The toxicity of lithium to human cardiomyocytes

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Research

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Abstract

Background: Lithium is widely used in the electronic consumer market and electric vehicles and has a great contribution in the world economy, resulting in large quantities of lithium waste in the environment. Yangtze River basin is one of the most developed areas in China. However, the environment influence of lithium in the Yangtze River basin and its roles in cardiomyocytes has not yet been clarified.

Results: Here we found that the concentration of lithium in the water environment is very high in Shanghai, as well as in tap water, which might be cause by the pollution of lithium batteries. Lithium inhibits not only cell viability of human cardiomyocytes, but also cell proliferation. Moreover, lithium promotes cell apoptosis of cardiomyocytes significantly. And we found that lithium controls cardiomyocytes' functions through regulating Gsk3β signaling.

Conclusions: This study reveals that the water environment of Shanghai might be polluted by the lithium batteries; and the enrichment of lithium may cause damage to human cardiomyocytes; and it is imperative to detect lithium concentration in the water environments (such as tap water and irrigation water) and effectively recycle lithium batteries in the future.

Background

Lithium is the first element in the alkali metal group and the lightest metal. It is very active; therefore it exists mainly in the form of compounds (such as Apatite or Aluminum silicide) in the environment [1–4]. The other two elements in the alkali metal group, sodium and potassium, account for more than 2% in the earth's crust; however, in contrast to them, the existence of lithium on the earth is scarce, counting only 0.0065% [5, 6]. Although lithium may also be enriched in several mines and salt lakes, it is usually widely spread in trace amounts in rocks and soils, as well as in water, including surface water, groundwater, and seawater [7]. For example, the content in seawater is very low, only 0.17 mg/L; In freshwater, the content is even lower, less than 0.04 mg/L [7]. The lithium compounds are highly soluble, and the main form of lithium in water is ion [8]. Because the concentration of lithium in the natural environment is generally very low, the lithium does not cause damage to the environment.

However, since Sony Corporation invented the first commercial lithium battery in 1991, it has rapidly become popular in the market because of its high-energy density, low sensitivity to temperature variation, and no memory-effect [9, 10]. It has been used widely in the consumer electronics field. In the 1990s, digital cameras and personal notebook computers were the earliest large-scale applications of lithium-ion batteries [10] Since the 21st century, with the booming of consumer electronics such as smartphones, tablet computers, and electric vehicles, the use of lithium batteries has begun to erupt. For example, since Steve Jobs invented the smartphone in 2007, the global sales of the smartphone in 2016 sharply swell to more than 1.5 billion. Since Company Tesla launched the first successful commercial electric vehicle - model S in 2009, the sales of electric vehicles have also been rapidly increasing. The facts above suggest that lithium has an excellent contribution to the world economy [11]. From unknown to known all over the

world, lithium batteries use less than 30 years. However, it might cause lots of lithium pollution. In cities, lithium batteries are routinely discarded in the environment, along with other solid garbage [10]. The lithium batteries are still cheap enough to throw old batteries and to obtain the virgin material [10]. Therefore the recycling rate is meager, even in developed countries that do well in environmental protection [10]. The rapid increase in consumption and the serious shortage of recycling might lead to the continuous accumulation of lithium in the economically developed areas where lithium is broadly used.

As one of the most economically developed regions in China, the Yangtze River Basin has a GDP accounting for two-fifths of China [12]. It contains a series of megacities, including Shanghai, Suzhou, Changsha, Nanjing, Wuhan and Chongqing. This region has a well-developed electronic consumer market and rapid economic growth, which continues to promote the rapid increase in lithium consumption. However, the research on the impact of lithium pollution in the Yangtze River Basin has not yet been reported to date. Shanghai, at the estuary of the Yangtze River, is the leader of the Chinese economy. Shanghai is one of the biggest consumer electronics market in China. Besides, Shanghai is actively promoting the popularization of electric vehicles these years [13]. Tesla built its super factory in Shanghai in 2019. The facts above increase substantially the use of lithium batteries, which accumulates the lithium pollution. Due to the discharge of large quantities of lithium resources, lithium pollution is growing rapidly and imposing severe threats to the environment and humans [14].

Though the low concentration of lithium has no harmful effect on the environment, the high level of lithium may cause considerable damage to the aquatic and terrestrial environment [15]. For example, a small dose of lithium has a significant inhibition effect on the proliferation and growth of aquatic organisms including Pimephales promelas, Ceriodaphnia dubia, and Elimia clavaeformis [16]. Also, lithium in water can accumulate in plants and cause damage to plant growth and development [17, 18]. For example, 60 mM of lithium can damage the growth of sunflower; the same concentration can also affect the growth of corn [17]. Lithium can be enriched in animals by food chains, and high concentrations of lithium can also cause severe damage to animals [19]. For instance, rats were treated with small doses of lithium for seven weeks (every alternate day) [20]. The epithelium lining of renal tissue was injured, and some significant changes were observed in the glomerular region in corticomedulary region [20]. Moreover, high concentrations of lithium could cause severe damage to humans, including nervous system (including coarse tremor and hyperreflexia), kidney (including sodium-losing nephritis and nephrotic syndrome), and endocrine system (including hypothyroidism) [21–24]. The heart is one of the most important organs in our body, and cardiomyocytes play a critical role in contraction. However, the functions of lithium on the myocardium have not been studied yet. Our research detected that lithium concentrations in the Yangtze River and rivers in Shanghai are relatively high, and it is possible to cause harm to human health through food chains [25]. Then we found that lithium not only significantly inhibited the cell viability and cell proliferation of human cardiomyocytes, but also promoted the cell apoptosis. Finally, we found that these effects of lithium may be related to the regulation of GSK3β.

