Lidocaine Inhibited Migration of Non-Small-Cell Lung Cancer A549 Cells via the CXCR4 Regulation

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Research Article

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Abstract

Background

Lidocaineis a local anestheticthat wildly used in surgical treatment and postoperative medical care for lung cancers.We hypothesized thatlidocaine at clinical plasma concentrationcan inhibit CXCL12/CXCR4 axis regulated cytoskeletal remodeling thereby decrease migration ofNon-small-cell lung cancers (NSLC) cells.

Methods

We determined the effect of lidocaine at clinical plasma concentration on CXCL12-induced cell viability, apoptosis, cell death, monolayer cell wound healing rate, individual cell migration indicators, expression of CXCR4, CD44, and ICAM-1, intracellular Ca^{2+}level, and filamentous actin level alteration of NSLC cells A549 and CXCR4-knocked down A549 cells using CCK-8, Bcl-2 ELISA, Cell death ELISA, wound healing assay, chemotaxis assay, western blotting, QPCR, Fura-2-based intracellular Ca^{2+}assay, and Fluorescein Phalloidin staining respectively.

Results

Lidocaine did not affect cell viability, apoptosis, and cell death but inhibited CXCL12-induced migration,intracellular Ca^{2+} releasing, and filamentous actin increase. Lidocaine decreased expression of CXCR4, increased CD44, but had no effect on ICAM-1. CXCL12induced the increase of CD44 and ICAM-1 but did not affect CD44 in the presence of lidocaine.The knockdown of CXCR4 eliminated all the effects of lidocaine.

Conclusion

Lidocaine at clinical plasma concentrations inhibited CXCL12-induced CXCR4 activation, thereby reduced the intracellular Ca^{2+}-dependent cytoskeleton remodeling, resulting in slower migration of A549 cells.

1. Introduction

Lung cancer is one of the most fatal cancer types with the most global death number among males and the second most global death number among females [1]. As the medical care for lung cancer develops, the mortality of lung cancer decreased in the USA and UK has decreased in recent years. However, in many industrialized nations, the emerging social smoking culture has resulted in higher lung cancer rates in these areas [2]. Among all lung cancer cases, more than four-fifths of clinical lung cancer diagnosed are non-small cell lung cancers [3], a lung cancer type that has diverse pathological features and with undesirable prognoses.

Clinical medical advancements have improved cancer therapy extensively in recent years [4]. As the clinical diagnosis is critical for disease treatment [5], in-depth analyses of lung cancer subtypes have

been developed for potential targeting therapy and customized treatment according to their genetic and cellular heterogeneity [6]. Surgical treatment is the most applicable intervention for early-stage lung cancer diagnoses and is critical for the further prescription of proper therapeutic options [7]. One of the clinical factors that might affect the outcome of surgical treatments is the anesthesia during the surgery [8, 9]. Many local anesthetics have been found to affect multiple cancer cells [10]. A local anesthetic, lidocaine, has been wildly used in surgical treatment[11] and postoperative medical care [12, 13]. Preclinical studies have revealed that lidocaine can inhibit the proliferation and migration of many cancer types, including lung cancer [14, 15], breast cancer[16, 17], gastric cancer [18], colon cancer [19–21], etc.

However, the doses of lidocaine used in most previous studies are much higher than the concentration in plasma during clinical use of lidocaine (we mentioned it as “plasma concentration”). In this study, we focused on a lower concentration range of lidocaine that is correspondent to clinical plasma concentration. Lidocaine at this concentration range has been reported to inhibit cytoskeletal remodeling and migration of breast cancer cell MDA-MB-231 [22]. We proposed that a similar effect of lidocaine on cell migration is also present in non-small cell lung cancer cells.

The metastatic potential of non-small-cell lung cancers has been found to be strongly associated with the chemokine CXCL12 (C-X-C Motif Chemokine Ligand 12) and the activity of the CXCL12 receptor CXCR4 (C-X-C chemokine receptor type 4) [23]. CXCR4/CXCL12 axis controls the immunity of the body and the survival, invasion, and metastasis of cancer cells [24] [25]. CXCR4 has been found to be differently expressed in cancer cells and might contribute to the motility of cancer cells [26]. The CXCR4/CXCL12 axis has been proposed as a potential drug target for non-small-cell lung cancers [27]. In this study, we hypothesized that lidocaine at clinical plasma concentration can inhibit CXCL12/CXCR4 axis regulated cytoskeletal remodeling thereby decrease migration of Non-small-cell lung cancer cells. This investigation can provide a better understanding of the pharmacological effects of lidocaine at clinical plasma concentrations on the migration of non-small-cell lung cancer cells.

2. Methods And Materials

2.1. Cell line and cells culture

A549 cells were purchased from Biofeng (China). Cells were cultured using Ham's F12K + 2mM Glutamine + 10% Foetal Bovine Serum (FBS) in a cell culture incubator at 37◦C 5% CO_2. Cells were cultured in an FBS-free medium for 24 hours to Synchronize before any assay.

2.2. Cell transfection and knockdown

CXCR4 knockdown was achieved by transfecting the CXCR4 shRNA (TRCN0000256866) plasmid into A549 cells to silence the expression of CXCR4. The plasmid was purchased from Sigma-Aldrich (USA). The Sequences of shRNA oligonucleotides are as follows: 5’- TCCTGTCCTGCTATTGCATTA-3’. The transfection method was described previously [28]. The silencing was validated by western blotting experiments.

2.3. Testing reagents

Lidocaine HCl pre-made parenteral solution was purchased from Hospira Inc. (USA). Human CXCL12 and pertussis toxin were purchased from Sigma-Aldrich (USA). Fura-2 (Fura-2-acetoxymethyl ester) was purchased from Abcam (UK). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies, Inc (Kumamoto, Japan).

2.4. CCK-8 assay

The CCK-8 assay is used to determine cell overall viability and cytotoxicity. The CCK-8 assay was conducted as previously described [29]. Briefly, cells were seeded in 96 well plates. After the incubation with testing drugs, 10 μl of the CCK-8 labeling reagent was added to each well. The cells were further incubated for 4 hours. The absorbance at 450 nm was measured using a microplate reader.

2.5. Apoptosis and Cell death detection

Apoptosis and cell death detection were determined using Bcl-2 ELISA kit (Abcam, UK) and Cell Death Detection ELISA plus (Roche, USA) respectively. The method of ELISA was described previously [30, 31]. The positive control was induced by high-temperature culture (55 ◦C for 30 min) as a previous study [32].

2.6. Western blotting

Membrane protein expression was determined using the western blotting assay. Membrane proteins of A549 were extracted using Mem-PER™ Plus Membrane Protein Extraction Kit (Thermo Fisher Scientific, USA). The extraction was conducted as previously described[33, 34]. Subsequently, the western blotting assay was conducted as in previous studies [35, 36]. Briefly, SDS-PAGE gel electrophoresis was used to separate proteins and semi-dry protein transfer was conducted as described [37]. The membrane was then incubated with primary and secondary antibodies according to the recommended experimental condition. Na+/K + ATPase protein was used as an internal reference gene to normalized the data. Antibodies used including CXCR4 Antibody (PA3-305), Anti-Alpha 1 Na+/K + ATPase Antibody (#ANP-001), Recombinant Anti-CD44 antibody [EPR1013Y] (ab51037), Anti-ICAM1 antibody [EP1442Y] (ab53013), Goat Anti-Mouse IgG H&L (HRP) (ab6789), and Goat Anti-Rabbit IgG H&L (HRP) (ab6721). ECL reagent (Thermo Scientific, USA) was used to visualize the target protein.

2.7. QPCR

The CXCR4 mRNA level was determined using QPCR assay. The extraction was conducted as previously described [38, 39]. Briefly, Trizol buffer (Sigma-Aldrich, USA) was used to isolate cellular RNA. An Agilent Bioanalyzer (Agilent 2100; Agilent Technologies, Inc, USA) was used to assess the concentration and integrity of RNA extracted. QuantiTect Reverse Transcription Kit (QIAGEN, USA) and miRCURY LNA SYBR Green PCR Kit (QIAGEN, USA) were used to conduct RT-PCR. The protocol of PCR reaction was described previously [40]. The GAPDH gene was used as an internal reference gene to normalize the data. CXCR4 Human qPCR Primer Pair (NM_003467) and GAPDH Human qPCR Primer Pair (NM_002046) were purchased from OriGene Technologies (USA).

