Original Article

Lithium Increases Radiosensitivity by Abrogating DNA Repair in Breast Cancer Spheroid Culture

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Abstract

Background: Lithium chloride (LiCl), a drug for bipolar disorder, has antiproliferative and apoptotic effects on certain breast cancer cell lines. This study was conducted to determine the effect of LiCl on radiosensitivity in a human breast cancer cell line in monolayer culture and the more realistic tumor model, multicellular tumor spheroid.

Methods: Monolayer and spheroid cells were treated with LiCl (20 mM) for 24 hours. The clonogenic assay was used to indicate changes in survival after x-ray radiation. The percentage of apoptotic cells was determined by acridine orange/ethidium bromide double staining. The amounts of DNA damage and repair after exposure to ionizing radiation were assessed by comet assay. Mre11 mRNA level was determined by RT-PCR. GSK-3β and β-catenin protein levels were measured by Western blotting.

Results: Treatment with LiCl decreased surviving fraction at 2, 3 and 6 Gy doses of x-ray ($P < 0.01$). The sensitizer enhancement ratio was higher in spheroids than monolayer culture. LiCl also decreased DNA repair ($P < 0.05$) and Mre11 mRNA level ($P < 0.01$) in T47D cells. These decreases were more prominent in spheroids than monolayer culture.

Conclusion: Treatment of T47D cells with LiCl sensitized this breast cancer cell line to ionizing radiation in monolayer and especially in the tumor-like spheroid culture. This radiosensitization was attributed, in part, to decline in DNA repair. Decrease in Mre11 mRNA level upon LiCl treatment was suggested to be an important cause for the decreased DNA repair in T47D monolayer and spheroid cells.

Keywords: breast cancer, LiCl, Mre11, radiosensitizer, spheroid

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Introduction

Breast cancer is one of the most frequent and life-threatening types of cancer in women worldwide.[1] In Iran, breast cancer is the most common type of cancer among women[2,3] and occurs at a younger age compared to other countries.[2] Radiotherapy is a common approach to control breast tumors. However, some breast tumors resist radiotherapy and reoccur as invasive cancers.[4,5] Sensitizing radioresistant breast tumors to ionizing radiation (IR) can be a breakthrough.

DNA molecule is considered the most important target of ionizing radiation. IR-induced DNA double strand breaks (DSB) cause chromosomal aberrations that lead to mitotic cell death.[6] Cells use DSB repair pathways to survive IR-induced DNA damage. Some tumor cells are proficient in repairing DNA strand breaks and are therefore more radioresistant.[7,8] Attenuating DNA repair in these cells can make them more radiosensitive.[7–10]

T47D is an epithelial human breast cancer cell line obtained from a metastatic site of a ductal carcinoma. This cell line is a radioresistant breast cancer cell line[11,12] that can form multicellular tumor spheroids in non-adhesive culture conditions.[13] The *in vitro* multicellular tumor spheroids are similar to small tumors and provide more realistic tumor models than monolayer culture. Spheroids are similar to *in vivo* avascular tumors or micrometastatic regions in terms of cell shape, intercellular and cell-to-matrix interactions, oxygen, nutrient and pH gradients as well as drug- and radiation-sensitivity.[14–16]

The incidence of breast cancer is higher in women with bipolar disorder.[17,18] Lithium is an FDA-approved drug used for treatment of bipolar disorder more than 50 years.[19] In addition to antimanic properties, Lithium Chloride (LiCl) has anticancer effects in different types of malignancies.[20–23] The antiproliferative and apoptotic effects of LiCl are demonstrated in certain breast cancer cell lines.[24–26] However, the effect of LiCl on radiosensitivity of breast cancer cells has not been studied.

Glycogen synthase kinase-3beta (GSK-3β) is a well-known target of LiCl and inactivated by this drug.[27–29] However, it is observed that LiCl increases GSK-3β protein level and activity in some breast cancer cell lines.[24] GSK-3β targets β-catenin protein for proteasomal degradation.[30] β-catenin is a co-activator of LEF1 transcription factor involved in the meiotic recombination 11 (Mre11) gene expression.[31] Therefore, GSK-3β can regulate Mre11 transcription.[31]

The Mre11 protein is an important component of Mre11/Rad50/Nbs1 (MRN) complex. This complex plays pivotal roles in sensing and repairing DSBs.[32–34] It is demonstrated that high Mre11 expression is correlated with high tumor recurrence after radiotherapy in breast cancer patients.[35] Inherited mutations of Mre11 lead to ATLD (ataxia-telangiectasia-like disorder) accompanied by high sensitivity to IR.[36] The influence of Mre11 level on DNA repair and radiosensitivity of cancer cell lines has been confirmed: knockdown of Mre11 decreased DSB repair and increased radiosensitivity in human cervix and nasopharyngeal cancer cell lines.[31]

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Furthermore, overexpression of Mre11 increased DNA repair and radioresistance in MCF-7 human breast cancer cell line.[35]

We have studied the effect of LiCl treatment on radiosensitivity and DNA repair in a simple and a more realistic model of breast cancer tumor (monolayer and spheroid cultures of T47D breast cancer cell line). In addition, the relationship between LiCl treatment and Mre11 expression was investigated in both types of culture for the first time.

Materials and Methods

Chemicals were obtained from Merck Company (Germany) unless mentioned otherwise.

Monolayer cell culture and treatment

The T47D breast cancer cell line was obtained from National Cell Bank of Iran (Pasteur Institute, Iran). Cells were maintained in RPMI-1640 medium (Gibco) and 10% FBS (Gibco) supplemented with Penicillin and Streptomycin antibiotics (Sigma). Cells were incubated at 37°C in a humidified atmosphere with 5% CO_2 and were passaged using trypsin (Gibco)/EDTA in phosphate-buffered saline (PBS) solution. T47D cells were cultured at 2×10^4 cells/cm^2 density. Three days later, exponentially growing cells were treated with LiCl (0 or 20mM) for 24 hours.