Materials And Methods

Detection of the concentration of lithium in water

The water samples were obtain from the places that were shown in Fig. 1e. The concentration of lithium in water samples was detected with K-Lite8F (Cornley, Meizhou, China) according to the manual. To evaluate the effects of lithium batteries to water, two disable Apple 6 s plus batteries and two disable Huawei P20 pro batteries were used in these study. Each battery was placed in a beaker, soaked in ultrapure water, and then the battery is punctured with a clean needle, then added ultrapure water to 2 liter. After three days, the polluted water was filtered by filter paper. The concentration of lithium in these samples was detected with K-Lite8F (Cornley) as well.

Cell culture and treatment

Human AC16 cardiomyocyte line was cultured in high-glucose Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (Gibco) and 1% (v/v) penicillin and streptomycin (Gibco). Cells were grown in a humidified atmosphere of 5% CO_2 at 37 °C. LiCl (Sinopharm Chemical Reagent, Shanghai, China) or Li_2SO_4 (Sinopharm Chemical Reagent, Shanghai, China) was reconstituted in ddH_2O. AC16 cells were incubated with LiCl or Li_2SO_4 at different concentrations (0.2 mM, 1 mM, 5 mM or 25 mM) for 48 hours as specified in figure legends.

Cell Viability Assay

The cell viability was tested with CellTiter-Lumi™ Luminescent Cell Viability Assay Kit (Beyotime, Nantong, China). The AC16 cells were seeded into 96-well plates (1×10^3 cells/well). After 48 hours treatment with Control(ddH_2O), LiCl, NaCl or Li_2SO_4 at different concentrations (0.2 mM, 1 mM, 5 mM or 25 mM), the cells were assessed. 100 μl CellTiter-Lumi™ reagent was added into each well of the plate. Then the plate was incubated at 37˚C for 10 min. The luminometer was subsequently recorded with SpectraMax M5 plate reader (Molecular Devices).

The cell viability was measured via the Cell Counting Kit-8 (CCK-8) assay as well. AC16 cells were seeded into 96-well plates (1×10^3 cells/well). After 48 hours treatment with Control, LiCl, NaCl or Li2SO4 at different concentrations (0.2 mM, 1 mM, 5 mM or 25 mM), the cells were assessed. 10 μl CCK-8 reagent (Dojindo, Kumamoto, Japan) was added into each well of the plate. Then the plate was incubated at 37˚C for 2 h. The absorbance at 450 nm was subsequently recorded with SpectraMax M5 plate reader (Molecular Devices).

Cell Apoptosis Assay

AC16 cells were treated with Control, 5 mM LiCl or 2.5 mM Li_2SO_4 for 48 hours. To quantify the cell apoptotic degree, the harvested cells were stained with annexin V-FITC/PI Cell Apoptosis Kit (Keygen,

Nanjing, China) according to the manufacturer's instructions. After incubation for 30 min at 4 °C, the cells were analyzed using FCM (FACS Canto; BD Biosciences).

EdU proliferation assay

5-Ethynyl-2'-deoxyuridine (EdU) is a synthetic thymidine analog which can incorporate into newly synthesized DNA during S phase. Therefore, EdU detection can be used for tracking DNA replication directly. The immunofluorescence staining of EdU was performed with BeyoClick™ EdU-555 proliferation kit (Beyotime) followed the Kit manual. Briefly, cells were cultured in 24-well plates, fixed in 4% paraformaldehyde after 48 hours treatment with Control, 5 mM LiCl or 2.5 mM Li_2SO_4, and permeabilized with 0.2% Triton X-100 for 15 min. Cells were counterstained with Hoechst for 5 min, then were washed with and imaged in PBS. At last, the image were taken using fluorescence microscopy (Nikon).

Western Blotting

Cultured AC16 cells were lysed in strong RIPA buffer containing Halt Protease Inhibitor Cocktails (Thermo, Waltham, MA). Protein concentrations were measured using a BCA protein assay kit (Pierce, Rockford, IL). Primary antibodies targeting proliferating cell nuclear antigen (PCNA) (ab29, abcam, Cambridge, UK), tumor protein p53(TP53) (ab1101, abcam), CYCLIN E (ab33911, abcam), glycogen synthase kinase 3 beta (Gsk3β) (ab32391, abcam), GSK3β (P-Ser9) (ab131097, abcam) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (ab9485, abcam) were incubated with proteins overnight at 4 °C, followed by incubation with the appropriate HRP (horseradish peroxidase) conjugated secondary antibodies(BA1038 and BA1039, Boster, Wuhan, China). Detection of HRP was performed using the ECL assay kit (Beyotime) and an ImageQuant LAS 4000 mini (GE, Boston, Unite States).

Statistical analysis

GraphPad Prism 7.0 software was used for statistical analysis. Statistical significance between two groups was determined using an unpaired two-tailed Student's t test. Data are presented as mean ± SD (standard deviation) or mean ± SEM (standard error of the mean) as indicated in the figure legends. P values were considered statistically significant if $P < 0.05$.

Results

The enrichment of lithium in Shanghai water environment

To investigate the potential pollution of lithium in China, we evaluated the electric vehicle market in 2018. Surprisingly, China accounts for more than half of the global market (Fig. 1a). At the same time, the electric vehicle market in China is growing rapidly these years (Fig. 1b). China has count 91% of the world's lithium battery demand in 2017 due to the huge electric vehicles, electric bikes and electric buses (Fig. 1c). Shanghai is one of the most active cities to promote the development of electric vehicles. The number of electric vehicles was more than 100,000 in 2016 in Shanghai, making it the largest electric vehicle city that time (Fig. 1d).