2.8. Wound healing assay

Cells migration was determined using the wound healing assay. The method was described previously [41, 42]. Briefly, cells were grown in six-well plates at over 90% confluency. A scratch wound was created in the cell monolayer using a 200-μl pipette tip. The cells were cultured with testing reagents and images were recorded every 3 or 6 hours. The wound distance was analyzed using Fiji16 software.

2.9. Chemotaxis assay

Real-time cell migration of individual A549 cells was recorded using the m-Slide chemotaxis system (ibidi, Germany). The method was described previously [43, 44]. Briefly, A549 cells were cultured on the central channel of the chemotaxis slide at 10% confluency for 8 h to allow adherence. Testing drugs were applied when the assay started and the images of cells were recorded for 15 hours with a time-lapse Micro-Imager. Single-cell tracking was analyzed using the ImageJ software. Spider plots representing the aggregated trajectories of cells. Forward migration indexes and cell velocity were analyzed using the Ibidi software.

2.10. Fura-2-based intracellular Ca^{2+} assay

Fura-2-based fluorescence was used to determine the intracellular Ca^{2+} level. Fura-2 has been wildly used in the indication of intracellular Ca^{2+} [45, 46]. A549 cells were cultured on poly-D-lysine coated TC-treated Cell Culture Dishes (Falcon) with lidocaine or vehicle for 24 hours. Cells of the Gai blocker group were pretreated with 1 mg/ml pertussis toxin for 2 hours before the Fura-2-loading. Cells were loaded with Fura-2 loading buffer (1 mM Fura-2, 140 mM NaCl, 5 mM KCl, 20 mM HEPES, and 1 mM $CaCl_2$, pH 7.4.) for 20 min at 37 ◦C. Then the loaded cells were gently washed with a washing buffer (140 mM NaCl, 5 mM KCl, 20 mM HEPES, and 1 mM $CaCl_2$, pH 7.4.). Images were recorded real-timely on an Axiovert 200 inverted microscope with an excitation at 340 and 380 nm. CXCL12 was perfused 50 s after the recording start and the monitoring continued until 150 s. The F340/380 ratio was calculated and plotted against time to indicate the changes in intracellular Ca^{2+} level.

2.11. Actin polymerization detection

The cytoskeleton remodeling was indicated by the determination of actin polymerization using Fluorescein Phalloidin staining. The method was described previously [47]. Briefly, cells were fixed in 4% paraformaldehyde for 10 min at 4 ◦C, then were permeabilized with 0.01% Triton-X-100 for 5 min. Filamentous actin was stained using Fluorescein Phalloidin (F432, Thermo Fisher Scientific, USA) for 40 min. Images of fluorescence were recorded and the fluorescence intensity was analyzed using the Fiji software.

2.12. Plotting and statistical analysis

Means and standard deviations are displayed in the figures. A T-test or ANOVA was used to analyze the significance of the difference ($p < 0.01$). Dunnett's post hoc tests were used to test the difference between groups. GraphPad Prism (version 8) was used to plot the data and calculate statistics. Figure 7 was plotted using the BioRender online tools.

3. Results

3.1. Lidocaine did not affect A549 cell viability at the clinical plasma concentrations.

The anti-arrhythmia plasma concentration and approximately equipotent nerve block concentrations of lidocaine are around 10 μM [48]. We first evaluated the toxicity of lidocaine at the clinical plasma concentration range (1-100 μM) on non-small-cell lung cancer A549 cells. Results showed that at these concentrations, lidocaine did not affect cell viability, apoptosis, and cell death (Fig. 1ABC). Therefore, we suggested that Lidocaine at clinical plasma concentrations did not inhibit proliferation or induce apoptosis or necrosis of A549. In addition, CXCL12 at 100 nM also failed to affect A549 viability (Fig. 1D). In this study, we performed a knockdown experiment of CXCR4 in A549. Results showed that CXCR4 knockdown did not affect the sensitivity of A549 to lidocaine at the clinical plasma concentration range in terms of cell viability (Fig. 1E). As we focused on the effect of lidocaine at clinical plasma concentration on the chemokinesis or chemotaxis of A549 cells, 100 μM lidocaine was used in the subsequent study.

3.2. lidocaine decreased CXCR4 expression.

We extracted the membrane expression of CXCR4 in A549 and observed the effect of lidocaine on the surface expression of CXCR4 in A549 cells. Results showed that lidocaine significantly decreased the surface expression of CXCR4 and the total expression of CXCR4 mRNA (Fig. 2). In the CXCR4 knockdown study, we knocked down over 80% CXCR4 mRNA (Fig. 2C) and the surface CXCR4 expression was very low that was almost not detectable in the western blotting (Fig. 2AB). After CXCR4 knockdown, the effect of lidocaine on CXCR4 expression was eliminated (Fig. 2). Thus, we suggested that lidocaine down-regulated CXCR4 expression when it was highly expressed, but did not affect CXCR4 when it was at a low expression level.

3.3. lidocaine inhibited CXCL12 induced migration.

CXCL12 is the agonist of the CXCR4 and has been wildly used for studying chemokinesis or chemotaxis of cells [49]. To tested if lidocaine affected CXCL12 induced migration of A549 cells, we performed both the wound healing assay to observed the migration of monolayer A549 cells as a group and in vitro chemotaxis assay to observe the migration of individual A549 cells. Results showed that CXCL12 significantly increased A549 migration as demonstrated by a faster wound healing rate and the higher

migrate index and velocity of individual cells. Lidocaine did not affect A549 cell migration but blocked the stimulation of CXCL12 (Fig. 3).

3.4. CXCR4 knockdown block lidocaine effect in migration

To investigate whether lidocaine affects migration through CXCR4, we also performed migration assays in CXCR4 knockdown A549 cells. Results showed that knockdown of CXCR4 eliminated the effects of CXCL12 or lidocaine on A549 migration, causing no significant difference in the wound healing speed and the migrate index and velocity of individual cells (Fig. 4). This suggested that CXCR4 was essential for the effects of CXCL12 and lidocaine.

3.5. Regulations of lidocaine on CD44 and ICAM-1.

To further explore the potential mechanisms for lidocaine action on A549 migration, we investigated two critical adhesion molecules on the epithelial cell membrane, CD44 and ICAM-1. Both CD44 and ICAM-1 levels were significantly increased by CXCL12. Lidocaine increased CD44 in both wild-type A549 and CXCR4 knockdown A549 cells in the absence or presence of CXCL12, but it did not affect ICAM-1 (Fig. 5). These results suggested that lidocaine can up-regulate CD44 but not ICAM-1. However, the regulation of lidocaine on CD44 was not essential for CXCL12 induced migration.

3.6. Lidocaine inhibited CXCL12-induced intracellular Ca^{2+} releasing and cytoskeleton remodeling.

Another potential mechanism we hypothesized underlying lidocaine's effect on migration is the regulation of the cytoskeleton remodeling. To test this hypothesis, we monitored the effect of lidocaine on the intracellular Ca^{2+} level alteration following the addition of CXCL12. The pertussis toxin was used as a control to block the Gai subunit of CXCR4 [47]. The CXCR4 knockdown A549 cells were used as another control. Results showed that lidocaine decreased the peak of the Ca^{2+} curve by about 50%. CXCR4 knockdown A549 cells reacted slightly (less than 5% of the peak of Ca^{2+} curve in the control group) to CXCL12 and pertussis toxin blocked almost all the effect of CXCL12 (Fig. 6A). To further study the CXCL12-induced cytoskeleton remodeling of A549 at the presence of lidocaine, we stained filamentous actin with phalloidin coupled to fluorescein isothiocyanate to observe the rearrangement of cortical actin fibers during CXCL12 stimulation. Results showed that lidocaine reduced cytoskeleton remodeling by about 50% and both the pertussis toxin group and CXCR4 knockdown group failed to show any reaction following CXCL12 stimulation. These results suggested that lidocaine inhibited CXCL12-induced intracellular Ca^{2+} releasing thereby reduced cytoskeleton remodeling.

4. Discussion

The therapeutic effect of surgery in metastatic non-small cell lung cancer has been controversial [50, 51]. During the surgery, many clinical factors might contribute to the metastasis and recurrence of lung cancer

surgery. The application of lidocaine during the surgery or preoperational treatment results in a plasma lidocaine micro-environment for lung cancer cell migration and survival. Hence, the potential impact of lidocaine on metastasis and recurrence of lung cancer surgery should be further studied. However, so far, the effect of lidocaine at plasma concentration on cancers was less studied. The doses of lidocaine used in most previous studies are much higher than the clinical plasma concentration. Therefore, although many previous studies demonstrated the effect of lidocaine on lung cancer cells, they fail to convince clinical surgeons that lidocaine exerts a considerable impact on the surgery outcome. Lidocaine is known as a sodium channel blocker. Although the major target of lidocaine, the voltage-gated sodium channels, has been found to play a role in cancer developments [52], lidocaine might also affect cancer independent of sodium channel blockade.

