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Alpha-Lipoic Acid-Mediated Inhibition of LTB_4 Synthesis Suppresses Epithelial-Mesenchymal Transition, Modulating Functional and Tumorigenic Capacities in Non-Small Cell Lung Cancer A549 Cells

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ABSTRACT

Background: Leukotriene B_4 (LTB_4) plays a crucial role in carcinogenesis by inducing epithelial-mesenchymal transition (EMT), a process associated with tumor progression. The synthesis of LTB_4 is mediated by leukotriene A_4 hydrolase (LTA_4H), and it binds to the receptors BLT_1 and BLT_2. Dysregulation in LTB_4 production is linked to the development of various pathologies. Therefore, the identification or design of inhibitors of LTB_4 synthesis or receptor antagonists represents an ongoing challenge. In this context, our laboratory previously demonstrated that alpha-lipoic acid (ALA) inhibits LTA_4H. The objective of this study was to evaluate the effect of ALA on the expression of canonical EMT markers and the functional and tumorigenic capacities induced by LTB_4 in A549 cells.
Methods: The expression of $cPLA_2$, 5LOX, FLAP, LTA_4H, BLT1, and LTB_4 production in human adenocarcinomic alveolar basal epithelial A549 cells was assessed using Western blot, RT-qPCR, and ELISA, respectively. Subsequently, the expression of canonical EMT markers was evaluated by Western blot. Functional assays were performed to assess cell viability, proliferation, invasion, migration, and clonogenicity using MTT, Western blot, Transwell assays, and colony formation assays, respectively. Results were expressed as median with interquartile range ($n \geq 3$) and analyzed using the Kruskal-Wallis or Tukey multiple comparisons tests.
Results: A549 cells express key proteins involved in LTB_4 synthesis and receptor binding, including LTA_4H and BLT_1, and ALA inhibits the production of LTB_4. Additionally, LTA_4H and BLT1 were detected in lung adenocarcinoma tissue samples. LTB_4 was found to induce EMT, whereas ALA treatment enhanced the expression of epithelial markers and reduced the expression of mesenchymal markers. Furthermore, ALA treatment resulted in a decrease in LTB_4 levels and attenuated the functional and tumorigenic capacities of A549 cells, including their viability, migration, invasion, and clonogenic potential.
Conclusions: These findings suggest that ALA may offer therapeutic potential in the context of lung cancer, as it could be integrated into conventional pharmacological therapies to enhance treatment efficacy and mitigate the adverse effects associated with chemotherapy. Further studies are warranted to confirm the clinical applicability of ALA as an adjunctive treatment in lung cancer.

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Introduction

Lung cancer (LC) is the leading cause of cancer-related mortality worldwide, with 2.5 million new cases reported in 2022 [1]. LC is classified into two major histological subtypes: small cell lung carcinoma (SCLC), which accounts for 15% of cases, and non-small cell lung cancer (NSCLC), which represents 85% of cases [2]. Chronic inflammation is a well-established risk factor for the development of NSCLC [2–4]. This inflammatory response is driven by cytokines, chemokines, prostaglandins, and leukotrienes, which are secreted by immune cells, tumor cells, and other cell types [3,5–8]. Previous studies have demonstrated that leukotriene B_4 (LTB_4) is closely associated with the induction of a pro-tumorigenic microenvironment, supporting the survival of malignant cells [9,10].

Various stimuli, including antigens and cytokines, can trigger the synthesis of LTB_4 [11,12]. These stimuli lead to the release of arachidonic acid (AA) from membrane phospholipids, catalyzed by calcium-dependent cytosolic phospholipase A_2 ($cPLA_2$) [12,13]. AA is then oxidized to leukotriene A_4 (LTA_4) by 5-lipoxygenase (5-LOX), an enzyme that requires activation by 5-lipoxygenase-activating protein (FLAP) [12,13]. LTA_4 is subsequently converted to LTB_4 by leukotriene A_4 hydrolase (LTA_4H) [11–13]. LTB_4 is then exported from the cell to the extracellular medium by transporters from the ATP-binding cassette (ABC) family [12,13]. Once released, LTB_4 binds to two types of receptors: leukotriene B_4 receptor type 1 (BLT1) and leukotriene B_4 receptor type 2 (BLT_2).

Studies suggest that LTB_4 plays a critical role in carcinogenesis [8,14,15]. Elevated levels of LTB_4 have been observed in various human cancers, including colon and prostate cancer, and the expression of LTB_4 receptors is upregulated in pancreatic cancer cells [8,16]. Moreover, inhibition of LTB_4 synthesis by bestatin has been shown to significantly reduce proliferation and colony formation in colorectal cancer cells *in vitro* [17].

LTB_4 promotes tumor progression, in part, by activating epithelial-mesenchymal transition (EMT) [18]. EMT is characterized by morphological and gene expression changes, including decreased expression of E-cadherin and increased expression of mesenchymal markers such as vimentin. These changes are regulated by transcription factors, including ZEB-1, SNAIL, and SLUG [18]. For example, You Ri Kim et al. demonstrated that LTB_4 reduces E-cadherin expression and increases N-cadherin and vimentin levels in BLT2-mediated PANC-1 cells, which enhances cell invasion and migration [19].

Given the pathological effects associated with LTB_4, various strategies have been explored to modulate its production [12,20,21]. Our laboratory has previously shown that alpha-lipoic acid (ALA) inhibits the enzymatic activity of human recombinant LTA_4H [22].

ALA is an endogenous compound synthesized in the mitochondria, primarily in its R configuration, and functions as a cofactor for several multienzyme complexes [23,24]. It participates in the chelation of divalent metals, as well as exhibiting antioxidant properties, modulating stress responses, and influencing inflammatory pathways [25].

The aim of this study was to evaluate the effect of ALA on the expression of canonical EMT markers and the functional and tumorigenic capacities mediated by LTB_4 in A549 non-small cell lung cancer cells.

Materials and Methods

Materials

Chemicals and materials. Alpha lipoic acid (Cat N° 1368201) and LTA_4H selective inhibitor, SC-57461A (Cat N° PZ0110) were purchased from Sigma-Aldrich;Merck KGaA (Burlington, Massachusetts). PMA (phorbol 12-myristate 13-acetate), Cat N° 10008014, leukotriene B_4 EIA Kit (Cat N° EHLTB4) and LTB_4 (Cat N° 20110) were obtained from Cayman Chemical Company (Ann Arbor, Michigan, USA). IL-6 (R&D SYSTEMS, Cat N° 206-IL) was acquired from GenexPress, Life Science Business (Santiago, Chile). Hank's Balanced Salt Solution (HBSS) (Cat N° 14025076) was obtained from ThermoFisher Scientific, Inc (Waltham, Massachusetts) . The salts, alcohols (ethanol and isopropanol), chloroform and dimethylsulfoxide were acquired from Winkler, Ltd (Lampa, Santiago, Chile)

Methods

Cell culture

Adenocarcinomic human alveolar basal epithelial cells (A549) were purchased from the American Type Culture Collection (ATCC, Gaithersburg, Maryland). They were maintained in DMEM/F12 medium (Gibco; Thermo Fisher Scientific, Inc. Cat N° 12400024, Waltham, Massachusetts) supplemented with 10% fetal bovine serum (FBS) (GenexPress; Life Science Business, Hyclone Cat N° SH30396.03, Santiago, Chile) and 1% penicillin/streptomycin (Andes Import, Cat N° 03-031-5B, Santiago, Chile), at 37°C in a humidified atmosphere with 5% CO_2. Before each experiment, cells were washed three times with sterile 1X PBS, and replaced with DMEM/F12 in the absence of serum for 24 hours.

LTA_4H inhibitors and stimuli with PMA, LTB_4 and IL6

A549 cells were preincubated with different concentrations of ALA (0.01 and 10 µMQ1) or 0.01 µMQ1 of SC57461A (potent and selective LTA_4H inhibitor) for 30 minutes at 37°C. Subsequently, the cells were stimulated with 5 nMQ1 of PMA for 15 minutes, LTB_4 (10, 50 and 100 nMQ1), 50 ng/mL of IL-6 or IL-6/LTB_4 for 12 or 48 hours. In all experiments the concentration of DMSO used was 1%.

Samples from patients with lung adenocarcinoma

FFPE (formalin-fixed, paraffin-embedded) biopsy sections were obtained from six lung adenocarcinoma samples from female and male patients with an age range of 61 to 68 years (Table 1). The biopsy files were obtained from the Department of Pathological Anatomy, Research Histopathology Core of Pontificia Universidad Católica de Chile, Santiago, Chile. The inclusion criterion of the samples was: confirmed lung adenocarcinoma. All use and processing protocols have been approved by the Ethical-Scientific Committee of the Faculty of Medicine of the Pontificia Universidad Católica de Chile (ID 201007026). For the diagnosis, patients were asked to sign an informed consent. For the use of samples from the pathology service, authorization was obtained from the Department of Pathological Anatomy, Research Histopathology Core of Pontificia Universidad Católica de Chile (Table 1).

Immunohistochemistry

The sections were mounted to electrically charged slides and had a thickness of 3 mm. The samples were dewaxed with xylene and hydrated with ethanol to distilled water. Subsequently, antigenic retrieval was carried out in 10 mM sodium citrate buffer pH 6.0 at 95°C for 30 minutes [26]. Then, endogenous peroxidase was blocked with 0.3% v/v hydrogen peroxide in methanol for 20 minutes and washed with 1X PBS. Blocking of nonspecific binding was performed with BSA (bovine serum albumin) for 30 minutes and subsequently the sections were incubated at 4°C overnight [26] with the primary anti-LTA4H (D-6) antibody (mouse anti-human, 1:100, sc-390567, Santa Cruz Biotechnology, INC, Dallas, Texas) or with the anti-LTB4-R1 (BLT1) antibody (rabbit anti-human, 1:200, BS-2654R, Bioss Antibodies, Waltham, Massachusetts). The universal secondary antibody and the ABC complex from the VECTASTAIN® Elite® ABC-HRP kit (PK-7200, Vector Laboratories) were used sequentially, 30 minutes of incubation

Table 1
Samples from patients with lung adenocarcinoma.

