Abstract

Lysophosphatidic Acid Promotes Hepatocellular Carcinoma Cell Invasion through EGFR, Cytokeratin 19 Expression and NF-κB Activation

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Background: Lysophosphatidic acid (LPA) is a lipid mediator that has diverse biological activities associated with cell proliferation, migration and survival. LPA has been implicated in the etiology of cancer due to its involvement in tumor growth, invasion and metastasis. The aim of this study was to investigate if LPA promotes invasion of hepatocellular carcinoma (HCC) cells and to elucidate which signaling pathway is modulated by LPA under hypoxic condition.

Methods and Results: We performed *in vitro* experiments using human HCC cell line (SNU761). Invasion of cells was assessed using invasion assay kit and the phenotypic changes after LPA treatment were examined using immunoblot analysis. The cells were treated with AG 1478 and guggulsterone, which are inhibitors of epidermal growth factor receptor (EGFR) and nuclear factor kappa B (NF-κB) activation, respectively. Treatment of LPA significantly augmented CK 19

expression leading to acceleration of invasion of HCC cells under hypoxic condition (1% O_2, 5% CO_2, 94% N_2). Knockdown of CK 19 by siRNA transfection under hypoxia attenuated invasion of HCC cells stimulated by LPA. AG 1478 attenuated invasion of HCC cells and CK 19 expression augmented by LPA. AG 1478 and guggulsterone showed additive effect on attenuation of invasion of HCC cells stimulated by LPA.

Conclusions: Collectively, these results suggest that LPA promotes invasiveness of HCC cells by augmenting EGFR transactivation, CK 19 expression and NF-κB activation under hypoxic condition. Therefore, selective blockade of LPA receptor may have therapeutic potential in CK 19-positive HCCs, particularly in the tumors exposed to hypoxic environments.

Keywords: lysophosphatidic acid; EGFR; CK19; hepatocellular carcinoma; invasion

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers, and was the third most common cause of death worldwide in 2008 (1). HCCs show vascular invasion in earlier stages, leading to intrahepatic and extrahepatic spreads of cancer cells. Therefore, it is critical to investigate the mechanisms of invasion and metastasis of HCCs to develop effective treatments of HCCs. Lysophosphatic acid (LPA) is a simple phospholipid activated by autotaxin (ATX), which was determined to promote invasion and metastasis in many cancers (2, 3). So signaling pathways modulated by LPA can be a novel therapeutic target to prevent invasion and metastasis in cancer therapy. Several studies demonstrated that LPA promotes invasion and metastasis of HCC cells (4, 5). LPA signaling pathway starts by binding to G protein-coupled receptors (GPCRs) on the surface of cells (6).

Cytokeratin 19 (CK19) is generally known as a biomarker for epithelial cells originated in biliary tracts. CK19-positive HCCs showed poor clinical outcomes (7) including high recurrence rates (8), and high expression of CK19 was associated with augmentation of tumor angiogenesis (9). But the underlying mechanisms are still poorly understood, and there were no previous studies on relationship between CK19 expression and invasion of HCC cells promoted by LPA. Epidermal growth factor receptor (EGFR) pathway can be activated by LPA and GPCRs (10), and previous studies showed that CK19 expression was augmented through EGFR in human HCC cells (11).

Nuclear factor kappa B (NF-κB) is a well-known transcription factor which has critical roles in inflammation, cell proliferation, and survival. While it was demonstrated that NF-κB was activated by LPA through GPCR pathway in a previous study (12), the effect of LPA on NF-κB activation has not been studied in HCC. Therefore, we aimed to investigate whether LPA promotes invasion of HCC cells and to examine the molecular mechanisms involved.

Materials and Methods

Cell cultures

Human HCC cell line used in our study was SNU761, which was derived from a Edmondson Steiner's classification grade III/IV HCC (13). Cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum, 100,000 U/l penicillin and 100 mg/l streptomycin. In all experiments performed in this study, cells were serum-starved overnight to avoid the effects of serum-induced signaling. According to the experimental design, the cells were subsequently incubated under standard culture conditions (20% O_2 and 5% CO_2, at 37 °C) or hypoxic culture conditions (1% O_2, 5% CO_2 and 94% N_2, at 37 °C).