Although a previous study suggested that lidocaine at the “mM” concentration range inhibited proliferation [15] and induced apoptosis of A549 [53], our result showed that, at plasma concentration, lidocaine had almost no effect on cell proliferation, apoptosis, and cell death. The previous study also suggested that the migration of A549 was inhibited by lidocaine at 8 mM, but as shown by our data, the effect of lidocaine on migration was not significant when the doses of lidocaine decreased to 100 μM. Lidocaine at clinical plasma concentrations only reduced CXCL12-induced migration in A549. The western blotting assay showed that lidocaine decreased the expression of CXCR4 especially the membrane expression of CXCR4. We suggested that the reduction of CXCR4 on membrane surface impaired the sensitivity of cells to CXCL12 stimulations. A previous study has suggested that lidocaine can regulate CXCR4 sensitivity to CXCL12 in breast cancer cells MDA-MB-231 [22], which was similar to A549 according to our results.

Different membrane surface proteins expressed on lung cancer cells as adhesion molecules can be critical in the migration of cells [54]. CD44 has been reported to play roles in the metastasis of non-small cell lung cancer cells [55]. In this study, the expression of CD44 was promoted by CXCL12 stimulation. Our results also showed that the surface expression of CD44 on A549 was up-regulated by lidocaine. However, the lidocaine did not further increase the CD44 expression at the presence of CXCL12 and the increase of CD44 in the lidocaine alone group did not affect cell migration. Thus, we suggested that CD44 was directly up-regulated by lidocaine bypassing the CXCL12/CXCR4 axis but the increase of CD44 was not essential for the migration of A549. In addition, we also determined another critical adhesion molecule for migration, the ICAM-1. The expression of ICAM-1 has been associated with lung cancer progression and prognosis [56]. A previous study reported that lidocaine affects migration of a lung cancer cell line H838 by reducing ICAM-1 [57]. However, in this study, the lidocaine at plasma concentration did not affect ICAM-1 expression. The ICAM-1 expression was up-regulated by CXCR4 activation by CXCL12, hence, we suggested ICAM-1 might be a potential downstream target of lidocaine/CXCR regulation (Fig. 7). Further validation is required in the future.

Another activity that might impact the migration of A549 was the remodeling of the cytoskeleton. The activation of CXCR4 by CXCL12 can trigger intracellular Ca^{2+} releasing [58]. Subsequently, the released intracellular Ca^{2+} facilitated many Ca^{2+}-dependent activities that are involved in cytoskeleton remodeling

[59, 60]. Results showed that lidocaine significantly reduced the intracellular Ca^{2+} releasing triggered by CXCL12 stimulated CXCR4 activation. We further determined the level of filamentous actin to observe the rearrangement of cortical actin fibers during CXCL12 stimulation. Actin is an important part of the cytoskeleton in most eukaryotic cells [61]. There are two types of actin: globular G-actin and filamentous F-actin[61]. The G-actin can polymerize into the actin filaments, the F-actin polymer filaments that form the cytoskeleton[61]. Thus, the level of F-actin can indicate the rate of cytoskeleton rearrangement. Our results showed that lidocaine reduced cytoskeleton remodeling by about 50%. Therefore, we suggested that lidocaine inhibited cytoskeleton remodeling via suppressing CXCL12-induced intracellular Ca^{2+} releasing (Fig. 7). However, lidocaine, as a multiple channel blocker, might have other effects on intracellular Ca^{2+} level. Many cancer-related ion channels, such as TRP[62] and TPCs [63], might contribute to lidocaine effects on cancer cells and need further investigation.

5. Conclusion

This study demonstrated that lidocaine at clinical plasma concentrations showed a significant inhibition effect on CXCL12-induced CXCR4 activation, thereby reduced the intracellular Ca^{2+} dependent cytoskeleton remodeling, resulting in slower migration of A549 cells. This investigation can provide a better understanding of the pharmacological effects of lidocaine at clinical plasma concentrations on the migration of non-small-cell lung cancer cells and can optimize the clinical application of lidocaine in lung cancer surgery.

6. Abbreviations

NSCLC: Non-small cell lung cancers

CXCL12: C-X-C Motif Chemokine Ligand 12

CXCR4: C-X-C chemokine receptor type 4

7. Declarations

Ethics approval and consent to participate

This work was approved and consented by the Ethical Committee of Changzhi Medical College AffilicatedHeping hospital.

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This study receivedfunding from the Changzhi Medical College AffilicatedHeping hospital (No. is not avaiable).

Availability of data and materials

The raw data of this study are provided from the corresponding author with reasonable request.

Authors' contributions

Baichun Xing contributed to the design of the study and most experimental work. **Baichun Xing** and **Linlin Yang** contributed to the data analysis and the drafting the manuscript. **Yanan Cui** supervised the project. All author had given final approval of the version to be published.

Competing interests

The authors claimed that there is no conflict of interest.

Consent for publication

All the author consent for this publication.

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8. References

1. Torre LA, Siegel RL, Jemal A: **Lung Cancer Statistics**. *Advances in experimental medicine and biology* 2016, **893**:1-19.
2. Barta JA, Powell CA, Wisnivesky JP: **Global Epidemiology of Lung Cancer**. *Annals of global health* 2019, **85**(1).
3. Skřičková J, Kadlec B, Venclíček O, Merta Z: **Lung cancer**. *Casopis lekaru ceskych* 2018, **157**(5):226-236.
4. Liu H: **Effect of Traditional Medicine on Clinical Cancer**. *Biomedical Journal of Scientific & Technical Research* 2020, **30**(4):23548-23551.
5. Haixia W, Shu M, Li Y, Panpan W, Kehuan S, Yingquan X, Hengrui L, Xiaoguang L, Zhidi W, Ling O: **Effectiveness associated with different therapies for senile osteopo-rosis: a network Meta-analysis**. *J Tradit Chin Med* 2020, **40**(1):17-27.
6. Chen Z, Fillmore CM, Hammerman PS, Kim CF, Wong KK: **Non-small-cell lung cancers: a heterogeneous set of diseases**. *Nature reviews Cancer* 2014, **14**(8):535-546.
7. Hoy H, Lynch T, Beck M: **Surgical Treatment of Lung Cancer**. *Critical care nursing clinics of North America* 2019, **31**(3):303-313.
8. Li R, Huang Y, Liu H, Dilger JP, Lin J: **Comparing volatile and intravenous anesthetics in a mouse model of breast cancer metastasis**. In., vol. 78: American Association for Cancer Research; 2018: 2162.