Sample	*Sex/Age*	*Histological Characteristics*
68G	Male / 67 years old	Invasive adenocarcinoma with 40% poorly differentiated component, 30% papillary component, 20% lepidic component and 10% micropapillary component. Size 2.4 × 2.5 cm.
11B	Male / 68 years old	Well-differentiated adenocarcinoma (2.3 cm) in situ (lepidic pattern).
32E	Female / 61 years old	Well-differentiated adenocarcinoma in situ (lepidic pattern 65% aporx). Size: 1.8 cm.
07F	Female / 62 years old	Invasive, mixed adenocarcinoma predominantly enteric (80%) with mucinous component (15%), acinar (5%) and extensive lepidic growth pattern. Size 4.5 cm.
08E	Female / 66 years old	Invasive adenocarcinoma, with predominantly lepidic growth pattern. Size 4.1 cm.
23C	Female / 65 years old	Well-differentiated acinar-type adenocarcinoma with lepidic component (25%). Size: 3.7 cm.

at room temperature each. Specific signal revealed was performed with 3,3′-diaminobenzidine (DAB; DAKO North America Inc.) [26] and nuclear contrast was performed with Mayer's hematoxylin (ScyTek Laboratories, Logan, Utah). After staining, the slides were dehydrated in ethanol, cleared with xylene and mounted with EntellanTM. Digital images were obtained with a Leica DM2500 microscope (Leica, Wetzlar, Germany). The images were processed with ImageJ 1.51w software (NIH, Bethesda, MD).

RNA extraction and quantification

Total RNA was extracted from A549 cells by centrifugations using TRIzol® (Thermo Fisher Scientific, Inc, Waltham, Massachusetts), chloroform for phase separation, isopropanol for RNA precipitation, and 75% ethanol for washes. Nuclease-free molecular biology water (Thermo Fisher Scientific, Inc, Waltham, Massachusetts) was then used to resuspend the extracted RNA. The determination of RNA concentration and purity was evaluated through spectrophotometry at 260/280 nm using a BioTeK Synergy HT plate reader (BioTek Instruments, Inc.) [27].

Reverse transcription-quantitative PCR (RT-qPCR)

A total of 1000 ng of total RNA was used to obtain cDNA synthesis using the 5X All-In-One RT MasterMix kit (25°C for 10 minutes, 42°C for 15 minutes and 85°C for 5 minutes, 1 cycle; Applied Biological Materials Inc, Vancouver, Canada). Next, for mRNA quantification, 50 ng/mL was amplified by qPCR using the Brilliant II SYBR Green qPCR Master Mix kit (95°C for 10 minutes, 1 cycle; 95°C for 15 min, 60°C for 15 minutes and 72°C for 15 min, 40 cycles; 95°C for 15 minutes, 65°C for 15 minutes and 95°C for 15 minutes, 1 cycle; Agilent Technologies, Inc.) and their respective pair of primers. Table 2 shows the sequence of the oligonucleotides used. The data were normalized with the housekeeping gene pumilio and the results obtained were analyzed by the ΔCq method [27,28].

Western blotting

The proteins obtained from A549 cells were extracted using RIPA buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% v/v NP-40, 1% w/v sodium deoxycholate, 2.5 mM Na_3PO_4, 1 mM b-glycerophosphate and 1 mM Na_3VO_4, pH 7.4) with a protease inhibitor cocktail (Roche Diagnostics) at 4°C. Subsequently, the homogenate was kept at 4°C for 15 minutes, and centrifuged at 16,708 g for 15 minutes at 4°C. The supernatant (protein) was quantified using the Bradford method at 595 nm. Loading buffer was added to 25 µg of proteins, and it was denatured at 95°C for 10 minutes [26]. Electrophoresis was performed using 10% SDS-PAGE gels and transferred to nitrocellulose membranes via wet transfer at constant 400 mA for 90 minutes. The membranes were blocked with 5% BSA (Winkler, Ltd. Cat N° BM-0150) in 0.2% TBS-Tween for 90 minutes [26] and incubated with the primary antibodies (Table 3) overnight at 4°C. Next, washes were performed with 0.2% TBS-Tween and incubated with secondary antibodies conjugated with the HRP enzyme (Table 4) for 90 minutes at room temperature. Detection of 5-lipoxygenase pathway proteins was determined using the EZ-ECL chemiluminescence kit (Biological Industries, Cat N° 20-500-500, Israel) on a Vilber Lourmat instrument (Fusion FX5-XT 826. WL/superbright serial number 15200393; Vilber). The analysis of the area under the curve of the bands obtained was performed using ImageJ 1.51w software (NIH, Bethesda, MD, USA). The markers (BLT1, *ZEB-1*, *SNAIL*, *SLUG*, and β-actin) were evaluated after the striping procedure (Stripping Buffer of 25 mM glycine-HCl, pH: 2.0 for 6 minutes).

Table 2
Reverse transcription-quantitative PCR oligonucleotides.

Gene	*Forward (5′-3′)*	*Reverse (5′-3′)*
$cPLA_2$	CTGGACAACCTGTCACCTTTACT	TCAACCCAATCTGCAAACATCAG
5-LOX	GCCACAAGGATTTACCCCGT	ATGACCCGCTCAGAAATAGTGT
FLAP	CTGCCAACCAGAACTGTGTAGA	AAATATGTAGCCAGGGGTGCTC
LTA_4H	GCAGAGCAATCCTTCCTTGTCA	CAGGTAGCAGGGTATTGGAACTTT
BLT1	TGTGAGTGGGGTACATGTGC	CGGAATCATCTGGGATGGGG
Pumilio	CGGTCGTCCTGAGGATAAAA	CGTACGTGAGGCGTGAGTAA

$cPLA_2$; Cytosolic phospholipase A_2, 5-LOX; 5-lipoxygenase, FLAP; 5-lipoxygenase activating protein, LTA_4H; Leukotriene A_4 hydrolase, BLT1; Leukotriene B_4 type 1 receptor.

Determination of LTB_4 levels in cell culture medium

A549 cells were seeded in 6-well plates in DMEM/F12 medium with 10% FBS. The next day, it was replaced with HBSS, and the vehicle (DMSO) or the inhibitors 0.01 µM of SC-57461A, 0.01 µM of ALA and 10 µM of ALA were incorporated for 30 minutes at 37°C. Subsequently, solution was extracted and PMA was incorporated and incubated for 15 minutes at 37°C an atmosphere with 5% CO_2. Finally, the medium was removed and centrifuged at 1000 g for 5 minutes at room temperature. At the same time, the number of viable cells was quantified using the Trypan-blue method. To determine the production of LTB_4 in the supernatant, leukotriene B_4 EIA Kit (Invitrogen, cat number EHLTB4, Waltham, Massachusetts, USA) was used with the protocols established by the manufacturer. The immunoassay is based on a competitive technique to determine the concentration of LTB_4. 100 µL of the Standard Diluent (HBSS) was added to the non-specific binding (NSB) and maximum binding (B_0) wells. Subsequently, 100 µL of the standards or samples were incorporated to the corresponding wells. Next, we aggregated 50 µL of Reagent Diluent to the NSB well, and then added 50 µL of Blue LTB4-AP conjugate to each well, except for the blank, total activity (TA) and NSB. Then, we incorporate 50 µL of the yellow LTB_4 antibody to each of the wells, except in the blank, TA and NSB, incubating on a horizontal orbital microplate shaker at 450 rpm at room temperature for 2 hours. Subsequently, the wells were washed 3 times with 1X Wash Buffer, and then 5 µL of blue LTB4-AP conjugate was added to the TA wells, and 200 µL of substrate solution to each of the wells, and the plate was incubated at 37°C for 2 hours. Finally, we aggregated 50 µL of stop solution to each of the wells and the plate was read immediately by determining the absorbance through spectrophotometry at 405 nm using the BioTeK Synergy HT plate reader (BioTek Instruments, Inc.).

Table 3
Primary antibody for western blotting.

Primary antibody	*Supplier*	*Catalog number*	*Species*	*Dilution*
$cPLA_2$ (4-4B-3C)	Santa Cruz Biotechnology, Inc, Texas, USA	sc-454	Mouse	1:1000
5-LOX (33)	Santa Cruz Biotechnology, Inc, Texas, USA	sc-136195	Mouse	1:1000
FLAP	ThermoFisher Scientific, Inc, Waltham, MA, USA	bs-7556R	Rabbit	1:1000
LTA_4H (D6)	Santa Cruz Biotechnology, Inc, Texas, USA	sc-390567	Mouse	1:1000
BLT1	ThermoFisher Scientific, Inc, Waltham, MA, USA	bs-2654R	Rabbit	1:1000
E-cadherin	Abcam, Cambridge, USA	ab8978	Mouse	1:1000
Vimentin	Abcam, Cambridge, USA	ab8979	Mouse	1:1000
ZEB-1	Santa Cruz Biotechnology, Inc, Texas, USA	sc-515797	Rabbit	1:1000
β-actin	MP Biomedicals, LLC, California, USA	691002	Mouse	1:10.000

$cPLA_2$; Cytosolic phospholipase A_2, 5-LOX; 5-lipoxygenase, FLAP; 5-lipoxygenase activating protein, LTA_4H; Leukotriene A_4 hydrolase, BLT1; Leukotriene B_4 type 1 receptor, ZEB-1; Zinc finger E-box binding homeobox, PCNA; proliferating cell nuclear antigen.

Table 4
Secondary antibody for western blotting.