The previous results suggest that Shanghai could become a region with lithium pollution, therefore we detected the lithium concentration in different water environments (Fig. 1e, 1f). Though the concentration of lithium in Yangtze River (1.80 mg/L) was the lowest in the six environments (Fig. 1e, 1f), it was much higher than the normal concentration in freshwater (0.04 mg/L)[7]. The concentrations of lithium in other water environments were much higher than in Yangtze River (Fig. 1e, 1f), and concentrations of lithium in the tip lake was the highest. Intriguingly, compared with in pure water (0 mM), the lithium concentration in tap water was a little high (0.37 mM). Lithium concentration in the water that were contaminated by lithium batteries were very high (Fig. 1g). These data indicated that the water environment in Shanghai could be polluted by lithium batteries.

Lithium inhibit the cell viability of human cardiomyocytes

To investigate the effects of lithium on cardiomyocyte, we evaluated the cell viability of AC16 cell under LiCl exposure at different concentrations (0 mM, 0.2 mM, 1 mM, 5 mM or 25 mM). Compared to the control (0 mM), the growth of AC16 cell was significantly inhibited by LiCl at concentrations of 5 mM and 25 mM with Luminescent assay, as well as CCK-8 assay (Fig. 2a-c). To evaluate whether Cl^- cause the inhibition effects, NaCl was added in the study. Interestingly, high dose of NaCl had no significant effect on cell viability of AC16 cell (Fig. 2d-f). Additionally, we observed that Li_2SO_4 at concentrations of 2.5 mM and 12.5 mM also obviously inhibited the cell viability of AC16 cell (Fig. 2g-i). Thus, these data suggested that lithium may exert adverse effects on cardiomyocytes to inhibit cell viability.

Lithium inhibit the cell proliferation of human cardiomyocytes

EdU proliferation assay showed that the EdU level in the 5 mM LiCl treated group were significantly reduced, suggesting that the cell proliferation rate of AC16 cell reduce sharply (Fig. 3a). Similarly, 2.5 mM Li_2SO_4 also inhibited AC16 cell proliferation dramatically (Fig. 3b). Then we evaluated the cell proliferation ability by cell counting. Consistently, the lithium repressed the AC16 cell proliferation significantly (Fig. 3c and 3d). Proliferating cell nuclear antigen (PCNA) is an essential co-factor for DNA polymerases during replication [26]. We showed that the protein expression of PCNA in AC16 cell reduced significantly after 48 hours treatment with 5 mM LiCl or Li_2SO_4 compared to the control (Fig. 3e and 3f).

Lithium triggers the cell apoptosis of human cardiomyocytes

To further evaluate the effects of lithium on cardiomyocyte, we test the cell apoptosis with AnnexinV-FITC/PI apoptosis assay. AC16 cell apoptosis increased significantly after 48 hours treatment with 5 mM LiCl compared to the control (Fig. 4a,4b). Consistently, we observed that 2.5 mM Li_2SO_4 also induced AC16 cell apoptosis (Fig. 4c,4d). These data suggest that lithium might promotes the cell apoptosis of human cardiomyocytes.

Lithium control human cardiomyocytes via Gsk3β signaling

TP53 is an apoptosis marker. Western-blot analysis revealed that 5 mM LiCl or Li_2SO_4 treatment for 48 hours significantly increased the expression levels of TP53 in AC16 cell (Fig. 5a,5b). Cyclin E is a proliferation marker. Cyclin E protein levels decreased after 5 mM LiCl or Li_2SO_4 treatment for 48 hours compared with the control (Fig. 5c,5d). It is reported that glycogen synthase kinase 3 beta (Gsk3β) is active regulator of cell proliferation and apoptosis, therefore we test the change of Gsk3β after lithium treatment. We incubated AC16 cell with LiCl or Li_2SO_4 for 48 hours and examined the protein levels of Gsk3β and phosphorylated Gsk3β (S9) via western blotting. Interestingly, it showed that LiCl or Li_2SO_4 treatment for 48 hours elevated pGsk3β level significantly (Fig. 5e,5f). These results suggest that lithium could control human cardiomyocytes through Gsk3β signaling.

Discussions

Lithium pollution is a new environmental problem that appeared after the 1990s. Compared to heavy metals such as Gd and Hg, which could cause severe damage to human, the influence of lithium on the environment is much smaller; therefore the public is not known about it. The previous researches, together with our study, confirm that a high concentration of lithium could cause serious injury to the environment and human, which needs to be paid enough attention [27, 28, 17]. At present, the indicators of environmental monitoring, such as the detection of soil and water, do not include lithium in China. It needs to add lithium as an indicator as soon as possible, especially in the vulnerable areas. Additionally, the detection of agricultural irrigation water is particularly important because it is not only closely associated with the crops, but also with our health [27]. Lithium has been listed as a pollutant that leads to environmental harm in irrigation water supplies in Australia [10]. The concentration of lithium entering waterways should be less than or equal to 2.5 mg/L [10]. Besides, the tap water supply system in Shanghai monitors more than forty indicators, such as Al, Cu, Mn, Se, and Fe. However, lithium is not on the list. We found that the level of lithium in the tap water of Shanghai was high sometimes. Therefore, it should be added to the list as quickly as possible in future.