Spheroid cell culture and treatment

Spheroids were formed by the liquid-overlay technique.[37] Subconfluent monolayer cells were trypsinized with trypsin/EDTA in PBS solution. 10 mL medium containing 1.25×10^5 cells were put on 100 mm Petri dishes coated with 1% Bacto agar (Difco). Small spheroids were formed on day 3. Half of the medium was replaced from day 6 every 3 days. Spheroids of day 11 with average diameter of 115 μm were treated with LiCl (0 or 20 mM) for 24 hours.

Exposure to ionizing radiation

Intact spheroids and monolayer cultures were radiated with a linear accelerator x-ray machine (Siemens Primus) at 2 Gy/min dose rate and 6 MV energy.

MTT cell viability assay

Exponentially growing cells were trypsinized and plated at 2×10^4 cells/cm^2 density in 96 well plates. After 3 days, the cells were treated with 0, 10, 20, 50 or 100 mM concentration of LiCl or sodium chloride (NaCl) for 24 hours. MTT assay was performed as described previously.[38] Briefly, medium was removed from each well and 50 mL MTT solution (Sigma) (2 mg/mL PBS) was added to each well. Cells were incubated at 37 °C for three hours to let formazan crystals accumulate. Medium was removed and formazan crystals were solubilized through addition of 100 mL dimethyl sulfoxide to each well. Absorbance was determined at 540 and 630 nm wavelengths by BioTek plate reader. Difference of absorbance at the two wavelengths represented cell viability.

Comet assay (single cell gel electrophoresis)

Cells were treated with 0, 10, 20, 50 or 100 mM concentration of LiCl or NaCl for 24 hours. Comet assay was performed as described previously.[39] After trypsinization, approximately 2×10^4 cells were mounted in 0.5 % low melting point agarose (Sigma) on slides previously coated with 1% agarose. Slides were immersed in lysis buffer ((NaCl (2.5 M), EDTA (100 mM), Tris-HCl (10 mM), and Triton X-100 (1%); pH 10) for one hour. Denaturation buffer (NaOH (300 mM), EDTA (1 mM); pH 13) was put on slides for 30 minutes. Electrophoresis was performed in fresh denaturation buffer at 1 V/cm for 30 minutes. Neutralization buffer (Tris-HCl (400 mM); pH 7.5) was put on slides for 5 minutes. All steps were performed at 4°C. Slides were stained with ethidium bromide and observed under fluorescence microscope (Zeiss, Axioskop 2 plus) connected to a CCD camera. At least 200 cells in each sample were analyzed by CometScore software (version 1.5; TriTek Corp.).The percent DNA in tail of comets (%T) was used as an indicator of the amount of DNA damage.[40]

In radiation experiments, spheroid and monolayer cell cultures were treated with LiCl (0 or 20 mM) for 24 hours and then exposed to 0 or 3 Gy dose of x-ray. Cultures were trypsinized and comet assay was performed at 15 min and 45 min post-IR. In order to omit the background DNA strand breaks and to make monolayer and spheroid cell cultures comparable, relative %T (RPT) was used.

$$RPT = (X-C)/C;$$

Where X is %T of radiated cells and C is %T of corresponding control cells.

The difference in RPT at 15 and 45 minute post-IR was used as an indicator of DNA break repair.

$$\text{Repair (\%)} = [(RPT_{15\ min} - RPT_{45\ min})/RPT_{15\ min}] \times 100.$$

Clonogenic survival assay

LiCl-treated or control monolayer and spheroid cells were exposed to 0, 2, 3 and 6 Gy doses of x-ray. Cell cultures were trypsinized and incubated for about 8 minutes at 37°C. Cells were counted using a hemocytometer and appropriate numbers of cells were plated in 60 mm Petri dishes in triplicates. After 12 days of incubation, the colonies were fixed with formaldehyde (2%) and stained with crystal violet (0.5%). Colonies containing more than 50 cells were counted. Plating efficiencies were determined by dividing the number of colonies by the number of plated cells. Surviving fractions were calculated by dividing plating efficiency at each radiation dose by the plating efficiency of unirradiated cells. Change in surviving fractions was considered as the indicator of change in radiosensitivity. Data were fitted to the linear-quadratic model by OriginPro8 software. The sensitizer enhancement ratio (SER) was calculated by dividing the isoeffective dose at surviving fraction 0.5 in the absence of LiCl by the dose in the presence of LiCl.

Fluorescence microscopic analysis of apoptosis

Acridine orange/ethidium bromide (AO/EB) double staining was used to determine the percentage of apoptotic cells.[41] Control and LiCl-treated cultures were exposed to 0 or 3 Gy of ionizing radiation and incubated for 2 hours at 37°C. Cell cultures were trypsinized with trypsin/EDTA in PBS solution and washed with cold PBS. Cell density was adjusted at 2×10^4 cells/mL. 15 mL of the suspended cells was mixed with 1 mL of AO/EB solution containing 100 mg/mL concentration of each dye. Cells were observed under fluorescence microscope (Zeiss, Axioskop 2 plus) connected to a CCD camera. At least 800 cells were counted in each sample. Cells that contained organized chromatin structure

were classified as normal and cells with condensed or fragmented chromatin were classified as apoptotic.

Reverse transcription-polymerase chain reaction (RT-PCR)

Control and LiCl-treated monolayer and spheroid cultures were trypsinized. RNA was extracted from 10^6 cells using High Pure RNA Isolation Kit (Roche) in accordance with the manufacturer's protocol. cDNA synthesis and PCR were performed with 2-steps RT-PCR Kit (Vivantis) in the Thermo Hybaid PCR machine. We designed primers for a 362 bp fragment in exons 5 to 8 of Mre11 mRNA (Forward: 5'- GGCAATCATGACGATCCCACA-3', Reverse: 5'- TGTTCATGGCCCCAGATAACAA- 3') using Primer3 software.[42] β-actin primers (Forward: 5'- GGCGGCAC-CACCATGTACCCT- 3', Reverse: 5'- AGGGGCCGGACTC-GTCATACT- 3') amplified a 202 bp nucleotide sequence of b-actin mRNA.[43] Ethidium bromide-stained bands were analyzed by TotalLab software.