Secondary antibody	*Supplier*	*Catalog number*	*Dilution*
Anti-rabbit	Jackson ImmunoResearch Laboratories, Inc, Philadelphia, PA.	111-035-003	1:10.000
Anti-mouse	Jackson ImmunoResearch Laboratories, Inc, Philadelphia, PA.	115-035-003	1:10.000

Cell viability assays by MTT

A total of 2,000 (LTB_4 assay) or 10,000 (inhibitor assay) A549 cells were seeded per well in 96-well plates in DMEM/F12 medium with 10% FBS, maintained at 37°C in an atmosphere of 5% CO_2 for 24 hours. The next day, the medium was replaced with HBSS, and the vehicle or inhibitors 0.01 µM of SC-57461A, 0.01µM of ALA and 10 µM of ALA were added for 30 minutes at 37°C. Next, HBSS was removed, and 5 nM of PMA was added and incubated for 15 minutes at 37°C in HBSS. Finally, medium was replaced by DMEM/F12 culture medium for 12 hours. On the other hand, 10, 50, and 100 nM of LTB_4 were added to DMEM/F12 culture medium and maintained for 12 hours at 37°C. After 12 hours, the culture medium is replaced with 100 µL of MTT solution (Sigma-Aldrich, Burlington, Massachusetts). Diluted 10 times in DMEM/F12 medium without FBS (initial stock of 5 mg/mL MTT solution), incubating for 3 hours at 37°C. Finally, the solution was removed, and the formazan crystals were resuspended in 100 µL of a solution containing 20% DMSO and 80% isopropanol while stirring for 10 minutes at room temperature, in an orbital shaker [29]. Absorbance was measured at 570 nm on an infinite M200/Tecan plate reader (Tecan, Zurich, Germany).

Proliferation assay

A549 cells were seeded in 6-well plates in DMEM/F12 medium with 10% FBS, maintaining it at 37°C in an atmosphere with 5% CO_2 for 24 hours. The next day, the medium was replaced with HBSS, and the vehicle (DMSO) or the inhibitors 0.01 µM of SC-57461A, 0.01 µM of ALA and 10 µM of ALA were added for 30 minutes at 37°C. Subsequently, HBSS was extracted, and 5 nM of PMA was added and incubated for 15 minutes at 37°C in HBSS. Finally, solution is replaced with DMEM/F12 culture medium for 12 hours. After 12 hours, the proteins were extracted, and quantified and the expression of PCNA was determined by western blot, as described as previously described.

EMT assay

A549 cells were seeded in 6-well plates in DMEM/F12 medium with 10% FBS, maintaining it at 37°C in an atmosphere with 5% CO_2 for 24 hours. The next day, the medium was replaced with HBSS, and the vehicle (DMSO) or the inhibitors 0.01 µM of SC-57461A, 0.01 µM of ALA, and 10 µM of ALA were added for 30 minutes at 37°C. Subsequently, HBSS was extracted and 5 nM of PMA was added in HBSS or 100 nM of LTB_4, 50 ng/mL of IL-6 or IL-6/LTB_4 in DMEM/F12 culture medium and incubated for 15 minutes at 37°C. Finally, PMA is extracted from the corresponding wells and replaced with DMEM culture medium with 1% DMSO for 48 hours. After 48 hours, the proteins were extracted, and quantified and the expression of EMT markers was determined by western blot, as described as previously described.

Migration transwell

A549 cells were seeded in 6-well plates in DMEM/F12 medium with 10% FBS, maintaining it at 37°C in an atmosphere with 5% CO_2 for 24 hours. The next day, the medium was replaced with HBSS, and the vehicle (DMSO) or the inhibitors 0.01 µM of SC-57461A, 0.01 µM of ALA, and 10 µM of ALA were added for 30 minutes at 37°C. Subsequently, solution is extracted and 5 nM of PMA is added and incubated for 15 minutes at 37°C an atmosphere with 5% CO_2. Next, HBSS was removed and washed three times with 1X PBS, and 1X trypsin was added to dissociate and count the cells.

A total of 500,000 cells/mL were seeded in serum-free DMEM/F12 medium in each of the wells for the transmigration assay (Genexpress, Cat 2025-07-20, Santiago, Chile). Cell migration was carried out by adding 550 µL of DMEM/F12 medium with 10% FBS, maintaining it at 37°C in an atmosphere of 5% CO_2 for 6 hours. Cells that did not migrate were removed by washing twice with 1x PBS, and those that migrated were fixed with 100% cold methanol for 20 minutes at room temperature and stained with a 0.2% w/v solution of crystal violet, dissolved in methanol, at 10% v/v for 5 minutes. Migrating cells were counted and averaged. The number of migrating cells was expressed as the median with interquartile range.

Invasion transwell

A549 cells were seeded in 6-well plates in DMEM/F12 medium with 10% FBS, maintaining it at 37°C in an atmosphere with 5% CO_2 for 24 hours. The next day, the medium was replaced with HBSS, and the vehicle (DMSO) or the inhibitors 0.01 µM of SC-57461A, 0.01 µM of ALA and 10 µM of ALA were added for 30 minutes at 37°C. Subsequently, HBSS was extracted and 5 nM of PMA was added and incubated for 15 minutes at 37°C an atmosphere with 5% CO_2. Next, the solution was removed and washed three times with 1x PBS, and 1x trypsin was added to dissociate and count the cells. For the invasion assay, Matrigel Matrix Basement Membrane (Corning, Inc; Cat N° 356231, New York, USA) 1/50 diluted in DMEM/F12 medium without serum was added to each of the wells, allowing it to gel at 37°C by 6 hours before seeding. Next, the so-

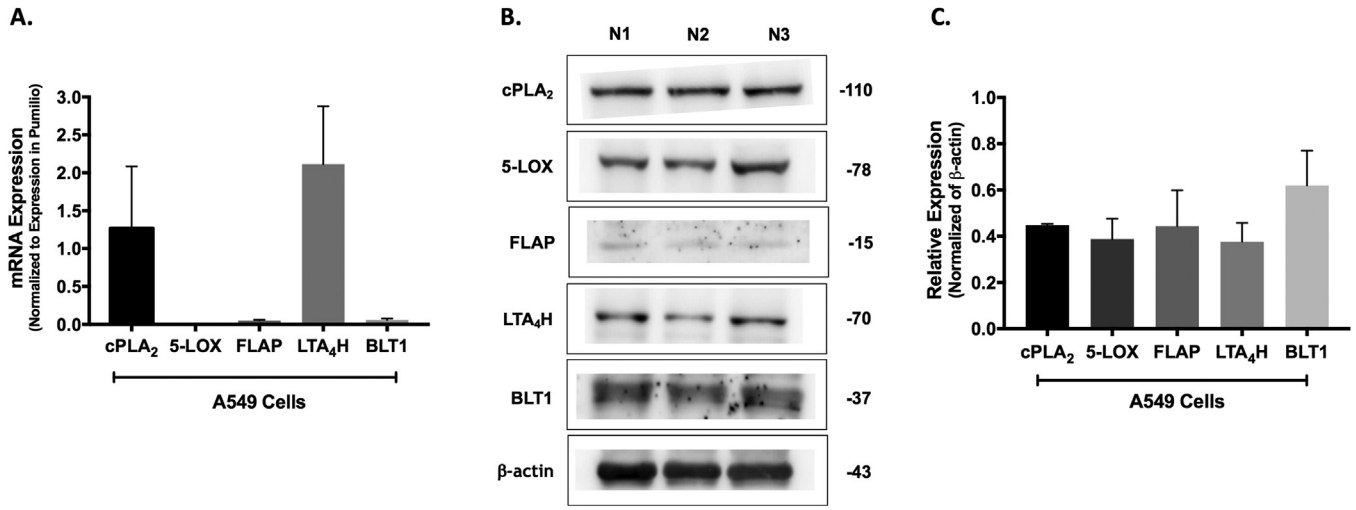

Figure 1. Protein expression of the 5-lipoxygenase pathway and the BLT1 receptor in A549 non-small lung cancer cells. (A) mRNA levels of $cPLA_2$, 5-LOX, FLAP, LTA_4H and BLT1 normalized with housekeeping (pumilio) in the cell line. (B) The protein lysate from A549 cells were analyzed by immunoblot and the membranes were incubated with primary antibodies against $cPLA_2$, 5-LOX, FLAP, LTA_4H, BLT1 and β-actin. **(C)** Semi-quantification of the relative protein expression of $cPLA_2$, 5-LOX, FLAP, LTA_4H and BLT1, normalized with β-actin. The values 110, 78, 15, 70, 37, and 43 correspond to mass units (kDa). $cPLA_2$, 5-LOX, LTA_4H, BLT1; n = 3; FLAP; n = 4

lution was extracted and washed once with 1x PBS, and then the cell suspension was added.

A total of 500,000 cells/mL were seeded in DMEM/F12 medium without serum in each of the wells for the trans-invasion assay (GenexPress; Life Science Business, Cat N° 2025-07-20, Santiago, Chile). The invasion of the cells was carried out by adding 550 µL of DMEM/F12 medium with 10% FBS, maintaining it at 37°C in an atmosphere with 5% CO_2 for 20 hours. Cells that did not invade were removed by washing twice with 1X PBS, and those that invaded were fixed with 100% cold methanol for 20 minutes at room temperature and stained with a 0.2% w/v solution of crystal violet, dissolved in methanol at 10% v/v for 5 minutes. Invasive cells were counted and averaged. The number of invasive cells was expressed as the median with interquartile range.