Recently we have transitioned from the 4G era the 5G era when various devices will communicate with each other [29]. Meanwhile, kinds of electronic devices such as sensors will continue to grow rapidly [29]. Moreover, electrification has become an irreversible trend in automotive [30–32]. For example, Paris plans to prohibit petrol vehicles in 2030, and France intends to prohibit petrol vehicles in 2040. Similarly, Britain will forbid new petrol vehicles after 2040. Electric vehicles currently only account for less 2% of all vehicles. If all the petrol vehicle are replaced by electric ones, it will be a very huge increase in lithium consumption. These could lead to more lithium pollution. In order to mitigate this problem, the following methods can be carried out. One possible method is to collect taxes on lithium batteries. The taxes might be collected for the treatment of lithium pollution. At the same time, it could raise the price of lithium batteries, which might improve the benefits of lithium recycling. Therefore it can not only reduce the waste of resources, but also increase environmental protection. In addition, garbage classification is

currently emerging in China, including Shanghai and Beijing. It is a fundamental method for garbage recycling. Thus, the classification and recycling of lithium batteries will significantly increase the efficiency of lithium resource and reduce environmental pollution.

Low concentrations of lithium have little impact on the environment and humans, and even lithium has an excellent therapeutic effect on some patients, such as bipolar disorder [33]. However, since it has strong side effects, patients who have taken it, must continuously be measured the lithium concentration in the blood [34]. Neural palsy, renal failure, and endocrine disorders are the most common side effects. Similar to nerve cells, the regenerative capacity of cardiomyocytes is feeble [35]. Damaged cells cannot be replaced by regeneration, so the damaged cells and tissues will accumulate year by year. We found that lithium could inhibit the activity of cardiomyocytes and promote their apoptosis. As people live longer, the possibility of heart damage also increases. Therefore, it is necessary to pay attention to the intake of lithium in the daily diet to effectively prevent its harm to the body. In addition, the large-scale clinical investigation and analyses to detect the damage of lithium to the heart.

There are some shortages that need to be improved in this study. For example, we have tested the concentration of lithium in the recent period. Because the water flow in the Yangzi River is constantly changing in each season, therefore we can detect changes in the four seasons in the future. Additionally, the lithium industry is developing rapidly, so it needs to be followed for many years. Besides, we found that lithium may affect the activity of cardiomyocytes by regulating the GSK3β pathway. It is reported that, because of the smaller radius and high polarizing strength, lithium exhibits high affinity and similarity for replacing some essential elements such as Na^+, K^+, and Mg^{2+} [14]. Therefore the effects of lithium on cardiomyocytes can be multifaceted.

Conclusions

In summary, here we found that the enormous consumption of lithium batteries could be serious threat to the environment of the Yangtze River Basin. At the same time, we found that certain concentrations of lithium will cause damage to human cardiomyocytes. It can not only inhibit the activity of cardiomyocytes, but also promote cardiomyocyte apoptosis. This study suggests that lithium in aqueous will harm human heart health if they enter the body through drinking water or food chain. Therefore, it is essential to detect lithium pollution in the environment (such as soil and water sources) and effectively recycle lithium batteries in the future.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

J.L. conceived and designed the research; J.S. and X.L. performed and designed experiments, analyzed results and wrote the manuscript; X.S. and W.W. collect clinical data and performed experiments; H.Z. and X.W. performed experiments and analyzed the data.

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Availability of data and materials

The data supporting the conclusions of this article are included within the article and its additional file.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

EdU: 5-Ethynyl-2'-deoxyuridine;

CCK-8: Cell Counting Kit-;

PBS: phosphate buffer saline;

PCNA: proliferating cell nuclear antigen;

TP53 tumor protein p53;

GAPDH: glyceraldehyde-3-phosphate dehydrogenase;

DNA: DeoxyriboNucleic Acid.