Western blot

The cultures were washed twice with ice-cold PBS. Lysis buffer (NaCl (200 mM), SDS (2 % w/v), Tris-HCl (50 mM), EDTA (2 mM), DTT (5 mM), and PMSF (1 mM); pH 8) was added and cells were scraped and kept on ice for 5 minutes. Lysates were vortexed and kept on boiling water for about 8 minutes and stored at -20°C. Bradford assay was used for determining total protein concentration. SDS polyacrylamide gel electrophoresis was performed on extracted lysates containing equal amount of proteins. Protein bands were transferred to PVDF membranes (BioRad). Specific antibodies were used against GSK-3β (Cell Signaling Technology), β-catenin (kindly gifted by Dr. S. Mahmoud A. Najafi, Department of Cell & Molecular Biology, Faculty of Biology, University of Tehran[44]) and Lamin B2 (Santa Cruz Biotechnology) proteins. These proteins were detected by enhanced chemiluminescence kit (Amersham Bioscience). Protein bands were analyzed by TotalLab software.

Statistical analysis

All data were represented as mean ± standard error of mean (SEM). Two- or one-way ANOVA followed by Tukey post-hoc analysis was performed using the OriginPro8 software to check the statistical significance of data. The significance of all results was determined by two-way ANOVA unless otherwise mentioned. *P* values less than 0.05 were considered statistically significant.

Results

Diameters of at least 200 spheroids were determined from day 5 every two days in three independent experiments (Figure 1). The mean geometric diameter of spheroids was calculated according to the equation: $(a \times b)^{1/2}$, where a and b are orthogonal diameters of spheroids.[45] Spheroids of day 11 with average diameter of 115 μm were chosen for treatment with LiCl. The probability of being hypoxic at this diameter is very low.[46] Furthermore, the distribution of diameters on day 11 was narrower than the distribution on day 13. The shape and diameter of spheroids were not affected by treatment with LiCl.

The MTT cell viability assay was performed on T47D cells after treatment with different concentrations of LiCl or NaCl (as the control salt) for 24 hours (Figure 2). Treatment with LiCl for 24 hours could not decrease viability up to 50 mM concentration. A significant difference between viabilities of cells treated with LiCl or NaCl was observed only at 100 mM concentration ($P < 0.05$).

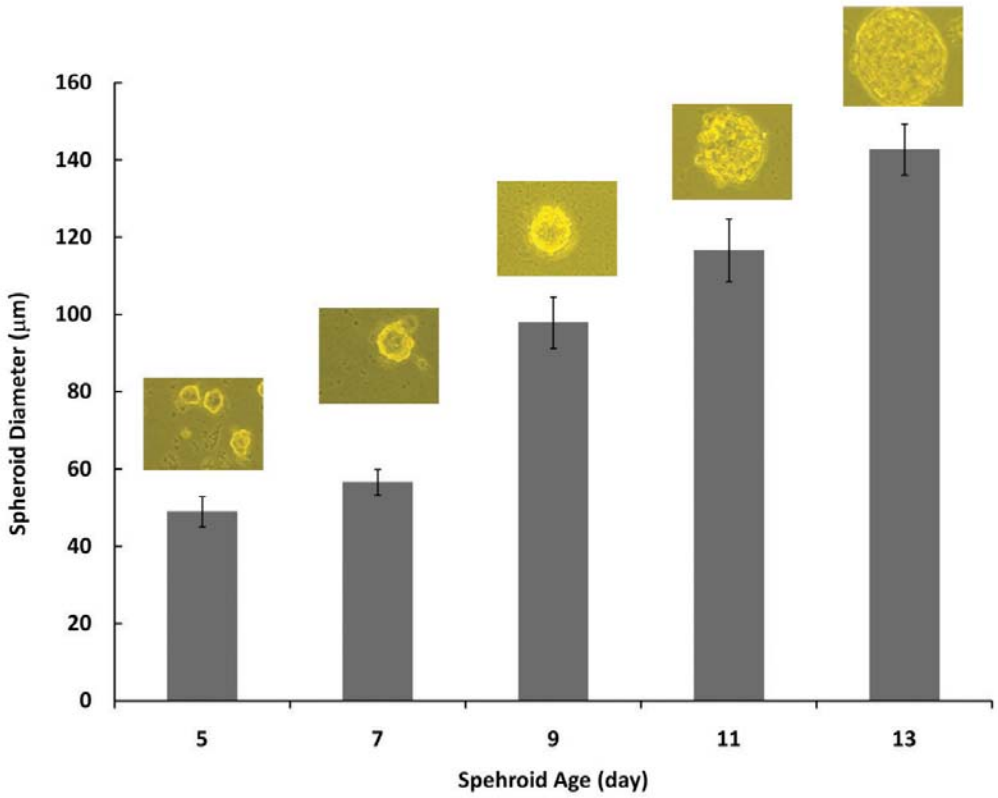

Figure 1. The spheroid growth curve. Spheroids were cultured as described in the Methods section. Spheroid diameters were determined from day 5 every 2 days. Data represent mean ± SEM of three independent experiments. A representative picture of spheroids of each day is shown on top.