Colony formation assay

A total of 2,000 A549 cells were seeded in 6-well plates in DMEM/F12 medium with 10% FBS, maintaining it at 37°C in an atmosphere with 5% CO_2 for 24 hours. Next, HBSS was added and the vehicle (DMSO) or the inhibitors 0.01 µM of SC-57461A, 0.01 µM of ALA and 10 µM of ALA were added for 30 minutes at 37°C. Subsequently, solution was extracted, and 5 nM of PMA was added and incubated for 15 minutes at 37°C an atmosphere with 5% CO_2. Finally, the hank solution is replaced with DMEM/F12 culture medium. The culture medium was replaced every 2 days for 6 or 9 days. After the test time, the culture medium was removed and washed three times with 1X PBS at 4°C. Colonies were fixed with fixation buffer (0.2 M of PB, 1 M of saccharose, 4% of paraformaldehyde, and water) for 40 minutes at room temperature. Finally, the fixative was removed, and the colonies were washed with 1X PBS 3 times for 5 minutes while stirring. The colonies were stained with 0.1% w/v crystal violet solution, dissolved in water for 3 minutes, after staining dye was removed with repeated washes of 1X PBS. The images were obtained through the Leica EZ4 microscope. The quantification of the colonies was carried out by visual counting, and the median with interquartile range was graphed.

Statistical analysis

Graphs were generated using GraphPad prism 7.1 software (Jolla, California) Results were expressed as the median with interquartile range of at least three independent experiments. The statistical analyzes that will be performed are Mann Whitney or Kruskal-Wallis Test. Differences will be considered statistically significant when $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$.

Statistical methods such as Student's T-test and analysis of variance are widely used in medical research, and they require a normal distribution of the data. However, if the sample distribution is skewed or the distribution is unknown due to the small sample size, parametric analysis cannot be used. Therefore, nonparametric tests are an excellent alternative [30].

Results

Expression levels of 5-lipoxygenase pathway proteins and BLT1 receptor in A549 cells

In the present study, the expression of proteins of the 5-lipoxygenase pathway ($cPLA_2$, 5-LOX, FLAP, LTA_4H) and the BLT_1 receptor was evaluated in the A549 cell line through RT-qPCR and western blot. It was shown that the A549 cell line expresses at the mRNA (Figure 1A) and protein (Figure 1B and C) levels all the proteins necessary for the synthesis of LTB_4. Additionally, it expresses the BLT_1 receptor (LTB_4-like receptor), with high affinity for LTB_4 (Figure 1). These results suggested to us that the A549 cell line is the optimal model for our study.

Localization of LTA_4H and BLT1 in samples from patients with lung adenocarcinoma

In the present study, a retrospective qualitative analysis of the localization of LTA_4H and BLT1 in samples from patients with lung adenocarcinoma was performed. Figure 2A; Table 1, showed the presence of LTA_4H mainly in the cytoplasm of cancer cells and stromal cells in all samples. Additionally, intense positivity was observed in the nucleus and slight immunolabeling at the plasma membrane level. However, in sample 07F, the presence of immunolabel was observed on the plasma membrane.

On the other hand, BLT1 (Figure 2B; Table 1) is in the cytoplasm, slight presence in the nucleus in tumor cells, and high presence of the receptor in stromal cells. We highlight that sample 32E showed clear positivity in the plasma membrane.

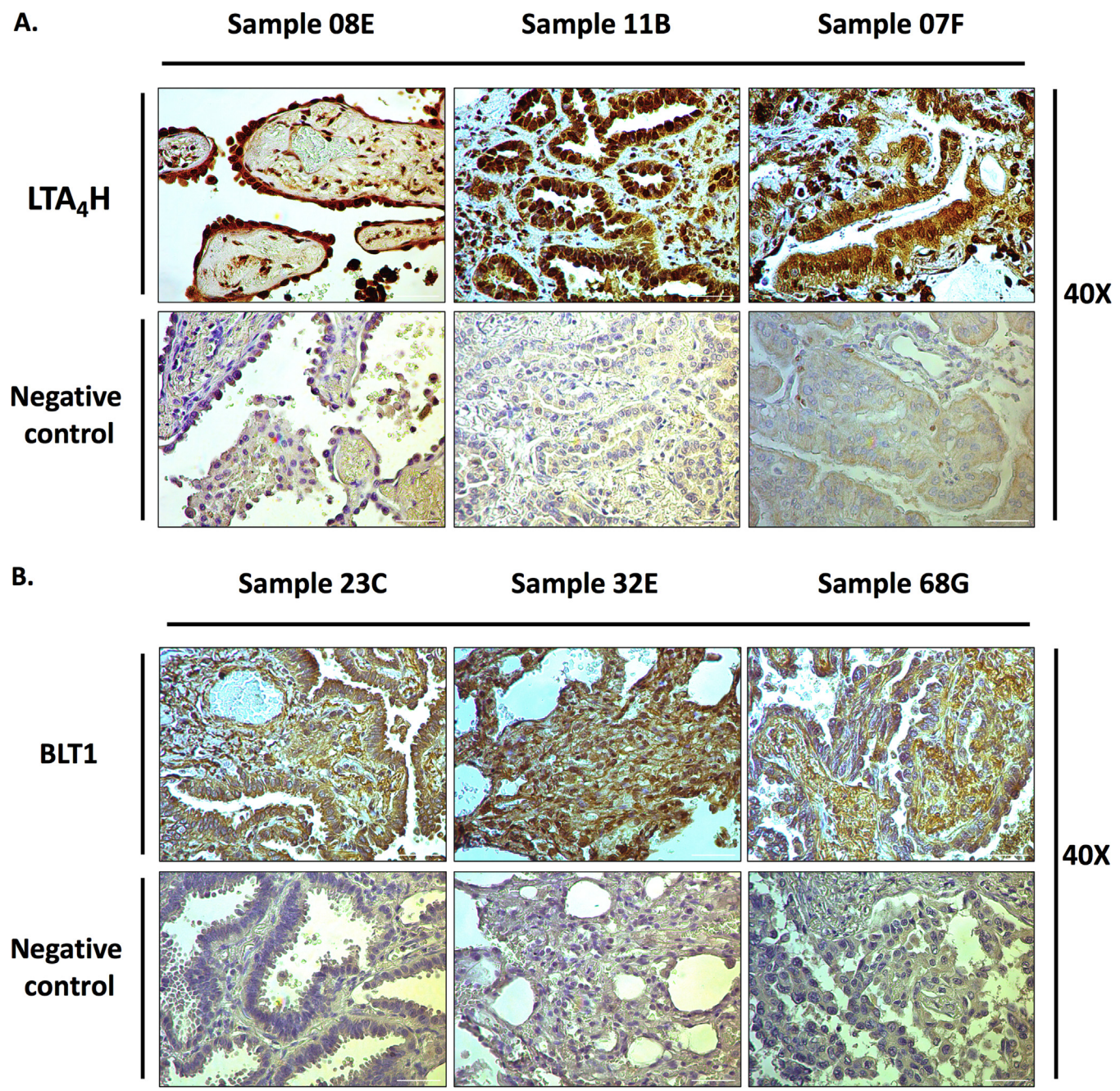

Figure 2. Localization of LTA_4H and BLT1 in samples from patients with lung adenocarcinoma. Images indicate the localization of LTA_4H and BLT1 in samples from patients with lung adenocarcinoma. (A) Immunohistochemistry analysis of LTA_4H. Scale bar: 50 µm. (B) Immunohistochemistry analysis of BLT1. Scale bar: 50 µm.

Effect of ALA on LTB_4 secretion in A549 cells

It was determined that A549 cells express proteins from the 5-LOX pathway and the BLT1 receptor. For this reason, the production of LTB_4 and the effect of ALA on the production of LTB_4 in the cell line were evaluated. The cells were stimulated with PMA, since studies have shown that in peritoneal macrophages increases the release of arachidonic acid and in polymorphonuclear leukocytes induces the release of LTB_4 [31].

Figure 3 shows that PMA stimulus tends to increase the production of LTB_4 ($P = 0.1024$) compared to the group control. On the other hand, 10 µM ALA reduces LTB_4 secretion in A549 cells (Kruskal-Wallis Test; $P = 0.0363$; Figure 3) compared to the group PMA. In addition, the cells were treated with 0.01 µM SC-57461A, a selective inhibitor of LTA_4H, the cells tend to reduce the production of LTB_4 (Kruskal-Wallis Test; $P = 0.1669$; Figure 3), compared to the group stimulated only with PMA. It showed no differences between the SC-57461A and 10 µM ALA groups.

Effect of LTB_4 on the expression of canonical markers of epithelial mesenchymal transition in A549 cells

IL-6, a pro-inflammatory cytokine, plays a crucial role in activating EMT [32–34]. In the same sense, previous studies have shown that LTB_4 induces morphological changes, decreased expression of E-cadherin and increased expression of N-cadherin and vimentin, in PANC-1 pancreatic cancer cell lines [19]. For this reason, the effect of IL-6 (positive control) and LTB_4 on the expression

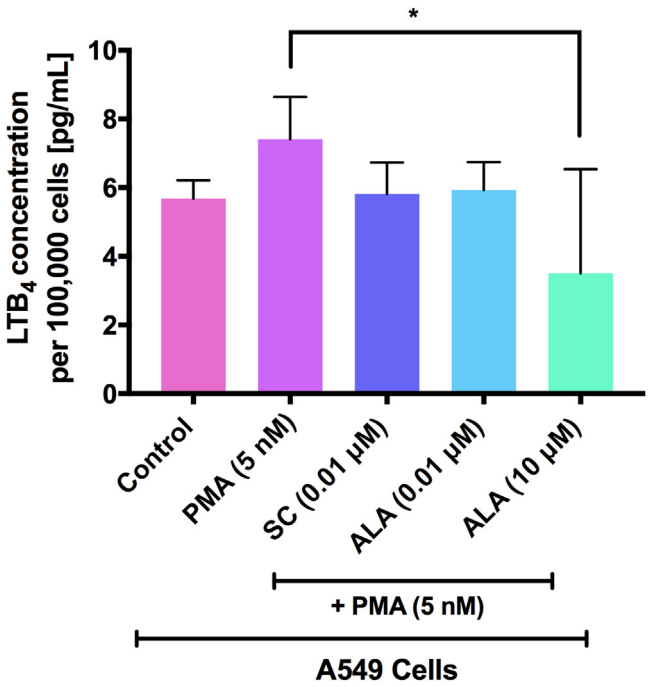

Figure 3. Effect of ALA on LTB_4 secretion in A549 non-small lung cancer cells. Cells were pre-incubated with SC (0.01 µM), ALA (0.01 µM), or ALA (10 µM) for 30 minutes, and then stimulated with PMA (5 nM) for 15 minutes. LTB_4 secretion was determined by ELISA Kit. Data are presented as the median with interquartile range (n = 3 independent experiments; $^*P \leq 0.05$; Kruskal-Wallis Test).