Materials and reagents

LPA were obtained from Avanti Polar Lipids (Alabaster, AL, USA). The EGFR kinase inhibitor, AG1478, was purchased from Calbiochem (La Jolla, CA, USA). Z-guggulsterone was obtained from Sigma–Aldrich (St. Luis, MO, USA), and CK19 small interfering RNA (siRNA), ANXA1 siRNA, and VMP1 siRNA were obtained from Santa Cruz biotechnology (Santa Cruz, CA, USA). The following primary antbodies were used: CK19 and Vimentin from BioGenex (Rocklin, CA, USA),

MMP9 and MMP2 from Cell Signaling Technology (Beverly, MA, USA) and E-cadherin and N-cadherin from Invitrogen (Carlsbad, CA, USA).

Cell proliferation

Cell proliferation was measured using CellTiter 96 Aqueous One Solution cell proliferation assays (Promega, Madison, WI, USA). This test is based on the cellular conversion of the colorimetric reagent MTS [3,4-(5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazoliumsalt] into soluble formazan by dehydrogenase enzymes, which are only found in metabolically active, proliferating cells. Following each treatment, 20 μL of dye solution was added to each well in a 96-well plate and incubated for 2 h. Subsequently, absorbance was recorded at 490 nm using an ELISA plate reader (Molecular Devices, Sunnyvale, CA, USA).

Immunoblot analysis

The cells were lysed for 20 min on ice with lysis buffer [50 mM tris–HCl (pH 7.4); 1 % Nonidet P-40; 0.25 % sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM phenyl-methylsulfonyl fluoride; 1 μg/mL aprotinin, leupeptin, and pepstatin; 1 mM Na3VO4; and 1 mM NaF] and centrifuged for 10 min at 14,000g at 4 °C. The samples were resolved via sodium dodecyl sulfate polyacrylamide gel

electrophoresis, transferred to nitrocellulose membranes, and blotted with the appropriate primary antibodies. The blots were then incubated with peroxidase-conjugated secondary antibodies (Biosource International, Camarillo, CA, USA). The bound antibodies were visualized using a chemiluminescent substrate (ECL; Amersham, Arlington Heights, IL, USA) and exposed to Kodak X-OMAT film. The images were detected using an image analyzer (LAS-1000; Fuji Photo Film, Tokyo, Japan).

Transfection of siRNA

The cells were seeded in a 6-well culture plate (2×10^5 cells/well) in 2-mL antibiotic-free medium supplemented with 10 % FBS. At 60–80 % confluence, the cells were transfected with siRNA using the siRNA Transfection Reagent (Santa Cruz Biotechnology, Santa Cruz, CA, USA) according to the manufacturer's instructions. The cells were treated with siRNA for 6 h at 37 °C, and growth medium containing 20 % FBS and antibiotics was subsequently added. After 18 h, the medium was aspirated and replaced with fresh medium containing 10 % FBS and antibiotics. Twenty-four hours after the transfection, the cells were treated for further experiments.

Invasion assay

In vitro invasion assay was performed using 24-well chambers. Inserts were transferred into wells and coated with Matrigel (BD Biosciences, Billerica, MA, USA) for 30 min at 37 °C. SNU761 cells were suspended in serum-free medium and seeded on the Matrigel-coated upper chambers (5×10^4 cells/chamber), and DMEM containing 10 % FBS was added to the lower chambers. The cells were incubated for 6 h at 37 °C and then either pretreated with AG 1478, gugglsterone or left untreated. After 1 h of pretreatment, the cells were incubated in the presence or absence of LPA (10 μM) for 24 h and then stained with 4 μg/mL calcein AM (BD biosciences, Billerica, MA, USA) in Hank's Balanced Salt Solution (HBSS) at 37 °C for 90 min. Invasion ability was determined as relative fluorescence units measured at an excitation of 494 nm and an emission of 517 nm using a multifunctional plate reader (EnVision Multilabel Reader; PerkinElmer, Waltham, MA, USA).

NF-κB activity assay

Cells were plated at 5×10^4 cells/well in 1 mL of media in a six-well plate and treated with or without guggulsterone. Nuclear fractions were extracted, and then NF-κB concentrations (p50/p65) were determined by TransFactor NF-κB p50/p65 chemiluminescent kits (Clontech, Mountain View, CA, USA).