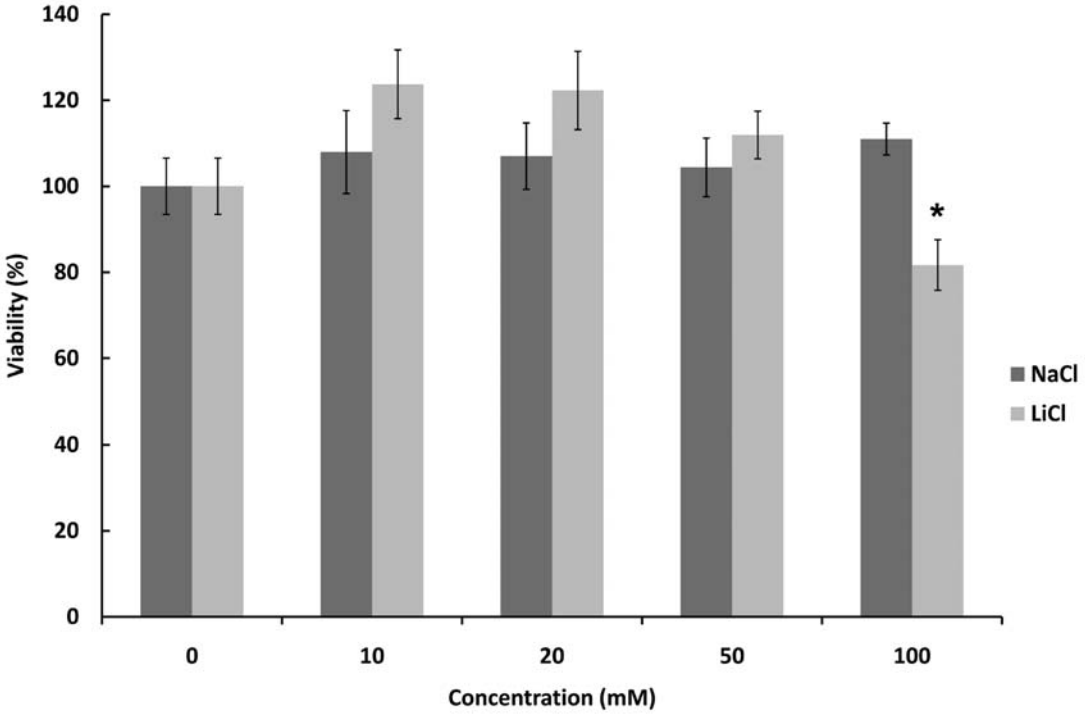

Figure 2. LiCl treatment did not decrease cell viability up to 50 mM concentration. Viabilities of cells treated with LiCl or NaCl were reported as percent with respect to control. Error bars indicate SEM of three independent experiments. *: $P < 0.05$ compared to cells treated with the same concentration of NaCl.

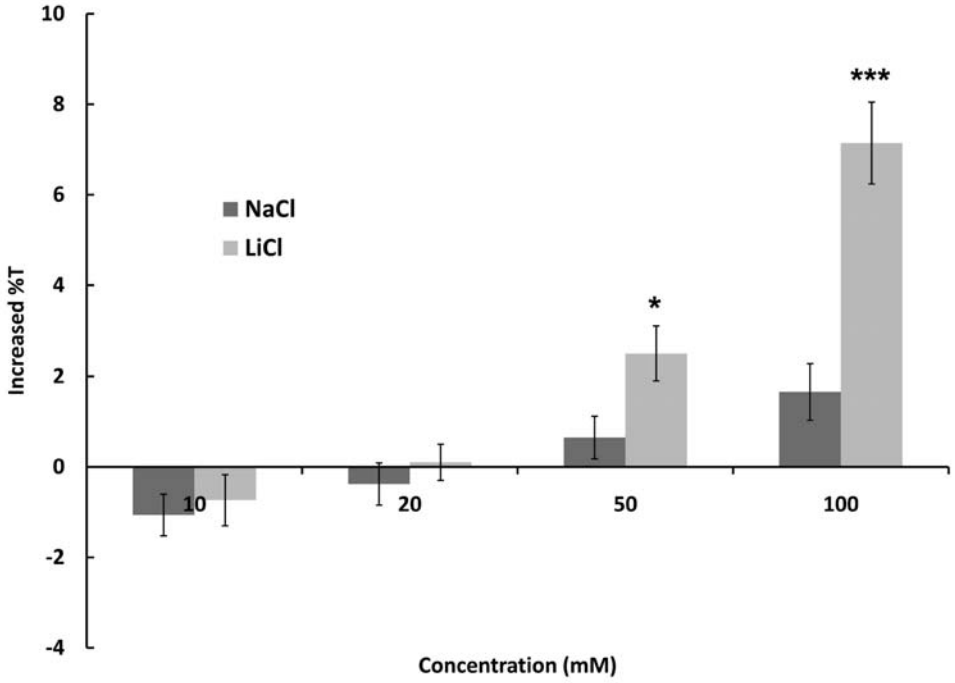

Figure 3. LiCl did not induce DNA strand break up to 20 mM concentration. Comet assay was performed on cells treated with different concentrations of LiCl or NaCl. %T of control was subtracted from %T of treated cells. Error bars indicate SEM of three independent experiments. *: $P < 0.05$ and ***: $P < 0.001$ compared to cells treated with the same concentration of NaCl.

Comet assay was performed on T47D cells treated with 0 to 100 mM concentrations of NaCl or LiCl (Figure 3). Treatment with LiCl could not induce DNA damage up to 20 mM concentration. Therefore, the 20 mM concentration of LiCl that did not decrease cell viability (Figure 2) and did not induce DNA breaks (Figure 3) was chosen for treatment of T47D cells with LiCl.