of canonical EMT markers was evaluated. Figures 4A and B show that IL-6 reduces the expression of E-cadherin (Kruskal-Wallis Test; $P = 0.0127$) and LTB_4 did not show significant changes compared to the control. However, at a concentration of 50 nM of LTB_4, a trend towards reduced expression levels of E-cadherin was observed (Kruskal-Wallis Test; $P = 0.2575$; Figures 4A and B) compared to the control. On the other hand, IL-6 and LTB_4 do not induce a change in vimentin expression (Kruskal-Wallis Test; $P > 0.05$; Figures 4A and C). Additionally, 50 nM of LTB_4 showed a tendency to increase the expression levels of the transcriptional factor *ZEB-1* (Kruskal-Wallis Test; $P = 0.0700$; Figures 4A and D) compared to the control and increases the expression of *ZEB-1* compared to the IL-6 group (Kruskal-Wallis Test; $P = 0.0415$; Figures 4A and D).

Effect of alpha lipoic acid-mediated LTB_4 decrease on the expression of canonical markers of epithelial mesenchymal transition in A549 cells

LTB_4 in A549 lung cancer cells was shown to induce EMT, modulating the expression of canonical EMT markers (**Figure 4**). On the other hand, we demonstrate that ALA reduced the production of LTB_4 (**Figure 3**). For this reason, we evaluated the effect of ALA on the expression of canonical EMT markers in A549 cells. The cells were preincubated with the respective inhibitors and stimulated with PMA. We demonstrated that preincubation with SC-57461A, a selective inhibitor of LTA_4H, increased the expression levels of E-cadherin compared to stimuli with LTB_4 (Kruskal-Wallis Test; $P = 0.0103$; Figures 5A and B), however, 10 µM of ALA showed a trend towards increased expression of the epithelial marker (Kruskal-Wallis Test; $P = 0.2633$; Figures 5A and B). On the other hand, ALA significantly decreases the expression levels of the transcriptional factor *ZEB-1* (Kruskal-Wallis Test; $P = 0.0253$; Figures 5A and D) compared to stimuli with LTB_4. No changes in Vimentin expression were observed with LTB_4 stimulus or with preincubation with 10 µM ALA.

Effect of LTB_4 on viability in A549 cells

LTB_4 plays a role in the survival of tumor cells of epithelial origin [8]. In the same sense, we evaluated the effect of LTB_4 on viability in A549 cells. The Figure 6A shows a tendency to increase viability as we expose the cells to 50 nM of LTB_4 (Kruskal-Wallis Test; $P = 0.1742$) compared to control. Although, at the concentration of 100 nM it did not show significance, a tendency to increase the viability of A549 cells was observed (Kruskal-Wallis Test; $P = 0.1410$; Figure 6A) compared to control.

Effect of alpha lipoic acid-mediated LTB_4 decrease on viability and proliferation

Studies have shown that LTB_4 plays a role in the proliferation and survival of tumor cells of epithelial origin, through the MAPK and PI3K signaling pathways [8].

In the same sense, we have previously shown that LTB_4 at a concentration of 100 nM tends to increase viability in A549 cells (Figure 6). For this reason, we evaluated the effect of ALA on viability and proliferation in A549 cells. The cells were preincubated with the respective inhibitors and stimulated with PMA. We demonstrate that preincubation with SC-57461A, a selective inhibitor of LTA_4H, significantly reduces the viability of A549 cells compared to the control (Kruskal-Wallis Test; $P = 0.0106$; Figures 6B). On the other hand, preincubation with 0.01 µM ALA showed a tendency to reduce cell viability compared to the control (Kruskal-Wallis Test; $P = 0.0828$; Figures 6B), however, at a concentration of 10 µM significantly reduces the viability of A549 cells compared to the control (Kruskal-Wallis Test; $P = 0.0225$; Figures 6B). Additionally, preincubation of 10 µM ALA significantly reduces the expression of proliferating cell nuclear antigen (PCNA) compared to the control and PMA, indicating a decrease in the proliferation of A549 cells with ALA exposure (Kruskal-Wallis Test; $P = 0.0019$; Figures 6C and D; control) and (Kruskal-Wallis Test; $P = 0.0198$; Figures 6C and D; PMA).

Effect of alpha lipoic acid-mediated LTB_4 decrease on invasive capacities, motility, and colony formation in A549 cells

EMT activation promotes functional abilities such as; proliferation, survival, invasion, migration, clonogenicity, and metastasis [18,35,36]. Our results showed that LTB_4 induces EMT (Figure 4) and that ALA by reducing LTB_4 levels attenuates EMT, reflected by a decrease in *ZEB-1* expression and a tendency to increase E-cadherin levels (Figure 5**A, B, and D**). Additionally, it reduces the proliferation of A549 cells, with a reduction in the expression levels of the PCNA marker (Figure 6**C and D**). For this reason, we evaluated the effects of ALA on certain functional capacities (migration, invasion, and clonogenicity) in A549 cells.

Migration assays were performed by transwell assay for 6 hours to then quantify the number of A549 cells that migrated. The results showed that PMA tends to increase cell mobility in relation to the control (Kruskal-Wallis Test; $P = 0.2211$; Figures 7A and B) and preincubation with 10 µM ALA tends to reduce cell mobility in comparison to the PMA group. (Kruskal-Wallis Test; $P = 0.2513$; Figures 7A and B).

Invasion assays were performed by transwell assay for 20 hours to then quantify the number of A549 cells that invaded. The results showed that PMA significantly increases cellular invasion compared to the control (Kruskal-Wallis Test; $P = 0.0022$; Figures 7C and D) and preincubation with 10 µM ALA in comparison to the PMA group tends to reduce it (Kruskal-Wallis Test; $P = 0.1687$; Figures 7C and D).

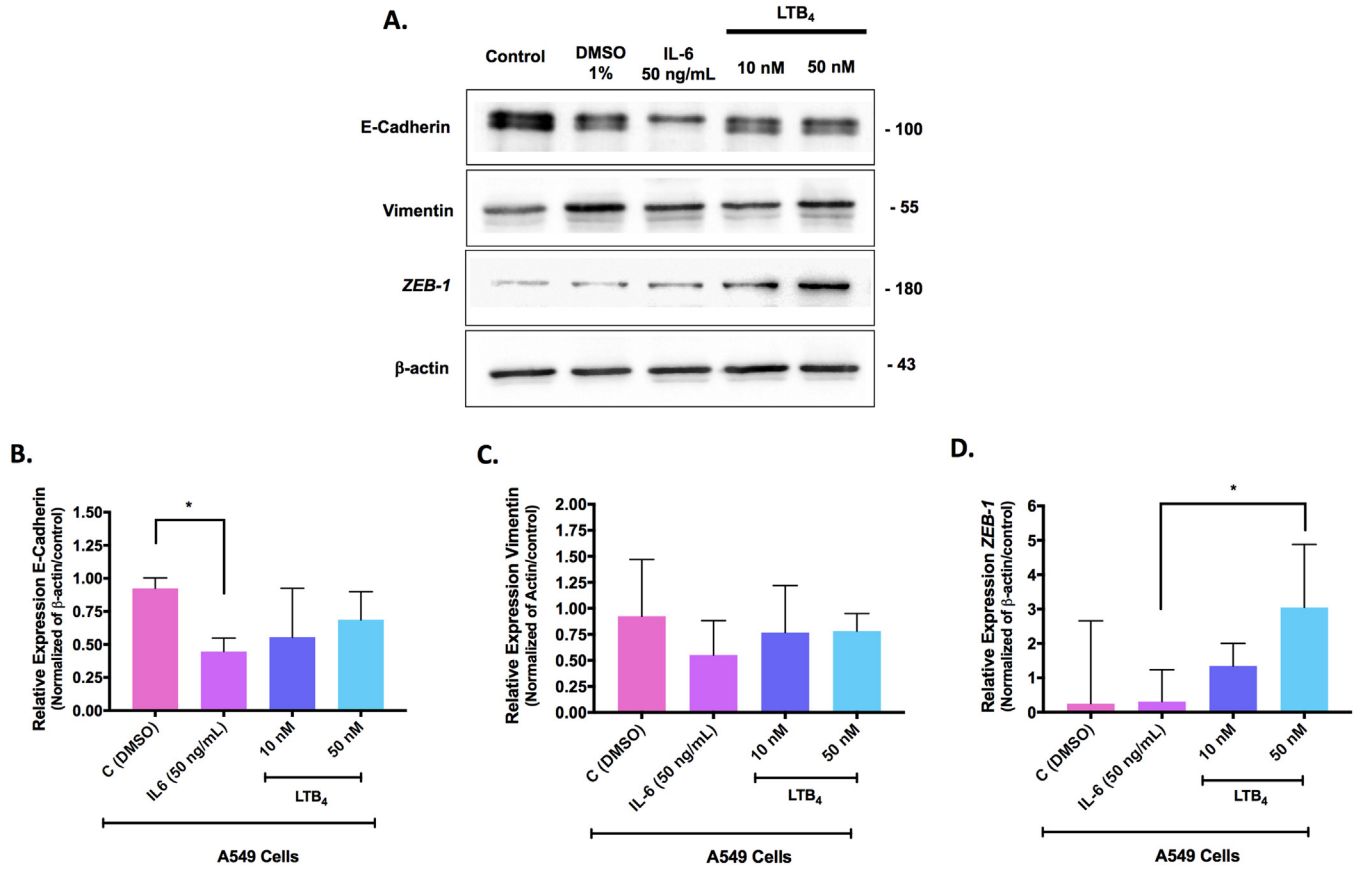

Figure 4. Effect of LTB_4 on the expression of canonical markers of epithelial mesenchymal transition in non-small cell lung cancer A549. (A) The cells were incubated with IL-6 or different concentrations of LTB_4 for 48 hours, subsequently the protein lysate was analyzed by immunoblot and the membranes were incubated with primary antibodies against E-cadherin, Vimentin, ZEB-1, and b-actin. (B) Semi-quantification of the relative protein expression of E-cadherin normalized with β-actin and the control. (C) Semi-quantification of the relative protein expression of Vimentin normalized with β-actin and the control. (D) Semi-quantification of the relative protein expression of ZEB-1 normalized with β-actin and the control. The values 100, 55, 180, and 43 correspond to mass units (kDa). Data are presented as the median with interquartile range (ZEB-1 (n = 3); Vimentin and E-cadherin (n = 4) independent experiments; $^{**}P \leq 0.01$; Kruskal-Wallis Test).