9. Li R, Liu H, Dilger JP, Lin J: **Effect of Propofol on breast Cancer cell, the immune system, and patient outcome**. *BMC anesthesiology* 2018, **18**(1):77.
10. Liu H, Dilger JP, Lin J: **Effects of local anesthetics on cancer cells**. *Pharmacology & Therapeutics* 2020, **212**:107558.
11. Liu H: **A clinical mini-review: Clinical use of Local anesthetics in cancer surgeries**. *The Gazette of Medical Sciences* 2020, **1**(3):030-034.
12. Akkuş M, Öner E: **Can local infiltration of lidocaine reduce the postoperative atrial fibrillation rate in patients undergoing lobectomy for lung cancer?***Acta chirurgica Belgica* 2020, **120**(4):265-270.
13. Watanabe SN, Imai K, Kimura T, Saito Y, Takashima S, Matsuzaki I, Kurihara N, Atari M, Matsuo T, Iwai H *et al*: **Effect of lidocaine cream analgesia for chest drain tube removal after video-assisted thoracoscopic surgery for lung cancer: a randomized clinical trial**. *Regional anesthesia and pain medicine* 2019.
14. Zhang L, Hu R, Cheng Y, Wu X, Xi S, Sun Y, Jiang H: **Lidocaine inhibits the proliferation of lung cancer by regulating the expression of GOLT1A**. *Cell Prolif* 2017, **50**(5).
15. Sun H, Sun Y: **Lidocaine inhibits proliferation and metastasis of lung cancer cell via regulation of miR-539/EGFR axis**. *Artificial cells, nanomedicine, and biotechnology* 2019, **47**(1):2866-2874.
16. Wall TP, Crowley PD, Sherwin A, Foley AG, Buggy DJ: **Effects of Lidocaine and Src Inhibition on Metastasis in a Murine Model of Breast Cancer Surgery**. *Cancers* 2019, **11**(10).
17. Liu H, Dilger JP, Lin J: **Lidocaine Suppresses Viability and Migration of Human Breast Cancer Cells: TRPM7 as A Target for Some Breast Cancer Cell Lines**. *Cancers* 2021, **13**(2):234.
18. Ye L, Zhang Y, Chen YJ, Liu Q: **Anti-tumor effects of lidocaine on human gastric cancer cells in vitro**. *Bratislavske lekarske listy* 2019, **120**(3):212-217.
19. Liu H: **A Prospective for the Potential Effect of Local Anesthetics on Stem-Like Cells in Colon Cancer**. *Biomedical Journal of Scientific & Technical Research* 2020, **25**(2):18927-18930.
20. Siekmann W, Tina E, Von Sydow AK, Gupta A: **Effect of lidocaine and ropivacaine on primary (SW480) and metastatic (SW620) colon cancer cell lines**. *Oncol Lett* 2019, **18**(1):395-401.
21. Bundscherer AC, Malsy M, Bitzinger DI, Wiese CH, Gruber MA, Graf BM: **Effects of Lidocaine on HT-29 and SW480 Colon Cancer Cells In Vitro**. *Anticancer Res* 2017, **37**(4):1941-1945.
22. D'Agostino G, Saporito A, Cecchinato V, Silvestri Y, Borgeat A, Anselmi L, Uguccioni M: **Lidocaine inhibits cytoskeletal remodelling and human breast cancer cell migration**. *Br J Anaesth* 2018, **121**(4):962-968.
23. Cavallaro S: **CXCR4/CXCL12 in non-small-cell lung cancer metastasis to the brain**. *International journal of molecular sciences* 2013, **14**(1):1713-1727.
24. Smith MC, Luker KE, Garbow JR, Prior JL, Jackson E, Piwnica-Worms D, Luker GD: **CXCR4 regulates growth of both primary and metastatic breast cancer**. *Cancer Res* 2004, **64**(23):8604-8612.
25. Bachelerie F, Ben-Baruch A, Burkhardt AM, Combadiere C, Farber JM, Graham GJ, Horuk R, Sparre-Ulrich AH, Locati M, Luster AD *et al*: **International Union of Basic and Clinical Pharmacology.**

[corrected]. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. *Pharmacological reviews* 2014, **66**(1):1-79.

26. Furusato B, Mohamed A, Uhlén M, Rhim JS: **CXCR4 and cancer**. *Pathology international* 2010, **60**(7):497-505.
27. Wang Z, Sun J, Feng Y, Tian X, Wang B, Zhou Y: **Oncogenic roles and drug target of CXCR4/CXCL12 axis in lung cancer and cancer stem cell**. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2016, **37**(7):8515-8528.
28. Li X, Peng B, Zhu X, Wang P, Xiong Y, Liu H, Sun K, Wang H, Ou L, Wu Z *et al*: **Changes in related circular RNAs following ERbeta knockdown and the relationship to rBMSC osteogenesis**. *Biochemical and biophysical research communications* 2017, **493**(1):100-107.
29. Wu Z, Ou L, Wang C, Yang L, Wang P, Liu H, Xiong Y, Sun K, Zhang R, Zhu X: **Icaritin induces MC3T3-E1 subclone14 cell differentiation through estrogen receptor-mediated ERK1/2 and p38 signaling activation**. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2017, **94**:1-9.
30. Liu X, Liu H, Xiong Y, Yang L, Wang C, Zhang R, Zhu X: **Postmenopausal osteoporosis is associated with the regulation of SP, CGRP, VIP, and NPY**. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2018, **104**:742-750.
31. Plested JS, Coull PA, Gidney MA: **ELISA**. *Methods in molecular medicine* 2003, **71**:243-261.
32. Zhang Y, Zhan X, Xiong J, Peng S, Huang W, Joshi R, Cai Y, Liu Y, Li R, Yuan K *et al*: **Temperature-dependent cell death patterns induced by functionalized gold nanoparticle photothermal therapy in melanoma cells**. *Sci Rep* 2018, **8**(1):8720.
33. Phuchareon J, McCormick F, Eisele DW, Tetsu O: **EGFR inhibition evokes innate drug resistance in lung cancer cells by preventing Akt activity and thus inactivating Ets-1 function**. *Proceedings of the National Academy of Sciences of the United States of America* 2015, **112**(29):E3855-3863.
34. Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H, Bertolotto C, Di Mario U, Harlan DM, Perfetti R: **Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets**. *Endocrinology* 2003, **144**(12):5149-5158.
35. Liu H, Xiong Y, Wang H, Yang L, Wang C, Liu X, Wu Z, Li X, Ou L, Zhang R *et al*: **Effects of water extract from epimedium on neuropeptide signaling in an ovariectomized osteoporosis rat model**. *Journal of ethnopharmacology* 2018, **221**:126-136.
36. Li L, He L, Wu Y, Zhang Y: **Carvacrol affects breast cancer cells through TRPM7 mediated cell cycle regulation**. *Life sciences* 2021, **266**:118894.
37. Mehta P: **Semi-dry protein transfer and immunodetection of P-selectin using an antibody to its C-terminal tag**. *Methods in molecular biology (Clifton, NJ)* 2009, **536**:229-235.
38. Liu H, Xiong Y, Zhu X, Gao H, Yin S, Wang J, Chen G, Wang C, Xiang L, Wang P *et al*: **Icariin improves osteoporosis, inhibits the expression of PPARgamma, C/EBPalpha, FABP4 mRNA, N1ICD and jagged1 proteins, and increases Notch2 mRNA in ovariectomized rats**. *Experimental and therapeutic medicine* 2017, **13**(4):1360-1368.

39. Song J, Song W, Zhang L: **LncRNA RP1-85F18. 6 affects osteoblast cells by regulating the cell cycle**. *Open Life Sciences* 2020, **15**(1):951-958.
40. Chen G, Wang C, Wang J, Yin S, Gao H, Xiang LU, Liu H, Xiong Y, Wang P, Zhu X *et al*: **Antiosteoporotic effect of icariin in ovariectomized rats is mediated via the Wnt/beta-catenin pathway**. *Experimental and therapeutic medicine* 2016, **12**(1):279-287.
41. Cappiello F, Casciaro B, Mangoni ML: **A Novel In Vitro Wound Healing Assay to Evaluate Cell Migration**. *Journal of visualized experiments : JoVE* 2018(133).
42. Ding M, Zhan H, Liao X, Li A, Zhong Y, Gao Q, Liu Y, Huang W, Cai Z: **Enhancer RNA - P2RY2e induced by estrogen promotes malignant behaviors of bladder cancer**. *International journal of biological sciences* 2018, **14**(10):1268-1276.
43. Pepperell EE, Watt SM: **A novel application for a 3-dimensional timelapse assay that distinguishes chemotactic from chemokinetic responses of hematopoietic CD133(+) stem/progenitor cells**. *Stem cell research* 2013, **11**(2):707-720.
44. Zantl R, Horn E: **Chemotaxis of slow migrating mammalian cells analysed by video microscopy**. *Methods in molecular biology (Clifton, NJ)* 2011, **769**:191-203.
45. Liu H, Dilger JP, Lin J: **The Role of Transient Receptor Potential Melastatin 7 (TRPM7) in Cell Viability: A Potential Target to Suppress Breast Cancer Cell Cycle**. *Cancers* 2020, **12**(1).
46. Roe MW, Lemasters JJ, Herman B: **Assessment of Fura-2 for measurements of cytosolic free calcium**. *Cell calcium* 1990, **11**(2-3):63-73.
47. Chazotte B: **Labeling cytoskeletal F-actin with rhodamine phalloidin or fluorescein phalloidin for imaging**. *Cold Spring Harbor protocols* 2010, **2010**(5):pdb.prot4947.
48. Li R, Xiao C, Liu H, Huang Y, Dilger JP, Lin J: **Effects of local anesthetics on breast cancer cell viability and migration**. *BMC cancer* 2018, **18**(1):666.
49. Zhou W, Guo S, Liu M, Burow ME, Wang G: **Targeting CXCL12/CXCR4 Axis in Tumor Immunotherapy**. *Curr Med Chem* 2019, **26**(17):3026-3041.
50. Le Pimpec Barthes F, Mordant P, Pricopi C, Foucault C, Dujon A, Riquet M: **[The place of surgery in metastatic non-small cell lung cancer]**. *Revue des maladies respiratoires* 2012, **29**(3):376-383.
51. Liu T, Liu H, Wang G, Zhang C, Liu B: **Survival of M1a Non-Small Cell Lung Cancer Treated Surgically: A Retrospective Single-Center Study**. *The Thoracic and cardiovascular surgeon* 2015, **63**(7):577-582.
52. Liu H: **Nav channels in cancers: Nonclassical roles**. *Global Journal of Cancer Therapy* 2020, **6**(1):5.
53. Wang HW, Wang LY, Jiang L, Tian SM, Zhong TD, Fang XM: **Amide-linked local anesthetics induce apoptosis in human non-small cell lung cancer**. *J Thorac Dis* 2016, **8**(10):2748-2757.
54. Yousefi M, Bahrami T, Salmaninejad A, Nosrati R, Ghaffari P, Ghaffari SH: **Lung cancer-associated brain metastasis: Molecular mechanisms and therapeutic options**. *Cellular oncology (Dordrecht)* 2017, **40**(5):419-441.
55. Guo JY, Chiu CH, Wang MJ, Li FA, Chen JY: **Proteoglycan serglycin promotes non-small cell lung cancer cell migration through the interaction of its glycosaminoglycans with CD44**. *Journal of*