Clonogenic survival assay was performed for monolayer and spheroid cultures as described in the Methods section. Plating efficiencies were similar for unirradiated monolayer and spheroid cell cultures. Also, LiCl treatment did not affect plating efficiencies. In order to compare the two different T47D cell cultures, colonogenic survival data were fitted to the linear-quadratic model (Figure 4). The α, β and SER parameters and surviving fractions at 2 Gy (SF2) and 3 Gy (SF3) are summarized in Table 1. The culture

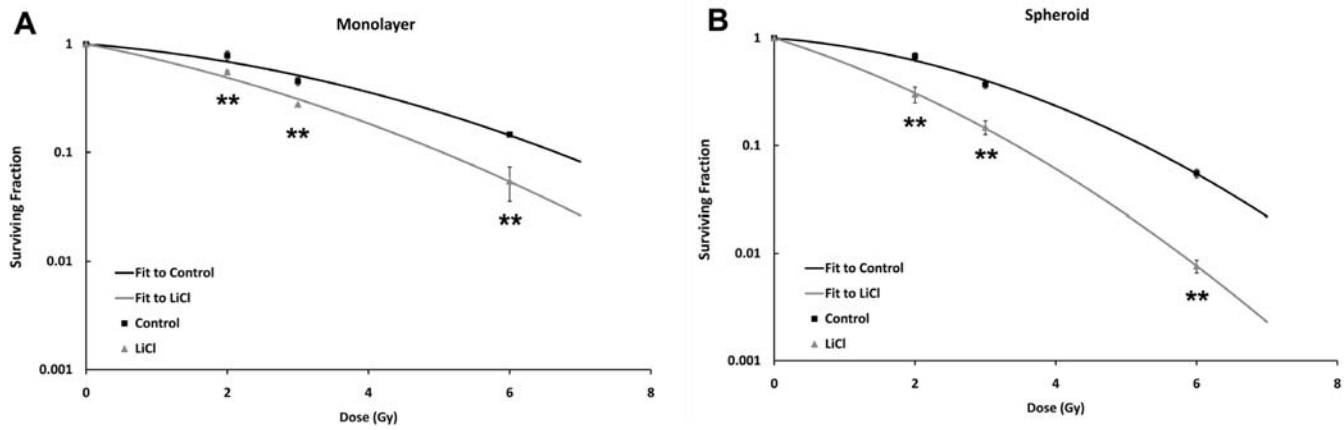

Figure 4. LiCl sensitized T47D monolayer and spheroid cultures to ionizing radiation. Exponentially growing monolayer cell cultures **(A)** and spheroids **(B)** were treated with LiCl (0 or 20 mM). 24 hours later cells were exposed to different doses of x-ray. Clonogenic survival assay was performed as described in the Methods section. Survival data were fitted to the linear-quadratic model. Error bars indicate SEM of three independent experiments and are depicted if they are larger than points. **: $P < 0.01$ compared to the corresponding control.

Table 1. Parameters obtained from fitting clonogenic survival data to the linear-quadratic model.

	α (Gy^{-1})	β (Gy^{-2})	SF2	SF3	SER
Monolayer					
Control	0.12	0.034	0.69	0.51	1
LiCl	0.29	0.033	0.49	0.31	1.58
Spheroid					
Control	0.12	0.061	0.62	0.40	1
LiCl	0.48	0.056	0.31	0.14	2.01

SF2: surviving fraction at 2 Gy; SF3: surviving fraction at 3 Gy; SER: sensitizer enhancement ratio.

model selectively influenced the β parameter. This parameter was higher in spheroids compared to monolayer cell culture. On the other hand, treatment with LiCl mainly affected the *a* parameter. LiCl increased the α parameter by 2.4 fold in monolayer and 4 fold in the spheroid model. Treatment with LiCl significantly decreased survival in monolayer and spheroid cultures ($P < 0.01$). The SER was higher in multicellular tumor spheroids than monolayer culture (Table 1).

AO/EB double staining apoptosis assay was performed on control and LiCl-treated culture models exposed to 0 or 3 Gy of x-ray (Figure 5). LiCl-treatment did not alter apoptosis significantly in unirradiated or radiated cells in either types of culture.

Using alkaline comet assay, the amount of DNA strand breaks was determined in monolayer and spheroid cell cultures at 15 and 45 minutes after exposure to 3 Gy of x-ray (Figure 6A). The radiation-induced DNA damage was reported as the increase in %T relative to control (RPT; see Materials and Methods). At both time points, RPT was dramatically higher in spheroid than monolayer cells ($P < 0.01$; Figure 6A). In addition, in both monolayer and spheroid cultures, RPT was higher in LiCl-treated cells than untreated cells at 45 minutes post-IR ($P < 0.001$ based on one-way ANOVA; Figure 6A). Although LiCl (20 mM) did not induce DNA breaks in unirradiated cells by itself (Figure 3), LiCl-treated cells, regardless of culture model, contained higher DNA breaks than untreated cells after exposure to IR. Comparison of RPT data at 15 and 45 minutes post-IR demonstrated that repair was significantly lower in LiCl-treated than untreated cells in both types of culture ($P < 0.05$; Figure 6B). Besides, DNA repair was higher in monolayer culture than in multicellular tumor spheroids ($P < 0.05$; Figure 6B).

The effect of LiCl treatment on mRNA level of Mre11 was studied in both monolayer and spheroid cell cultures (Figure 7). Treatment of T47D monolayer and spheroid cultures with LiCl (20 mM) for 24 hours decreased Mre11 mRNA level ($P < 0.01$). The decrease in Mre11 mRNA upon LiCl treatment was 21% and 33% in monolayer and spheroid cell cultures, respectively. Comparison of Mre11 mRNA basal level in spheroid and monolayer models indicated lower Mre11 mRNA in multicellular tumor spheroids than monolayer culture ($P < 0.01$).

GSK-3β and β-catenin protein levels were determined in monolayer cultures treated with LiCl (0 or 20 mM) for 24 hours. Treatment with LiCl increased GSK-3β protein level by 56% ($P < 0.01$ based on one-way ANOVA; Figure 8A) and decreased β-catenin protein level by 17% ($P < 0.05$ based on one-way ANOVA; Figure 8B).