The clonogenicity assay was carried out in two stages. After 6 days, colony formation was observed in the controls (Control and control with vehicle (DMSO)) with no differences between the groups (Mann-Whitney Test; $P = 0.100$; Figures 7E and F). However, both the stimulation with PMA and the preincubation with the inhibitors formed colonies of reduced size (Figure 7E). The test was carried out for 9 days, as seen in Figure 7G, the controls (control and control with vehicle (DMSO)) grew a large number of colonies, which did not allow them to be quantified. On the contrary, preincubations with SC-57461A and 10 µM ALA reduced colony number compared to the PMA group (SC-57461A; Kruskal-Wallis Test; $P = 0.0141$; Figures 7G and H, 10 µM ALA; Kruskal-Wallis Test; P=0.0156; Figures 7G and H).

Discussion

The role of leukotrienes, specifically LTB_4, in cancer progression remains relatively underexplored compared to other lipid mediators like prostaglandins [8]. However, emerging evidence highlights their potential involvement in carcinogenesis [8,14,15]. High LTB_4 levels have been detected in various cancers, including prostate, colon, and pancreatic cancers, with increased expression of its receptors (BLT_1 and BLT_2) contributing to tumor progression and metastasis [8,16]. Our study aimed to investigate the 5-LOX/LTB_4 pathway and the BLT1 receptor in a lung cancer model, using A549 cells as a cellular model. We confirmed that A549 cells express the key proteins necessary for LTB_4 synthesis and the BLT1 receptor, providing strong evidence that this pathway could play an important role in lung cancer progression (Figure 1).

In our investigation of LTA_4H localization, we observed that this key enzyme for LTB_4 synthesis (LTA_4H) was primarily located in cytoplasm, with some nuclear presence, particularly in tumor and stromal cells (Figure 2A). These findings align with studies in alveolar macrophages and type II epithelial cells, where LTA_4H was found to localize in both the nucleus and cytoplasm, suggesting its involvement in diverse cellular processes in the tumor microenvironment [22,37,38]. On the other hand, BLT1 is a G protein-coupled receptor expressed on several cells that include; neutrophils, eosinophils, activated T lymphocytes, tumor cells, among others [39]. It is located in the plasma membrane and cytoplasm in Human Umbilical Vein Endothelial Cells (HUVEC) [40]. Our results showed that the BLT_1 receptor was localized in the cytoplasm and the plasma membrane, particularly in stromal cells (Figure 2B), indicating that tumor and stromal cells can potentially engage in autocrine and paracrine signaling via the LTB_4/BLT1 axis, which could contribute to lung cancer progression.

Given the central role of LTB_4 in tumor biology, several strategies have been explored to regulate its synthesis and receptor activation. Although specific drugs targeting LTB_4 synthesis or BLT1 have not yet reached clinical use, research on LTA_4H inhibitors has shown promise [12,20,21,41,42]. In our study, we demonstrated that alpha-lipoic acid (ALA), is a compound synthesized endogenously in the mitochondria, it participates as a cofactor of multienzyme complexes [23,25,43] and inhibitor of LTA_4H, decreased LTB_4

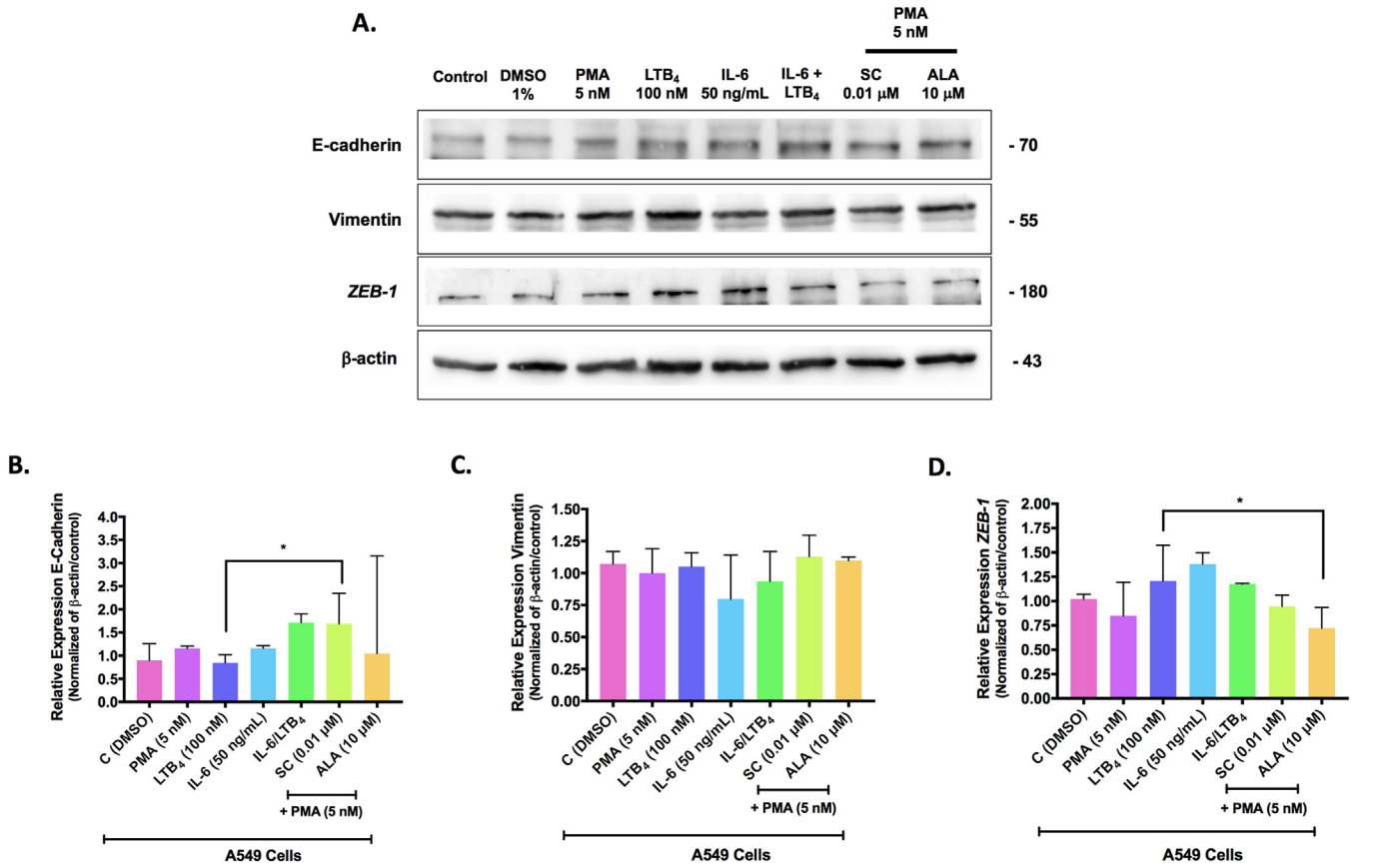

Figure 5. Effect of ALA-mediated LTB_4 decrease on the expression of canonical markers of epithelial mesenchymal transition in non-small cell lung cancer A549. (A) Cells were pre-incubated with SC (0.01 µM) or ALA (0.01 or 10 µM) for 30 minutes, and then stimulated with PMA (5 nM), for 15 minutes, LTB_4 (100 nM), 50 ng/mL IL-6 or IL-6/LTB_4 for 48 hours, subsequently the protein lysate was analyzed by immunoblot and the membranes were incubated with primary antibodies against E-cadherin, Vimentin, ZEB-1 and β-actin. (B) Semi-quantification of the relative protein expression of E-cadherin normalized with β-actin and the control. (C) Semi-quantification of the relative protein expression of Vimentin normalized with β-actin and the control. (D) Semi-quantification of the relative protein expression of ZEB-1 normalized with β-actin and the control. The values 70, 55, 180, and 43 correspond to mass units (kDa). Data are presented as the median with interquartile range (n = 3 independent experiments; $^*P \leq 0.05$; Kruskal-Wallis Test).

production in A549 cells (Figure 3). This finding is consistent with previous research showing that ALA inhibits LTA_4H activity [22]. Furthermore, it did not affect the aminopeptidase activity of thermolysin, a peptidase that has a structural similarity to the catalytic domain of LTA_4H [44], over a wide range of concentrations, suggesting that the inhibitory effect of ALA is selective to LTA_4H [22], making it a promising candidate for modulating LTB_4 levels in cancer. Additionally, ALA can be absorbed from the diet or from food supplements in intact form and stored in tissues [25]. For this reason, our objective was to study the effect of ALA on other inflammatory pathologies that include LC. In LC there is evidence in an orthotopic lung tumor model that elevated levels of LTB_4 are produced by tumor-associated neutrophils and macrophages [10,45]. In parallel, it has been shown that exhaled air condensate from lung cancer patients showed elevated concentrations of LTB_4 compared to healthy smoking/non-smoking controls [10,46,47].