biomedical science 2020, **27**(1):2.

56. Kotteas EA, Boulas P, Gkiozos I, Tsagkouli S, Tsoukalas G, Syrigos KN: **The intercellular cell adhesion molecule-1 (icam-1) in lung cancer: implications for disease progression and prognosis**. *Anticancer Res* 2014, **34**(9):4665-4672.
57. Piegeler T, Votta-Velis EG, Liu G, Place AT, Schwartz DE, Beck-Schimmer B, Minshall RD, Borgeat A: **Antimetastatic potential of amide-linked local anesthetics: inhibition of lung adenocarcinoma cell migration and inflammatory Src signaling independent of sodium channel blockade**. *Anesthesiology* 2012, **117**(3):548-559.
58. Princen K, Hatse S, Vermeire K, De Clercq E, Schols D: **Evaluation of SDF-1/CXCR4-induced Ca2+ signaling by fluorometric imaging plate reader (FLIPR) and flow cytometry**. *Cytometry Part A : the journal of the International Society for Analytical Cytology* 2003, **51**(1):35-45.
59. Ikebuchi NW, Waisman DM: **Calcium-dependent regulation of actin filament bundling by lipocortin-85**. *The Journal of biological chemistry* 1990, **265**(6):3392-3400.
60. van Vliet AR, Giordano F, Gerlo S, Segura I, Van Eygen S, Molenberghs G, Rocha S, Houcine A, Derua R, Verfaillie T *et al*: **The ER Stress Sensor PERK Coordinates ER-Plasma Membrane Contact Site Formation through Interaction with Filamin-A and F-Actin Remodeling**. *Molecular cell* 2017, **65**(5):885-899.e886.
61. Oda T, Iwasa M, Aihara T, Maéda Y, Narita A: **The nature of the globular- to fibrous-actin transition**. *Nature* 2009, **457**(7228):441-445.
62. Yang D, Kim J: **Emerging role of transient receptor potential (TRP) channels in cancer progression**. *BMB reports* 2020, **53**(3):125-132.
63. Liu H: **A prospective for the role of two-pore channels in breast cancer cells**. *Global Journal of Cancer Therapy* 2020, **6**(1):001–003.

Figures

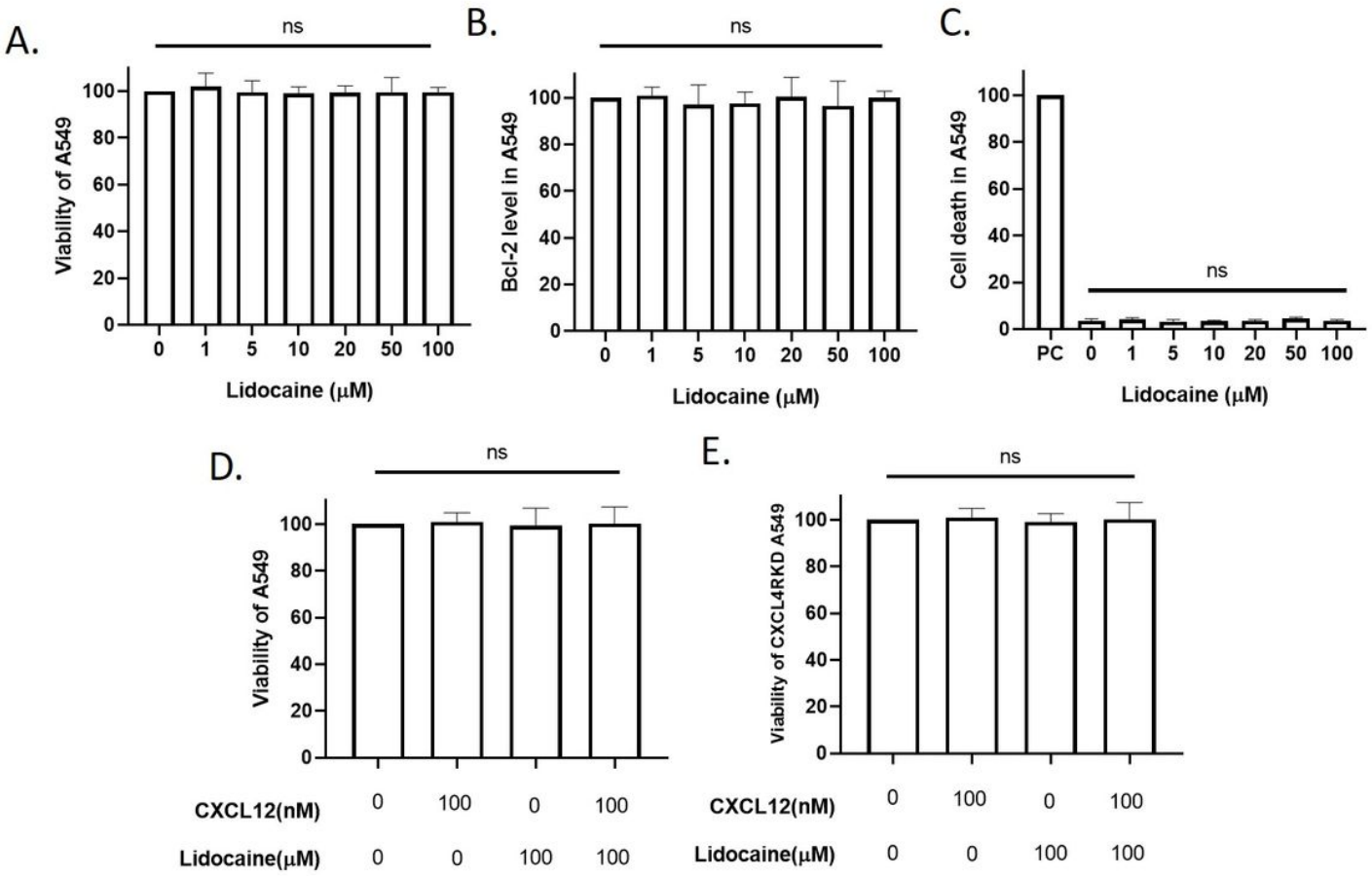

Figure 1

Effect of lidocaine at clinicalplasma concentration on A549 viability. A549 were exposed to tested drugs for 24 hours. A. Effect of clinical-plasma-dose lidocaine on A549 viability. Cell viability was determined using theCCK-8 assay. B. Effect of lidocaine on A549 apoptosis. Cell apoptosis was determined by testing anti-apoptosis factor Bcl-2 using ELISA. C. Effect of lidocaine on A549 cell death. Cell death was determined by monitoring DNA fragmentation using a Cell Death Detection kit. The cell death positive control was induced by high-temperature culture (55 ◦C for 30 min). D. Effect of CXCL12 and lidocaine on the viability of A549. E. Effect of CXCL12 and lidocaine on the viability of CXCR4 knocked down A549. (Significant differences are indicated by *p < 0.01, NS=not significant)