Discussion

The effect of spheroid cell culture and LiCl treatment on radiosensitivity and repair of IR-induced DNA damage was studied in the T47D cell line. Treatment with LiCl sensitized T47D cells to ionizing radiation. LiCl-induced radiosensitization was more prominent in multicellular tumor spheroids. This radiosensitization was accompanied by decreased DNA repair, especially in spheroid culture. Spheroid culture and LiCl also decreased the Mre11 mRNA level. The LiCl-induced decline in Mre11 mRNA level was in accordance with the observed increase in GSK-3β and decrease in β-catenin protein levels.

It has been indicated that in 40% of carcinoma cell lines, spheroids are more resistant to IR than monolayer culture (contact ef-

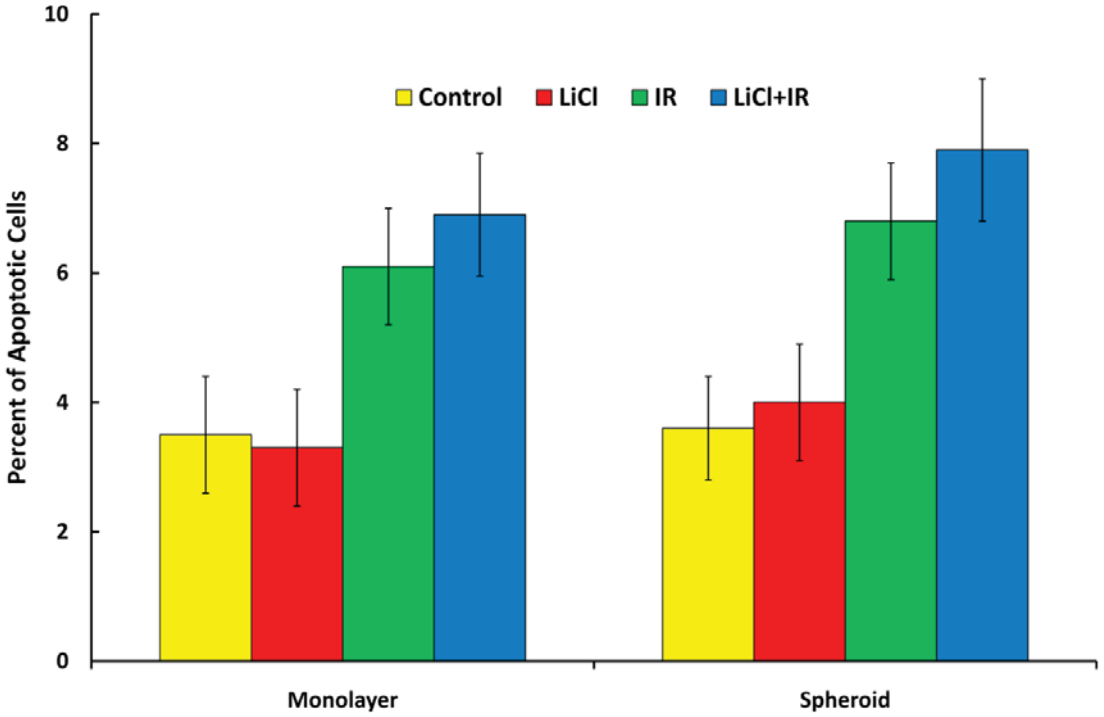

Figure 5. Apoptosis was not influenced by the culture model and LiCl treatment. Control and LiCl-treated monolayer and spheroid cultures were exposed to 0 or 3 Gy dose of x-ray. Cells were trypsinized after 2 hours of incubation. Percent of apoptotic cells was determined by AO/EB double staining. Data indicate mean ± SEM of three independent experiments.

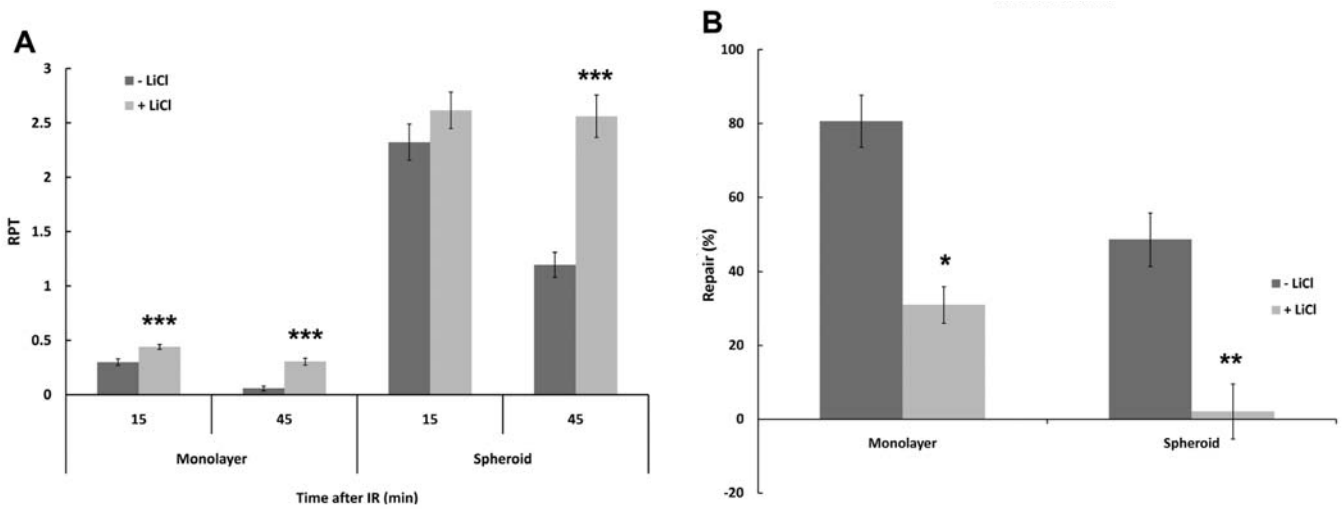

Figure 6. The repair of IR-induced DNA breaks was less in spheroid than monolayer and in LiCl-treated than control cells. Monolayer and spheroid cell cultures were treated with LiCl (0 or 20 mM) and exposed to IR (0 or 3 Gy). Comet assay was performed at 15 and 45 minutes post-IR. (A) RPT (indicator of the amount of DNA damages) and (B) Repair (%) were calculated as described in the Methods section. Data represent mean ± SEM of three independent experiments. *: $P < 0.05$, **: $P < 0.01$ and ***: $P < 0.001$ compared to the corresponding control.