The activation of epithelial-mesenchymal transition (EMT) is a hallmark of cancer progression, particularly in epithelial-derived cancers like lung cancer [18,35]. EMT enables cancer cells to acquire invasive and migratory properties, contributing to metastasis. IL-6, a pro-inflammatory cytokine, has been implicated in EMT activation in various cancers, including LC, through the activation of JAK/STAT3 signaling [32,34]. In our study, we confirmed that IL-6 induces a decrease in E-cadherin expression, a key epithelial marker, consistent with its role in EMT (Figure 4). Moreover, LTB_4 also showed a tendency to reduce E-cadherin expression, further supporting the idea that LTB_4 contributes to EMT activation in LC cells.

E-cadherin repression and the activation of mesenchymal markers, such as N-cadherin and vimentin, are regulated by transcription factors like ZEB-1, SNAIL, and TWIST [48]. Our results indicated that ALA treatment at 10 µM tended to increased E-cadherin expression and reduced ZEB-1 expression, suggesting that ALA might attenuate EMT in A549 cells by modulating the expression of these critical transcriptional regulators (Figure 5). This modulation of EMT markers indicates that ALA may help prevent the invasive and metastatic properties of LC cells, likely by inhibiting LTB_4-mediated signaling.

On the other hand, LTB_4 mediates the synthesis of IL-6 and IL-8, increasing the invasiveness of cancer cells [14], suggesting that this effect may be mediated directly by the activation of the LTB_4-mediated signaling pathway, or by the increase in IL-6 induced by LTB_4.

We also assessed the functional impact of ALA on lung cancer cell viability, proliferation, and invasion. LTB_4 has been shown to promote cell survival and proliferation in cancer through signaling pathways like ERK and Akt activation [8]. In our study, we observed that LTB_4 had a tendency to increase cell viability (Figure 6A), and that ALA preincubation significantly reduced both cell viability and proliferation (Figures 6B, C, and D). This suggests that

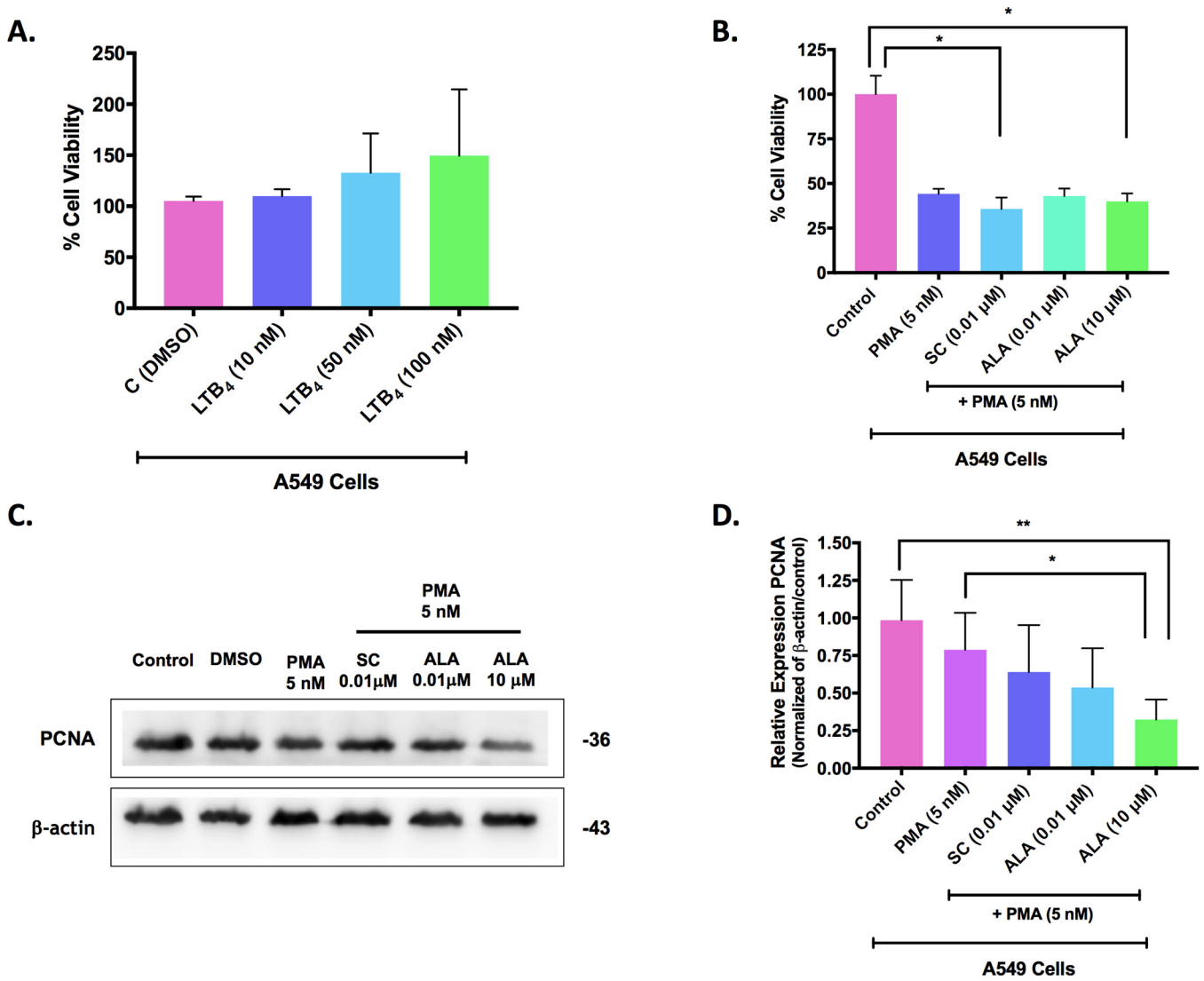

Figure 6. Effect of LTB_4 and ALA-mediated LTB_4 decrease on the viability and proliferation in non-small cell lung cancer A549. (**A**) The cells were stimulated with different concentrations of LTB_4 for 12 hours, subsequently the percentage of viable cells was evaluated through MTT. Cells were pre-incubated with SC (0.01 μM) or ALA (0.01 or 10 μM) for 30 minutes, and then stimulated with PMA (5 nM) for 15 minutes. (B) Percentage of viable cells by MTT. (C) Protein lysate was analyzed by immunoblot and the membranes were incubated with primary antibodies against PCNA and β-actin. (D) Semi-quantification of the relative protein expression of PCNA normalized with β-actin and the control. The values 36 and 43 correspond to mass units (kDa). Data are presented as the median with interquartile range (viability (n = 3); proliferation (n = 4) independent experiments; $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$; Kruskal-Wallis Test).

ALA might be effective in reducing cancer cell growth and viability, potentially making it a therapeutic adjunct in lung cancer treatment. Other studies have shown that ALA at concentrations in the order of mM activates the AMPK-p53 pathway in hepatocellular carcinoma cells, reducing invasion and migration [49]. In addition, in breast cancer cells from metastasis, ALA inhibits invasion and migration through the inhibition of the ERK1/2 and Akt pathway [50]. Regarding the invasiveness and migratory potential of A549 cells, our results showed that preincubation with ALA reduced cell invasion (Figures 7C and D) and migration (Figures 7A and B), although the effect was not highly significant. This suggests that at concentrations of 10 μM, ALA might reduce the aggressive behavior of A549 cells by attenuating EMT markers like ZEB-1, which has been linked to enhanced migration and invasion

Besides, EMT inducers can promote the expression of cancer stem cell (CSC) markers [48]. CSCs are defined by their ability to self-renew and asymmetric division which are responsible for cancer recurrence and metastasis [48,51]. On the other hand, Gisella Pérez, et al demonstrated that silencing ZEB-1 reduces the ability of cells to form colonies [51].

ALA's ability to decrease ZEB-1 expression is further supported by our colony formation assay, which showed that ALA reduced colony formation (Figures 7G and H), a hallmark of reduced stemness and clonogenic potential.

Our results show that ALA reduces LTB_4 levels, generating the changes described in the cellular model at micromolar concentrations. These results are favorable since pharmacokinetic studies report that ALA reaches plasma concentrations of 50 μM at therapeutic doses. On the other hand, it is an original experimental investigation since the reduction of LTB_4 synthesis mediated by ALA is a new mechanism of action not associated with its antioxidant capacity.