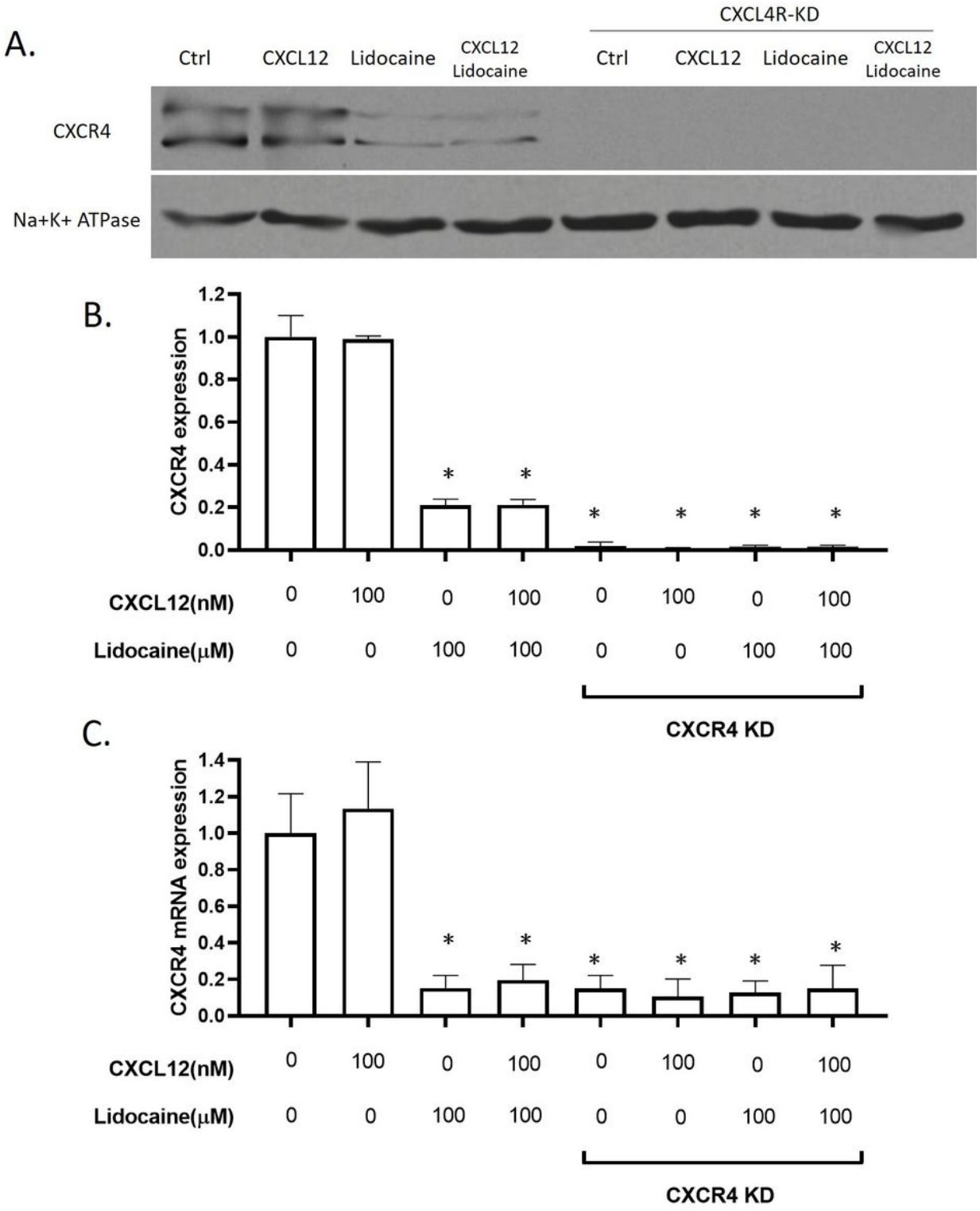

Figure 2

Effect of lidocaine on CXCR4 expression in A549. AB. membrane expression of CXCR4 protein in A549. Membrane proteins were extracted using a plasma membrane protein extraction kit. The CXCR4 protein level in membrane protein was determined using western blotting. C. CXCR4 mRNA expression in A549. CXCR4 mRNA expression was determined using QPCR.(Significant differences compared to both control (CXCL12 and lidocaine are 0) are indicated by *p < 0.01, NS=not significant)

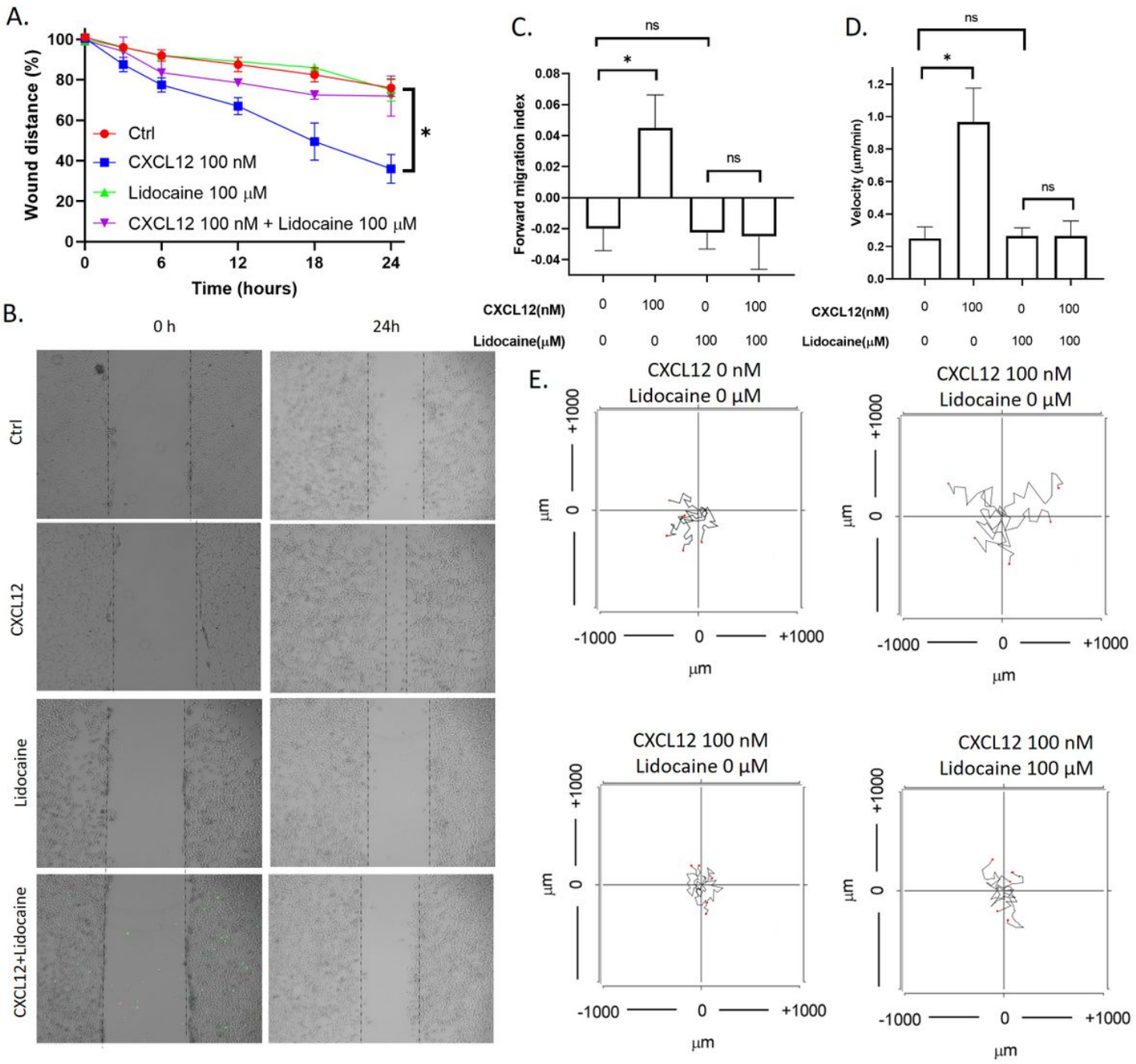

Figure 3

Effect of lidocaine on CXCL12-induced A549 migration. A. Effect of lidocaine on CXCL12-induced A549 monolayer wound healing. B. Representative images of wound healing assay. C. Effect of lidocaine on CXCL12-induced A549 forward migration index. Individual cell migration was determined using chemotaxis assay. D. Effect of lidocaine on CXCL12-induced A549 migration velocity. E. Aggregated trajectories of individual A549 cells at different experimental conditions (10 hours). (Significant differences are indicated by *p < 0.01, NS=not significant)