fect). However, the contact effect is not prevalent in all cell lines.[14] The contact effect-induced radioresistance was not observed in T47D multicellular tumor spheroids (Figure 4). Based on two-way ANOVA analysis on surviving fractions in control cultures, the radiosensitivity was affected by the culture model ($P < 0.05$). The β parameter was greater in T47D spheroid than monolayer (Table 1) and it implies that the repair of sublethal damage is lower in T47D spheroid than monolayer culture.[47]

The increase in α and decrease in SF2 parameter in LiCl-treated monolayer and spheroid cells introduced LiCl as an α-type sensitizer for T47D breast cancer cell line. These effects were prominent for cells in tumor-like spheroid culture (Figure 4 and Table 1). The sensitizer enhancement ratio also indicated that spheroids were sensitized more than monolayer culture upon LiCl treatment (Table 1).

Increase in the β parameter is a consequence of mitotic cell death. However, the α parameter is influenced by both mitotic and apoptotic cell death.[6] AO/EB apoptosis assay indicated that the LiCl-induced increase in the α parameter and decrease in SF3 parameter (Table 1) did not principally originate from induction of apoptosis in monolayer or spheroid cultures (Figure 5). Therefore, increment in the α parameter also originated mainly from

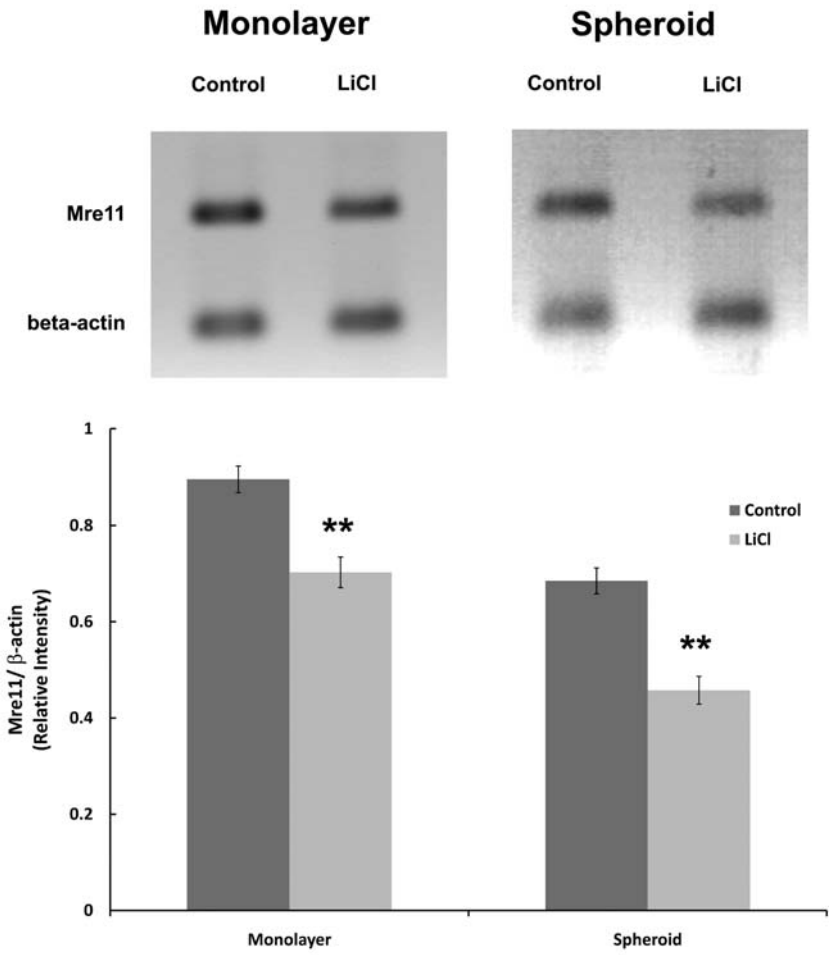

Figure 7. Mre11 mRNA level was lower in multicellular tumor spheroids than monolayer and in LiCl-treated than control cells. Monolayer (A) and spheroid (B) cell cultures were treated with LiCl (0 or 20 mM) for 24 hours. RNA was extracted and RT-PCR was performed to determine the β-actin (as loading control) and Mre11 mRNA levels. Data represent mean ± SEM of three independent experiments. **: $P < 0.01$ compared to control.

mitotic cell death in T47D cell line.

Based on the Tukey post-hoc mean differences' significance test at 0.01 level, suppression of repair by LiCl treatment is higher in spheroid culture in comparison to monolayer culture (Figure 6B). The higher sensitivity of T47D spheroids to radiation upon LiCl treatment with respect to control (Figure 4 and Table 1) might reflect the higher suppression of repair in spheroid than monolayer in response to LiCl treatment.