However, the study presents certain limitations such as the reduced number of samples and stimuli with PMA in cellular models that express enzymes such as leukotriene C_4 synthase that can induce the synthesis of cysteinyl leukotrienes (LTC_4). Additionally,

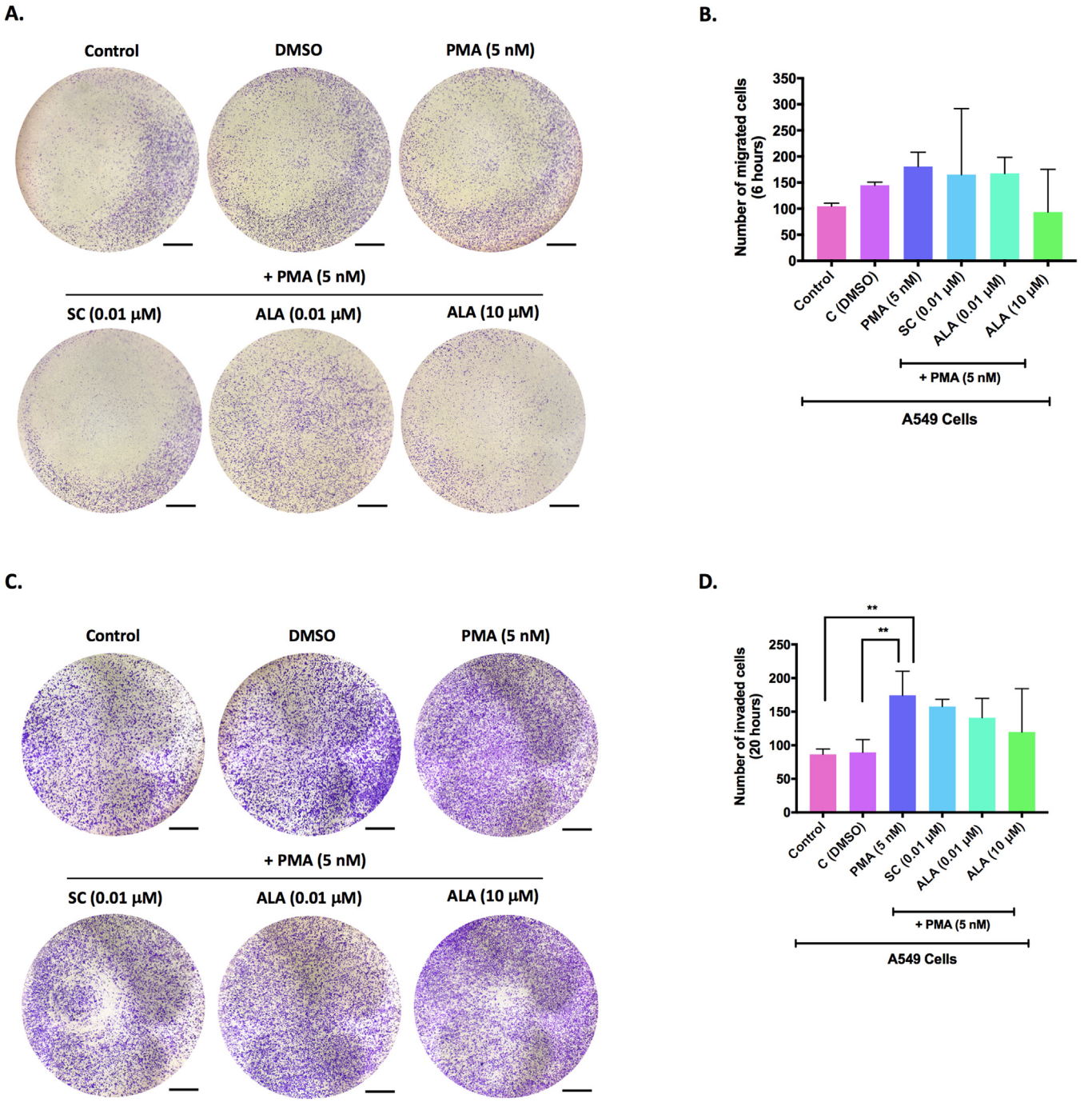

Figure 7. Effect of ALA-mediated LTB_4 decrease on invasive capacities, motility, and colony formation in non-small cell lung cancer A549. Cells were pre-incubated with SC (0.01 µM) or ALA (0.01 or 10 µM) for 30 minutes, and then stimulated with PMA (5 nM) for 15 minutes. (A) Illustration of the migration transwell test per 6 hours, Scale bar; 1 mm. (B) Number of cells migrated per 6 hours. (C) Illustration of the invasion transwell test per 20 hours, Scale bar; 1 mm. (D) Number of cells invaded per 20 hours. (E) Illustration of the colony formation assay after 6 days, Scale bar; 1 cm and 1 mm (zoom). (F) Quantification of the number of colonies after 6 days. (G) Illustration of the colony formation test at 9 days, Scale bar; 1 cm and 1 mm (zoom). (H) Quantification of the number of colonies after 9 days. Data are presented as the median with interquartile range (migration and invasion n = 3 independent experiments; $^{*}P \leq 0.05$; Kruskal-Wallis Test). colony formation (6 days; n = 3 independent experiments; $^{*}P \leq 0.05$; Mann-Whitney and 9 days; n = 4 independent experiments; $^{*}P \leq 0.05$; Mann-Whitney).

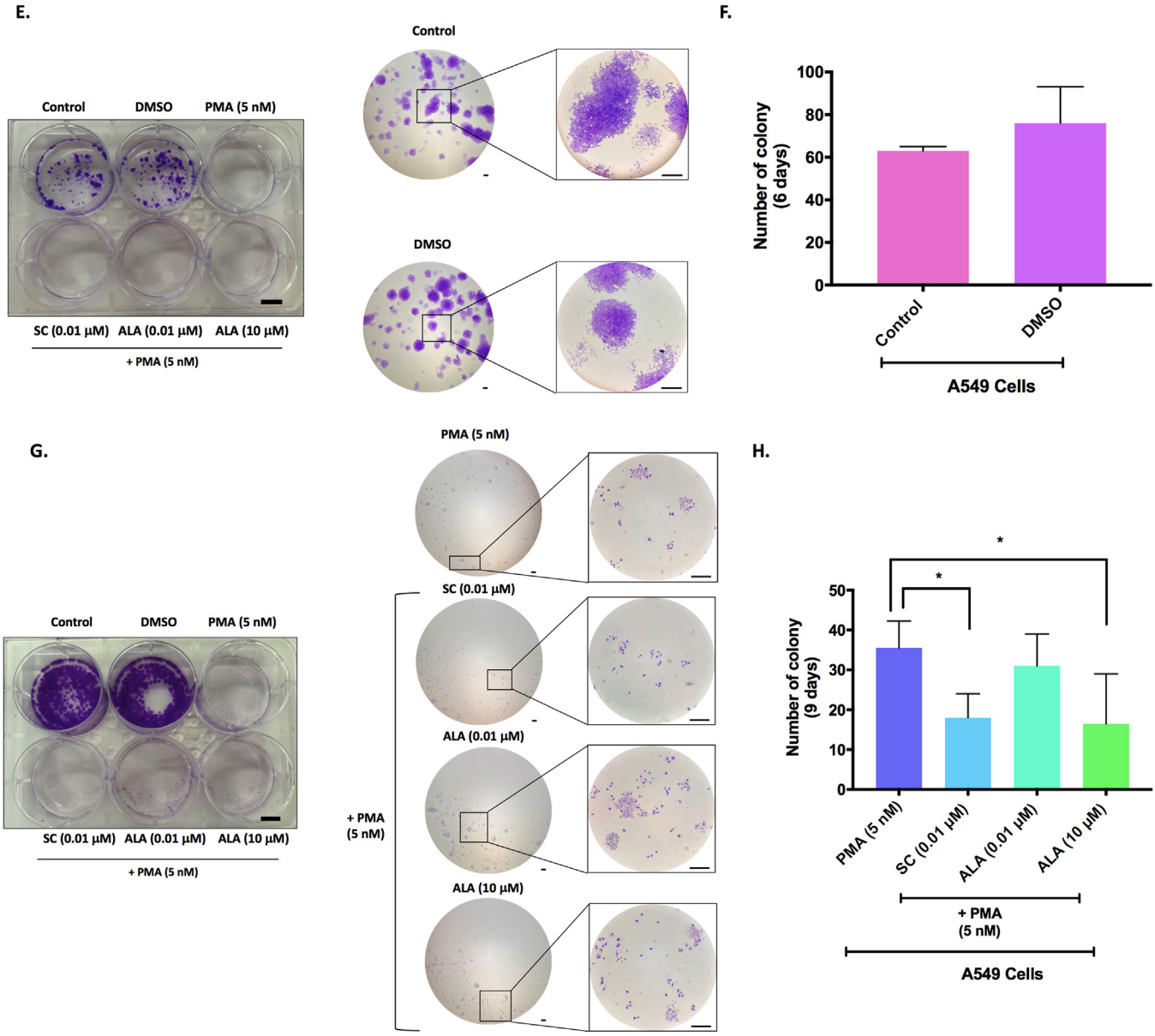

Figure 7. Continued

we must replicate our studies in an *in vivo model* that allows us to support future clinical trials.

Conclusions

In summary, our findings provide compelling evidence that ALA modulates the LTB_4/BLT_1 signaling axis, leading to decreased EMT, reduced cell viability, proliferation, invasion, and clonogenicity in A549 lung cancer cells. These results suggest that ALA could serve as an adjunctive therapeutic strategy in lung cancer by targeting key molecular pathways involved in tumor progression and metastasis. Given that ALA can be administered orally and reaches effective plasma concentrations, its use in clinical settings could complement conventional cancer therapies and help mitigate disease progression. Further studies, particularly in vivo models, are warranted to validate the therapeutic potential of ALA in lung cancer treatment.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

CRediT authorship contribution statement

María José Torres: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition. **Juan Carlos Ríos:** Methodology, Writing – review & editing. **Alexandra Valle:** Investigation. **Sebastián Indo:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Kevin Brockway GV:** Investigation. **Fernanda López-Moncada:** Visualization. **Mario Faúndez:** Methodology, Resources. **Enrique A. Castellón:** Resources, Writing – review & editing. **Héctor R. Contreras:** Resources, Writing – review & editing.

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Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. María José Torres confirms the authenticity of all raw data shown. All authors appearing in the manuscript read and approved the final writing.

Ethics Approval and Consent to Participate

This project has the approval of the following committees: Institutional Scientific Ethics Committee for Research Safety and Institutional Scientific Ethics Committee for UC Health Sciences (Exempt Resolution 012321), ID:201007026 of the Facultad de Medicina, Pontificia Universidad Católica de Chile.

Authorization for the collection of samples from patients with lung adenocarcinoma by the Departamento de Anatomía Patológica, Núcleo de Histología de Investigación de la Pontificia Universidad Católica de Chile, Santiago, Chile.

Patient Consent for Publication

No applicable.

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