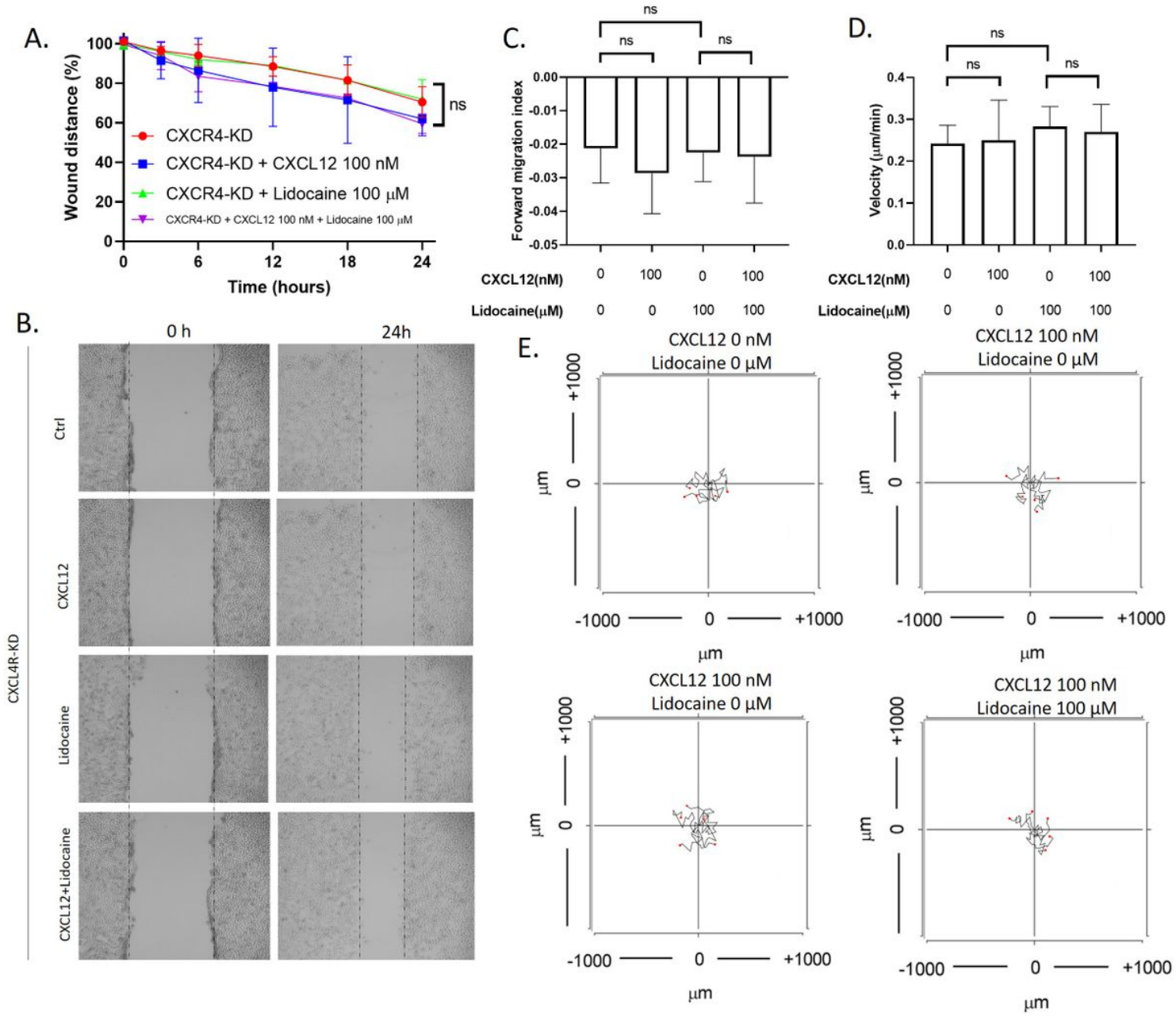

Figure 4

Effect of lidocaine on CXCL12-induced CXCR4knocked down A549 cells migration. A. Effect of lidocaine on CXCL12-induced CXCR4- knocked down A549 monolayer wound healing. B. Representative images of wound healing assay. C. Effect of lidocaine on CXCL12-induced CXCR4- knocked down A549 forward migration index. Individual cell migration was determined using chemotaxis assay. D. Effect of lidocaine on CXCL12-induced CXCR4- knocked down A549 migration velocity. E. Aggregated trajectories of individual CXCR4- knocked down A549 cells at different experimental conditions (10 hours).(Significant differences are indicated by *p < 0.01, NS=not significant)

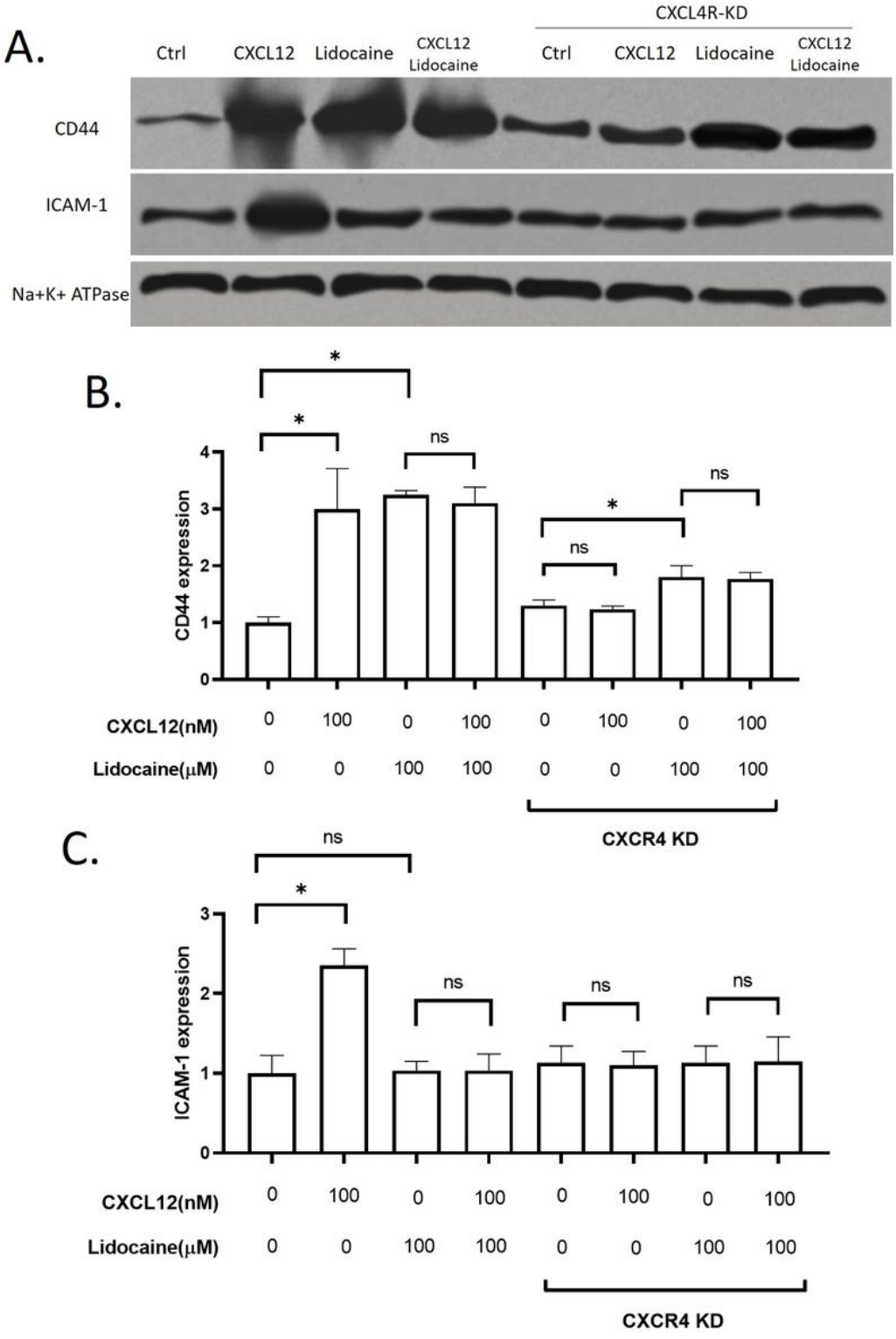

Figure 5

Effect of lidocaine on membrane expression of CD44 and ICAM-1 in A549. Membrane proteins were extracted using a plasma membrane protein extraction kit. Protein expression was determined using western blotting. A. Representative images of western blotting. B. membrane expression of CD44 protein in A549. C. membrane expression of ICAM-1 protein in A549. (Significant differences are indicated by *p < 0.01, NS=not significant)