In an extensive study, researchers reported a direct correlation between Mre11 mRNA and protein levels and suggested that Mre11 protein level is regulated by its mRNA level.[48] Decrease in Mre11 mRNA and protein level causes repression of two other components of the MRN complex leading to a decreased level of the MRN complex.[35,48,49] This complex is involved in both types of DSB repair: Non-homologous end joining (NHEJ) and homologous recombination repair (HRR).[32] MRN tethers the ends of DSBs together or to the template sequences.[50] This complex is necessary for clearing complex DSBs and preparing them for rejoining in canonical NHEJ.[51,52] MRN is also involved in end resection process in HRR[53] and another NHEJ process called alternative end joining.[54,55] Therefore, suppression of Mre11 hinders DSB repair processes. The Mre11 mRNA level was lower in multicellular tumor spheroids than monolayer cultures. In addition, LiCl decreased the Mre11 mRNA level in both models of cell culture (Figure 7). Hence, the higher amount of DNA damage and lower repair in spheroid compared to the monolayer (Figure 6) might be due to the lower level of Mre11 mRNA in multicellular tumor spheroids compared to monolayer culture. Besides, the decreased DNA repair in LiCl-treated cells (Figure 6B) might originate from the decreased Mre11 gene expression upon LiCl treatment.

MRN recognizes DSB soon after radiation[56] and causes checkpoint activation.[57–59] Cell cycle arrest provides an opportunity for irradiated cells to repair DNA DSBs before entering mitosis. Hence, Mre11 deficiency causes cells to neglect DNA breakage, accumulate more DSBs[60] and repair them later, enter mitosis, encounter chromosomal aberrations and undergo mitotic cell death.[6] Therefore, the observed decrease in Mre11 expression (Figure 7) may underlie the lower survival of LiCl-treated cells than untreated cells and of spheroid culture than monolayer culture (Figure 4).

GSK-3β protein level was increased and β-catenin protein level was decreased in LiCl-treated cells (Figure 8). Because β-catenin protein level is regulated by GSK-3β kinase activity,[30] decrease in β-catenin protein level together with increase in total level of GSK-3β protein implies increased GSK-3β activity in LiCl-treated cells. The increased GSK-3β total protein level and activity in response to LiCl was previously observed in some breast cancer cells.[24] LiCl is reported to target other proteins as well [29] that may have participated in changing the response to IR.

LiCl decreased β-catenin protein and Mre11 mRNA in T47D

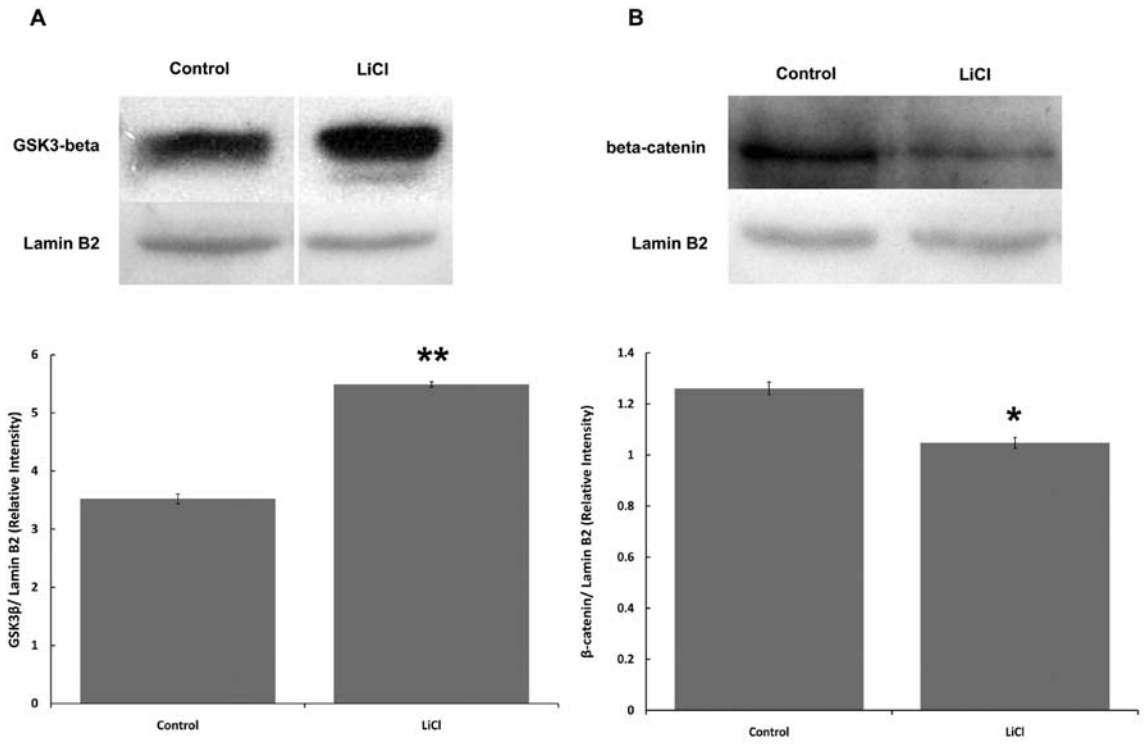

Figure 8. LiCl increased GSK-3β and decreased β-catenin protein levels in monolayer cell culture. Western blot analysis was performed on T47D cells treated with 0 or 20 mM concentration of LiCl. GSK-3β (A) and β-catenin (B) protein levels were determined. The Lamin B2 protein was detected as the loading control. Data represent mean ± SEM of three independent experiments. *: $P < 0.05$ and **: $P < 0.01$ compared to control.

cell line (Figure 7 and Figure 8B). A direct correlation between β-catenin protein level and Mre11 mRNA level has been demonstrated in cancer cells.[31] Therefore, the LiCl-induced decrease in Mre11 mRNA level might have occurred through the GSK-3β/β-catenin pathway.

In conclusion, LiCl was a potent radiosensitizer for T47D breast cancer cell line especially for the more realistic tumor model, spheroid cell culture. This radiosensitization was at least partially mediated by the decreased DNA repair. The decrease in DNA break repair and the radiosensitization of LiCl-treated T47D cells is probably mediated by suppression of Mre11; a significant protein in DSB repair processes.

Acknowledgments

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