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Figures

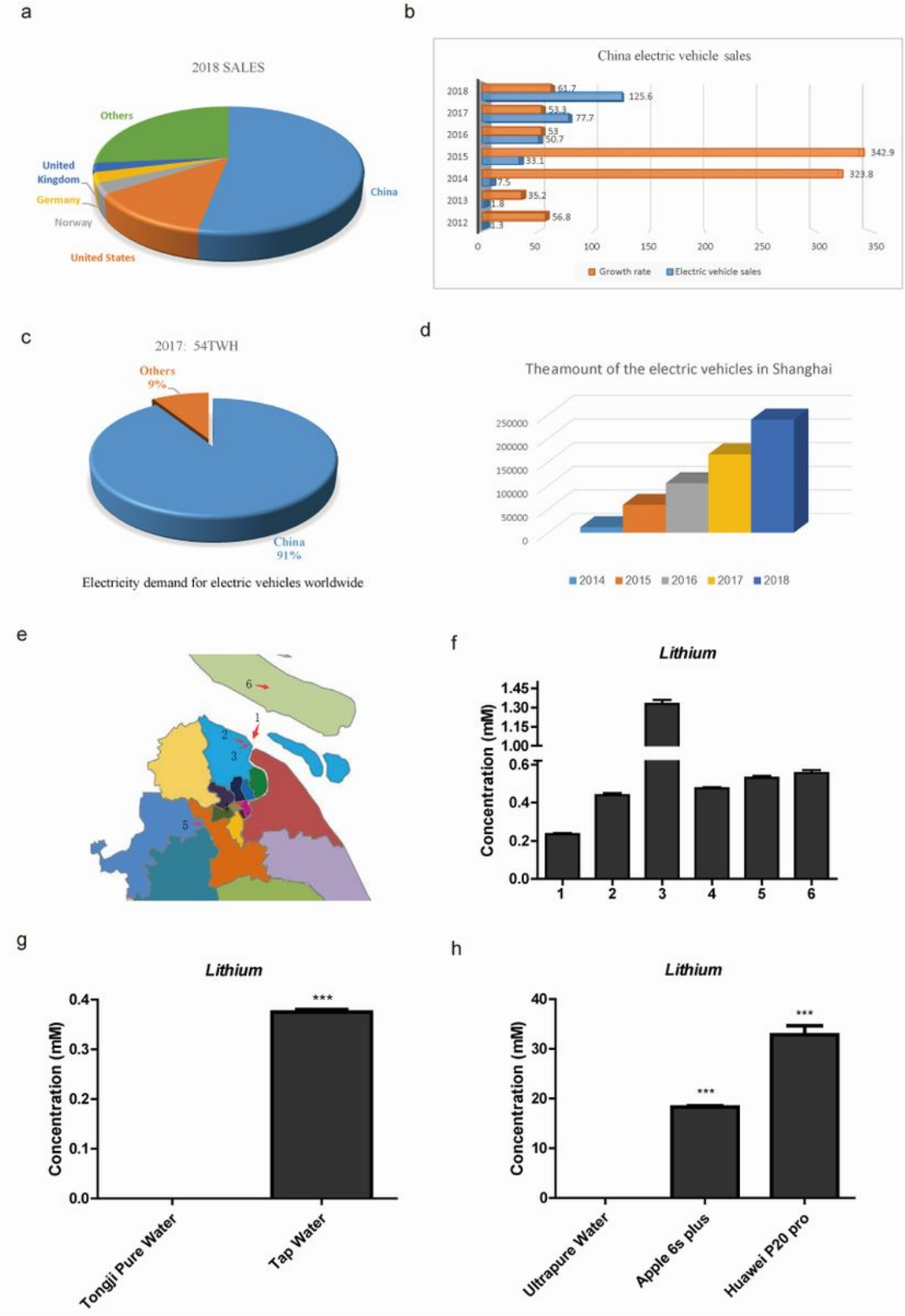

Figure 1

Lithium enrichs in Shanghai water environment a. Pie chart shows the sales of electric vehicle worldwide in 2018. b. Histogram shows the China electric vehicle sales and growth rate from 2012 to 2018. c. Pie chart shows the demand for electric vehicles worldwide in 2017. d. Histogram shows the electric vehicles in Shanghai from 2014 to 2018. e. Schematic diagram shows the six water environments. 1- Yangtze river, 2- Freshwater lake, 3- pit lake, 4- Suzhou River, 5- Dingpu River, 6- Chongming island. f. Histogram

shows the concentration of lithium in the six water environments. e. Histogram shows the concentration of lithium in Tongji Pure Water and Tap Water. f. Histogram shows the concentration of lithium in Ultrapure Water, Apple 6s plus and Huawei P20 pro.

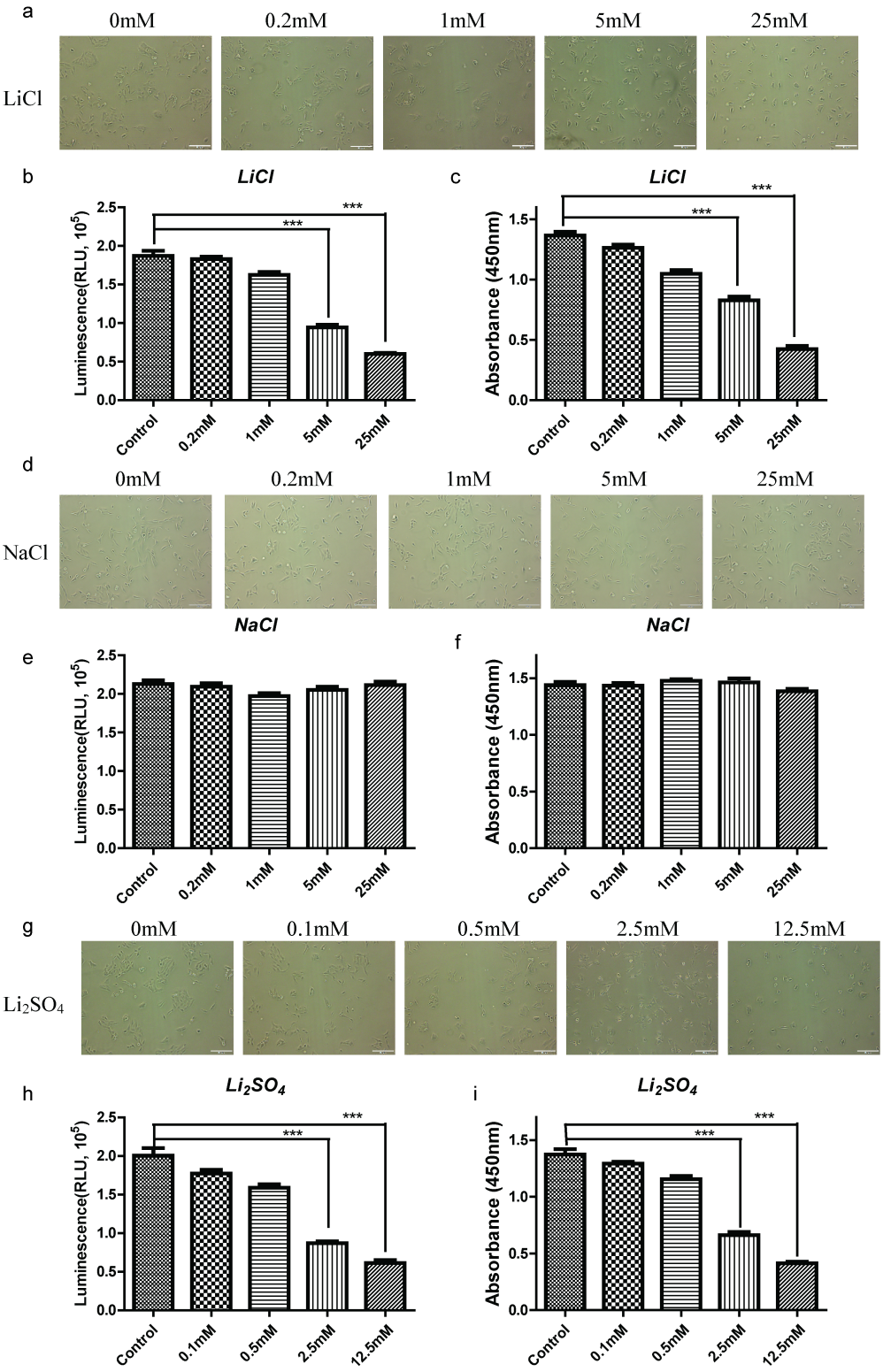

Figure 2

Lithium suppresses the cell viability of human cardiomyocytes a. Microscopic images of AC16 cells treated with Control (0mM) and LiCl at different concentrations (0.2mM, 1mM, 5mM or 25mM) for 48