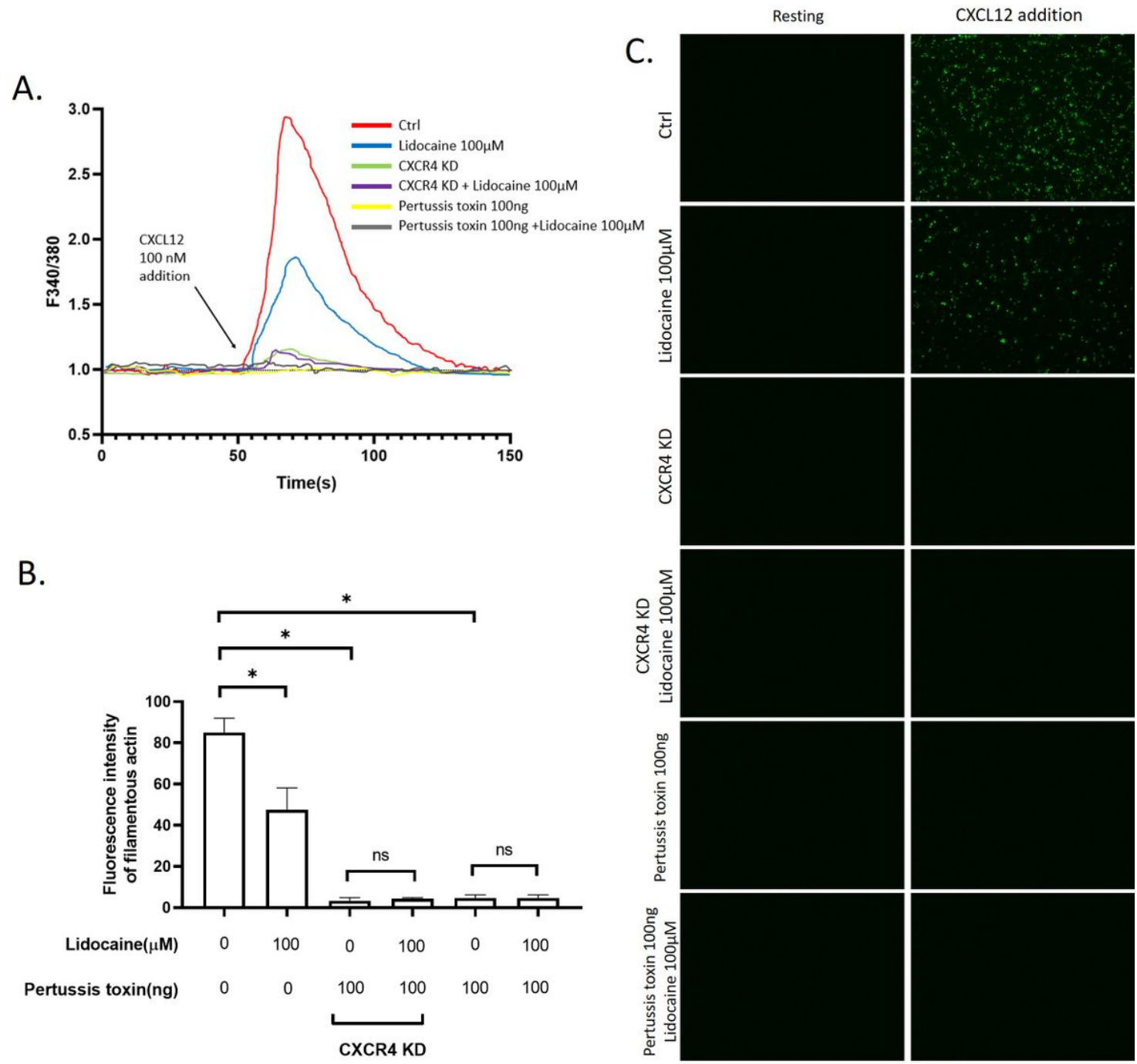

Figure 6

Effect of lidocaine on CXCL12-induced intracellular Ca2+ releasing and cytoskeleton remodeling. A. Real-time monitoring of the intracellular Ca2+ concentration in response to CXCL12 addition in A549 or CXCR4-knocked down A549 cells pre-incubated with testing reagents for 2 hours. B. Fluorescence intensity of filamentous actin before and after the exposure of CXCL12. Filamentous actin of A549 was stained using phalloidin coupled to fluorescein isothiocyanate (FITC). The data were displayed in the Relative Fluorescence Unit.C. Representative images of fluorescence of filamentous actin. (Significant differences are indicated by *p < 0.01, NS=not significant)

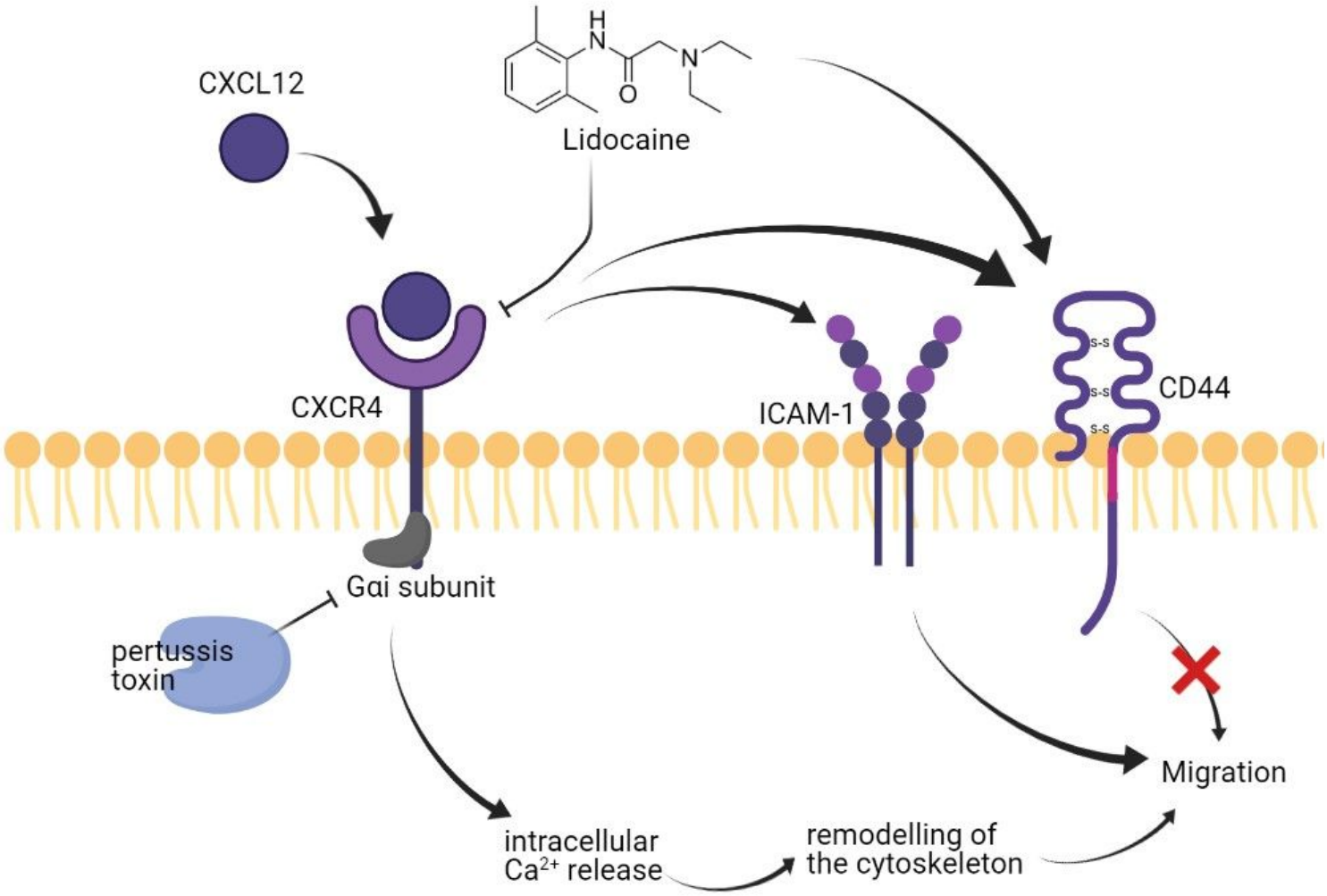

Figure 7

Effect of lidocaine on A549 migration.