Immunofluorescent microscopy

Cells were plated at 1×10^6 cells in 5 mL of media in a 100-mm culture dish and treated with or without guggulsterone for 18 h. After 18 h of incubation, cells were treated with LPA for 1 h and then stained with rabbit anti-NF-κB p65 (Santa Cruz Biotechnology) and incubated with CyTM3-conjugated AffiniPure goat anti-rabbit Immunoglobulin G (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). The immunostained cells were observed under a fluorescence microscope (Zeiss). NF-κB translocation was determined by counting nuclear NF-κB (p65)-stained cells out of the total cells counted.

Real-time PCR analysis

Total RNA was extracted by AxyPrep Multisource Total RNA Miniprep Kit according to the manufacturer's recommendations (Axygen Biosciences, Union City, CA, USA). cDNA templates were synthesized using oligo-dT random primers and Moloney Murine Leukemia Virus reverse transcriptase. The cDNA templates were used to determine gene expression levels by real-time PCR (Thermal Cycler Dice™ Real Time System; Takara Bio, Shiga, Japan) using SYBR green (Takara Bio).

Microarray

Gene expression profile was obtained using Illumina HumanHT-12 v4 Expression BeadChip (Illumina, Inc., San Diego, CA). Biotinylated cRNA were prepared from 0.55 ug total RNA using the Illumina TotalPrep RNA Amplification Kit (Ambion, Austin,TX) and following fragmentation, 0.75 ug of cRNA were hybridized.

Statistical analysis

All data are presented as mean ± standard deviation (SD) of at least three independent experiments, unless otherwise indicated. Statistical evaluations of numeric variables in each group were conducted using the Mann–Whitney *U* test. All statistical analyses were performed using PASW 21.0 for Windows (IBM, Chicago, IL, USA). Differences with a *P* value less than 0.05 were considered statistically significant.

Results

LPA induced cellular invasion without increasing proliferation

First, it was evaluated whether LPA promotes invasion of HCC cells. LPA enhanced cellular invasion under both normoxic condition (Fig. 1a, $P = 0.01$) and hypoxic condition ($P = 0.01$). We measured percent change increase of cell invasion in LPA treated cells and compared it with that in LPA untreated cells, enhancement of cell invasion was significantly higher under hypoxic condition compared to normoxic condition ($P = 0.04$), which suggests that LPA induces cellular invasion, especially under hypoxic condition. If LPA increases cell proliferation, false positive results of cell invasion assays can be obtained, leading to misinterpretation. Therefore, we performed MTS proliferation assay after LPA treatment. LPA was determined not to enhance cell proliferation under both normoxic condition and hypoxic condition at all of the tested conditions (Fig. 1b).

CK19 expression was augmented by LPA treatment, and CK19 siRNA counterbalanced invasion promoted by LPA at hypoxic conditions.

Next, signaling pathways, which are known to affect cell proliferation were investigated. LPA augmented CK19 expression under both normoxic condition and hypoxic condition, and the effect of LPA was more prominent under hypoxic

condition (Fig. 2a). We also demonstrated that LPA did not increase cellular invasion when CK19 expression was blocked by siRNA transfection (Fig. 2b).

EGFR inhibition and NF-κB inhibition reduced LPA induced cell invasion at hypoxic conditions

We hypothesized that EGFR may mediate LPA induced cellular invasion as upstream signal of CK19, since EGFR is known to affect CK19 expression and HCC cell growth (11). To verify this hypothesis we first measured invasion of HCC cells by percent change of fluorescence ratio of HCC cells treated with AG1478, an EGFR inhibitor, compared to that of control under hypoxic condition (Fig. 3a). After inhibition of EGFR, cellular invasion decreased although it was not statistically significant (Fig. 3a, $P = 0.16$). We also investigated if inhibition of EGFR affects CK19 expression under hypoxic condition. After inhibition of EGFR, CK19 expression, which was augmented by LPA treatment, decreased markedly (Fig. 3b).