hours. Bars = 200μm. b. Influence of LiCl at different concentrations (0mM, 0.2mM, 1mM, 5mM or 25mM) on cell viability of AC16 cell as measured by luminescence assay 48 hours post-cell seeding. The data are represented as means ± SEM (n = 3). ***p < 0.001. c. Influence of LiCl at different concentrations (0mM, 0.2mM, 1mM, 5mM or 25mM) on cell viability of AC16 cell as measured by CCK-8 assay 48 hours post-cell seeding. The data are represented as means ± SEM (n = 3). ***p < 0.001. d. Microscopic images of AC16 cells treated with Control (0mM) and NaCl at different concentrations (0.2mM, 1mM, 5mM or 25mM) for 48 hours. Bars = 200μm. e. Influence of NaCl at different concentrations (0mM, 0.2mM, 1mM, 5mM or 25mM) on cell viability of AC16 cell as measured by luminescence assay 48 hours post-cell seeding. The data are represented as means ± SEM (n = 3). ***p < 0.001. f. Influence of NaCl at different concentrations (0mM, 0.2mM, 1mM, 5mM or 25mM) on cell viability of AC16 cell as measured by CCK-8 assay 48 hours post-cell seeding. The data are represented as means ± SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001. g. Microscopic images of AC16 cells treated with Control (0mM) and Li2SO4 at different concentrations (0.1mM, 0.5mM, 2.5mM or 12.5mM) for 48 hours. Bars = 200μm. h. Influence of Li2SO4 at different concentrations (0mM, 0.1mM, 0.5mM, 2.5mM or 12.5mM) on cell viability of AC16 cell as measured by luminescence assay 48 hours post-cell seeding. The data are represented as means ± SEM (n = 3). ***p < 0.001. i. Influence of Li2SO4 at different concentrations (0mM, 0.1mM, 0.5mM, 2.5mM or 12.5mM) on cell viability of AC16 cell as measured by CCK-8 assay 48 hours post-cell seeding. The data are represented as means ± SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001.

