from our data (see Figure 4 and 5). The difference of the mutations might be the amino acid features as alanine is neutral but arginine is basic (the wild type LIF at 29th glutamine is acidic). We speculate that it might be the mutated alanine and the other mutation leading to the antagonist effect[18].

In conclusion, we reported a mutation of LIF at the 29th residue and provided evidences that this mutation totally abolished the normal functions of LIF on activation of STAT3 signal and inhibition of M1 cell proliferation. However, this mutation had no dominant negative effect on LIF-induced activity. We propose that the 29th glutamine is critical for maintaining the activity of this important factor.

Acknowledgements We thank Dr. Xinyuan Fu for supplying the HepG2 stable cell line with STAT3-luciferase reporter gene and helpful comments and suggestions. We gratefully acknowledge the gift of pGL2-APRE-Luc from Dr. Toshio Hirano, Division of Immunology, Osaka University, Japan.

References

1 Gearing D P, Gough N M, King J A, *et al*. Molecular cloning and expression of cDNA encoding a murine myeloid leukaemia inhibitory factor (LIF). EMBO J, 1987, **6** (13): 3995~4002

2 Hilton D J, Nicola N A, Metcalf D. Specific binding of murine leukemia inhibitory factor to normal and leukemic monocytic cells. Proc Natl Acad Sci USA, 1988, **85** (16): 5971~5975

3 Metcalf D, Hilton D J, Nicola N A. Clonal analysis of the actions of the murine leukemia inhibitory factor on leukemic and normal murine hemopoietic cells. Leukemia, 1988, **2** (4): 216~221

4 Williams R L, Hilton D J, Pease S, *et al*. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. Nature, 1988, **336** (6200): 684~687

5 Bhatt H, Brunet L J, Stewart C L. Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. Proc Natl Acad Sci USA, 1991, **88** (24): 11408~11412

6 Nachtigall M J, Kliman H J, Feinberg R F, *et al*. The effect of leukemia inhibitory factor (LIF) on trophoblast differentiation: a

potential role in human implantation. J Clin Endocrinol Metab, 1996, **81**(2): 801~806

7 Barasch J, Yang J, Ware C B, *et al*. Mesenchymal to epithelial conversion in rat metanephros is induced by LIF. Cell, 1999, **99** (4): 377~386

8 Koblar S A, Turnley A M, Classon B J, *et al*. Neural precursor differentiation into astrocytes requires signaling through the leukemia inhibitory factor receptor. Proc Natl Acad Sci USA, 1998, **95** (6): 3178~3181

9 Metcalf D. The unsolved enigmas of leukemia inhibitory factor. Stem Cells, 2003, **21** (1): 5~14

10 Kurek J B, Bower J J, Romanella M, *et al*. The role of leukemia inhibitory factor in skeletal muscle regeneration. Muscle Nerve, 1997, **20** (7): 815~822

11 Kurek J B, Radford A J, Crump D E, *et al*. LIF (AM424), a promising growth factor for the treatment of ALS. J Neurol Sci, 1998, **160** (Suppl 1): S106~113

12 Kurek J B, Bennett T M, Bower J J, *et al*. Leukaemia inhibitory factor (LIF) production in a mouse model of spinal trauma. Neurosci Lett, 1998, **249** (1): 1~4

13 Cui S, Selwood L. cDNA cloning, characterization, expression and recombinant protein production of leukemia inhibitory factor (LIF) from the marsupial, the brushtail possum (*Trichosurus vulpecula*). Gene, 2000, **243** (1~2): 167~178

14 Giess R, Tanasescu I, Steck T, *et al*. Leukaemia inhibitory factor gene mutations in infertile women. Mol Hum Reprod, 1999, **5**(6): 581~586

15 Stewart C L, Kaspar P, Brunet L J, *et al*. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature, 1992, **359** (6390):76~79

16 Hudson K R, Vernallis A B, Heath J K, Characterization of the receptor binding sites of human leukemia inhibitory factor and creation of antagonists. J Biol Chem, 1996, **271** (20):11971~11978

17 Hinds M G, Maurer T, Zhang J G, *et al*. Solution structure of leukemia inhibitory factor. J Biol Chem, 1998, **273** (22): 13738~13745

18 Fairlie W D, Uboldi A D, McCoubrie J E, *et al*. Affinity maturation of leukemia inhibitory factor and conversion to potent antagonists of signaling. J Biol Chem, 2004, **279** (3): 2125~2134

19 Bugga L, Gadient R A, Kwan K, *et al*. Analysis of neuronal and glial phenotypes in brains of mice deficient in leukemia inhibitory factor. J Neurobiol, 1998, **36** (4): 509~524

20 Robinson R C, Grey L M, Staunton D, *et al*. The crystal structure and biological function of leukemia inhibitory factor: implications for receptor binding. Cell, 1994, **77** (7):1101~1116

白血病抑制因子 LIF 第 29 位氨基酸突变导致其功能完全丧失 *

宁红秀[1,2)**] 陈 鈜[1)**] 张原江[1,2)] 戎 煜[1,2)] 吴晓君[3)] 张秀芳[1)] 常智杰[1,2)***]

([1)]清华大学生物科学与技术系，北京 100084；[2)]清华大学生命科学与医学研究院，北京 100084；
[3)]北京大学第三医院，北京 100083)

摘要 白血病抑制因子(LIF)为一种多功能细胞因子，在生物发育及维持正常生理功能中发挥着重要的作用. LIF 在所有的哺乳动物中都具有高度的保守性，某些氨基酸在不同物种中保持不变. LIF 中 29 位氨基酸在所有的哺乳动物中都是 Q. 为了研究 Q 对 LIF 功能的重要性，利用随机 PCR 的方法将 29 位氨基酸突变为 R，并将突变后的 LIF 克隆到真核表达载体 pcDNA6，在哺乳动物细胞中成功表达. 同时以克隆到的野生型 LIF 作为对照. 将分泌到细胞培养基中的野生型和突变 LIF 因子收集，通过 EMSA 实验以及荧光素酶报告系统检测，发现野生型 LIF 因子具有激活 STAT3 信号通路的生物学活性. 同时，^{3}H-TdR 掺入实验结果表明，野生型 LIF 因子能显著抑制鼠骨髓白血病细胞系 M1 的增殖。而 29 位氨基酸突变的 LIF 因子则完全丧失了以上功能，但所发现的突变没有显性负的作用. 结果充分说明，LIF 中高度保守的 29 位氨基酸对 LIF 功能的维持具有重要的作用.

关键词 白血病抑制因子(LIF)，突变，功能，细胞增殖

学科分类号 Q7

---

*国家重点基础研究发展规划项目(973)(2001CB510006, 2002CB51300)和国家自然科学基金资助项目 (39970369, 30070703, 30470888 和 30030050) .

同等贡献作者. * 通讯联系人. Tel: 010-62785076; Fax: 010-62773624, E-mail: zhijiec@tsinghua.edu.cn

收稿日期: 2004-10-14, 接受日期: 2004-12-08