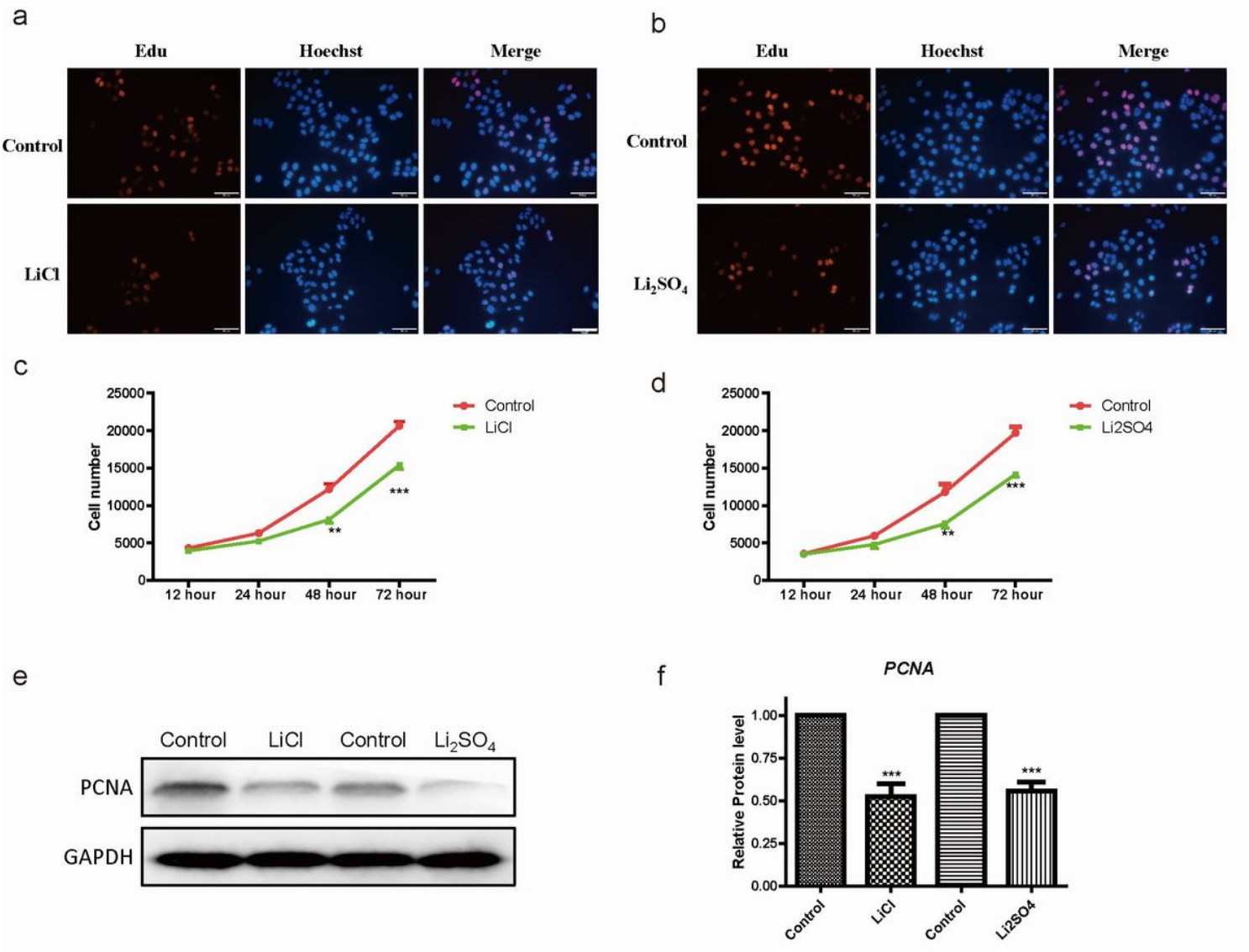

Figure 3

Lithium suppresses the cell proliferation of human cardiomyocytes a. EdU detection in AC16 cells treated with Control and 5mM LiCl for 48 hours. Nuclei were counterstained with DAPI. Bars = 200 μm. b. EdU detection in AC16 cells treated with Control and 2.5mM Li2SO4 for 48 hours. Nuclei were counterstained with DAPI. Bars = 200 μm. c. Cell number analyses of AC16 cells treated with Control and 5mM LiCl for 48 hours. The data are represented as means ± SEM (n = 3). **p < 0.01, ***p < 0.001. d. Cell number analyses of AC16 cells treated with Control and 2.5mM Li2SO4 for 48 hours. The data are represented as means ± SEM (n = 3). **p < 0.01, ***p < 0.001. e and f. Western blot analyses of the protein levels of PCNA in cells treated with Control, 5mM LiCl and 2.5mM Li2SO4 for 48 hours. The data are represented as means ± SEM (n = 3). ***p < 0.001.

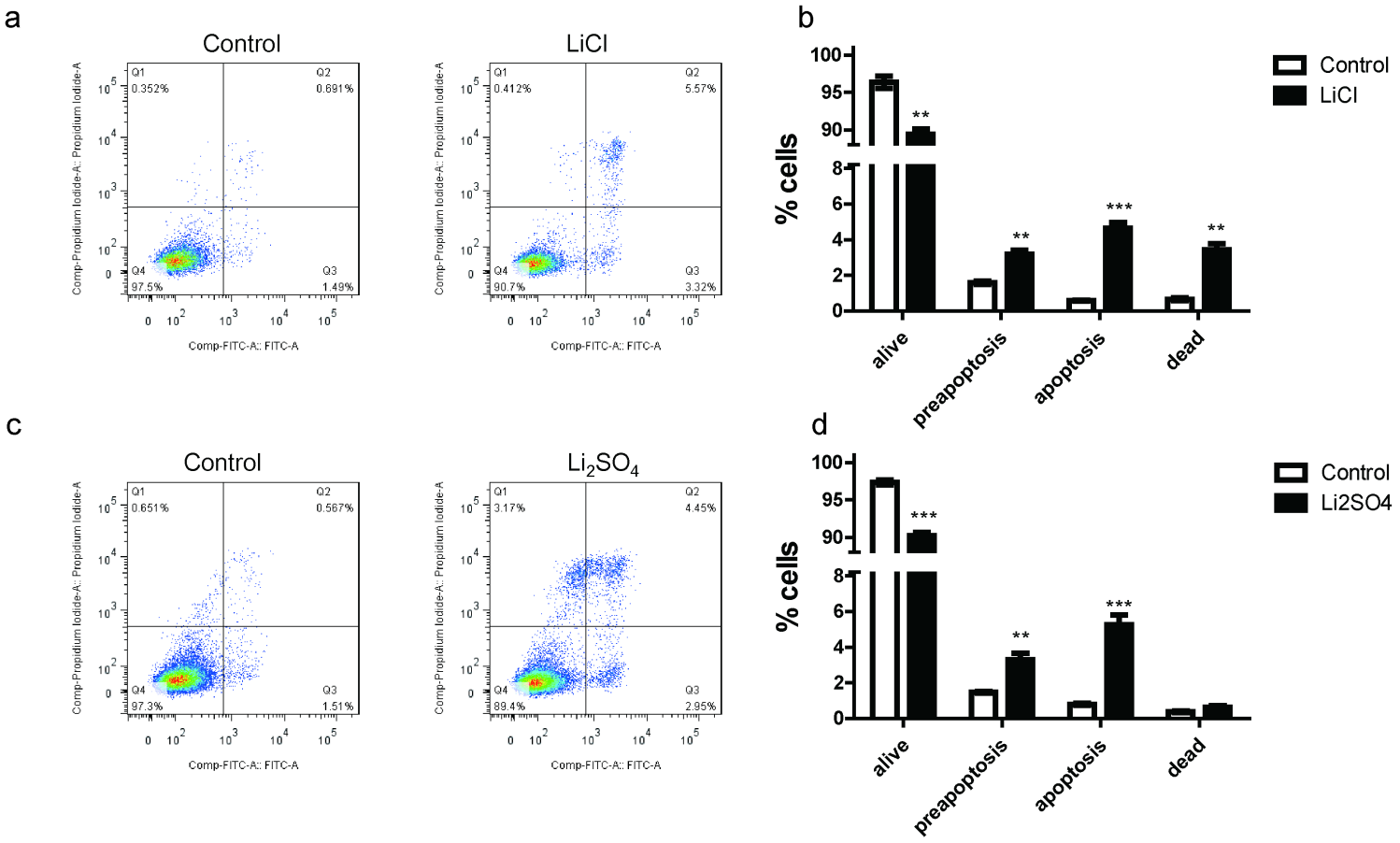

Figure 4

Lithium triggers the cell apoptosis of human cardiomyocytes a. Comparison of AC16 cell apoptosis treated with Control or 5mM LiCl for 48 hours via annexinV-FITC/PI apoptosis assay. b. Statistical analyses of the apoptotic cells in a. The data are represented as means ± SEM (n = 3). **p < 0.01, ***p < 0.001. c. Comparison of AC16 cell apoptosis treated with Control or 2.5mM Li2SO4 for 48 hours via annexinV-FITC/PI apoptosis assay. d. Statistical analyses of the apoptotic cells in c. The data are represented as means ± SEM (n = 3). **p < 0.01, ***p < 0.001.

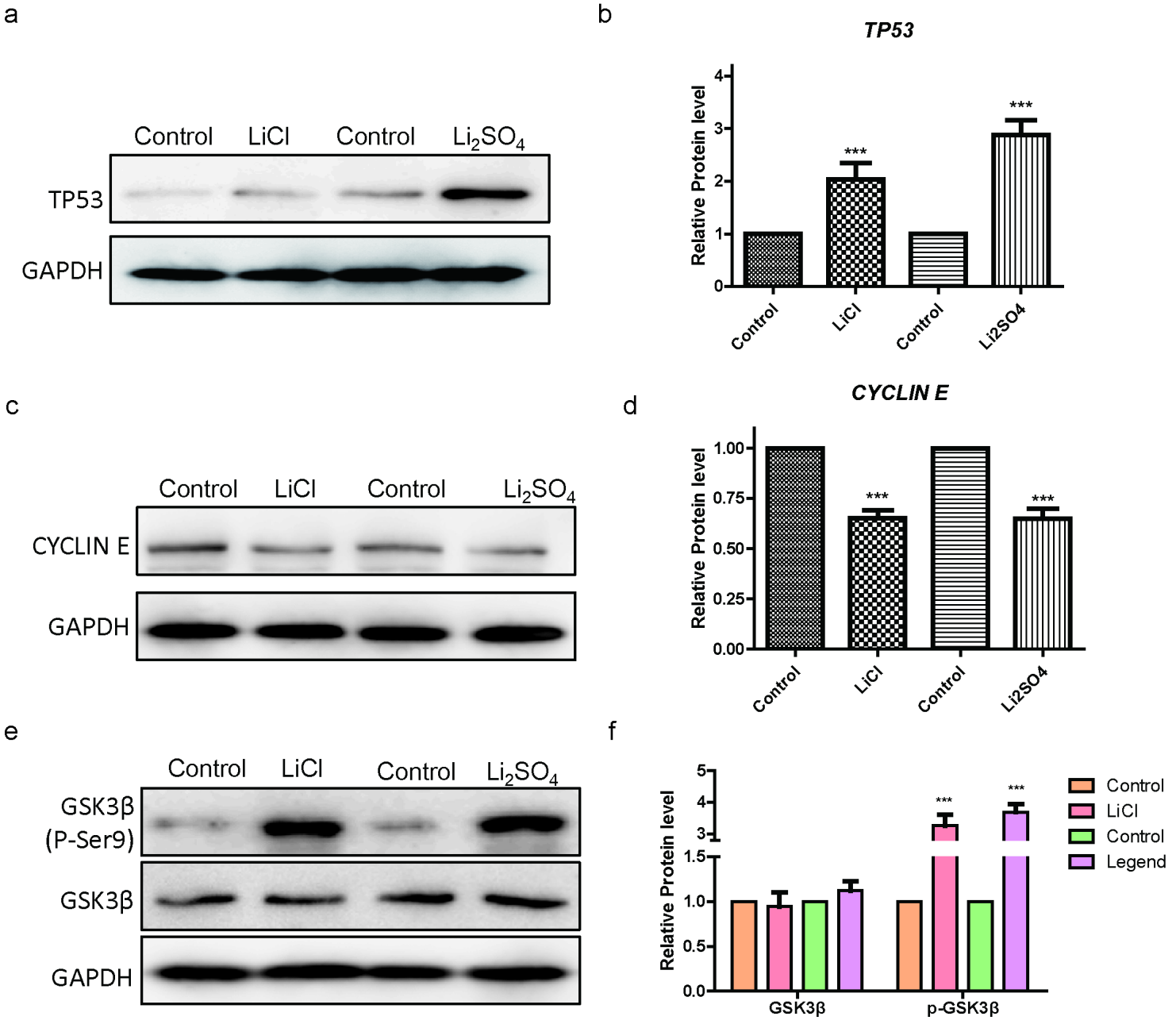

Figure 5

Lithium controls human cardiomyocytes by Gsk3β signaling a. Western blot analyses of the protein levels of TP53 in AC16 cells treated with Control, 5mM LiCl and 2.5mM Li2SO4 for 48 hours. b. Statistical analyses of the protein levels of TP53 in a. The data are represented as means ± SEM (n = 3). ***p < 0.001. c. Western blot analyses of the protein levels of Cyclin E in AC16 cells treated with Control, 5mM LiCl and 2.5mM Li2SO4 for 48 hours. d. Statistical analyses of the protein levels of Cyclin E in c. The data are represented as means ± SEM (n = 3). ***p < 0.001. e. Western blot analyses of the protein levels of Gsk3β and phosphorylated Gsk3β (P-Ser9) in AC16 cells treated with Control, 5mM LiCl and 2.5mM Li2SO4 for 48 hours. f. Statistical analyses of the protein levels of Gsk3β and phosphorylated Gsk3β (P-Ser9) in c. The data are represented as means ± SEM (n = 3). ***p < 0.001.