LPA was shown to mediate translocation of NF-κB to the nucleus in previous studies (12, 14). Therefore, we hypothesized that EGFR signaling pathway and NF-κB signaling pathway may have interaction in cell invasion stimulated by LPA. We measured invasion of HCC cells treated with AG1478 and guggulsterone, which was determined to inhibit NF-κB activation (15). Invasion of HCC cells decreased by inhibition of NF-κB activation, however, it was not statistically significant

compared to the control (Fig. 4a, $P = 0.25$). We found that inhibition of both EGFR and NF-κB activation yielded additive effect on reduction of cellular invasion (Fig. 4b). Further experiments were performed to determine interaction between EGFR signal and NF-κB signal, and we examined NF-κB nuclear translocation by immunofluorescent microscropy. The percent of nuclei stained with NF-κB (p65) after LPA treatment decreased significantly when HCC cells were treated with AG1478 under hypoxic condition (Fig. 4c, $P = 0.05$), whereas HCC cells without LPA treatment did not show statistically significant change after AG1478 treatment (Fig. 4c, $P = 0.51$).

ANXA1 and VMP1 may have roles in LPA induced cellular invasion.

Next we searched whether other mediators which might affect LPA-mediated cellular invasion. Among mediators increased by LPA ANXA1 plays a role in tumor metastasis in HCC (16), so we performed invasion assays with ANXA1 siRNA, under both normoxic and hypoxic conditions. Under both conditions, ANXA1 counterbalanced LPA induced cellular invasion (Fig. 5). We also planned further studies on VMP1 since several studies showed that down-regulation of VMP1 increases invasion in HCC (17, 18). First we quantified VMP1 mRNA expression with real-time PCR after LPA treatment. Under normoxic condition, LPA induced marginally significant down-regulation of VMP mRNA (Fig. 6a, $P = 0.08$). However, The expression of VMP1 mRNA was not changed after LPA

treatment under hypoxic condition (Fig. 6a, $P = 0.44$). After knockdown of VMP1 signal with siRNA transfection, cellular invasion augmented by LPA was counterbalanced under both normoxic and hypoxic conditions (Fig. 6b).

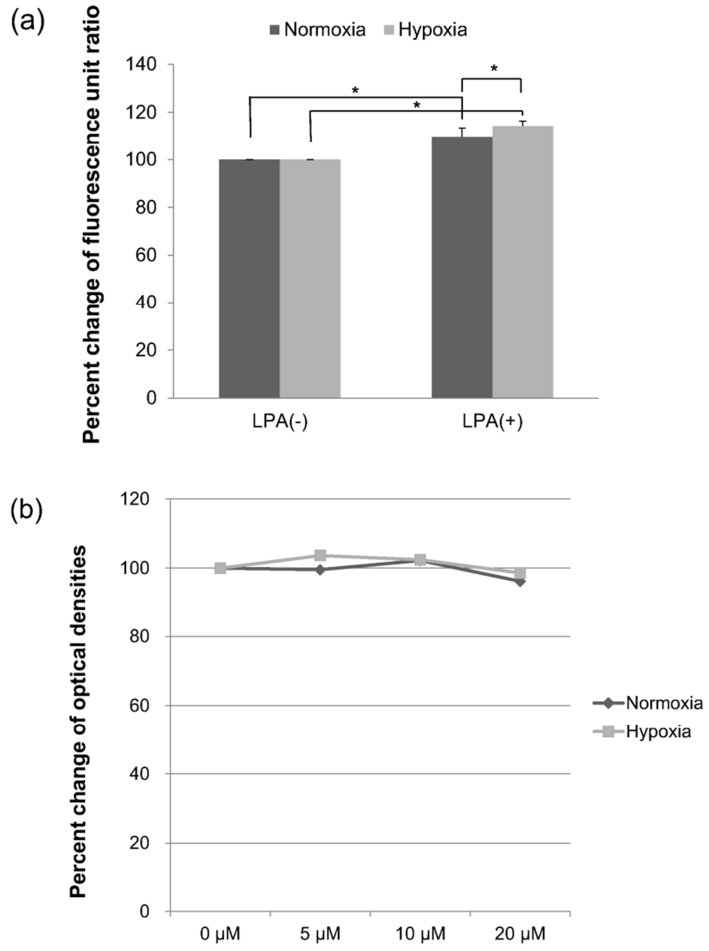

Figure 1. LPA enhanced cellular invasion of human HCC cells. (a) The invasion ability of SNU761 cells was measured by invasion assays. SNU761 cells were seeded on Matrigel-coated upper chambers (5×10^4 cells/chamber), and DMEM containing 10 % FBS was added to the lower chambers of a 24-well plate. Cells were cultured under normoxic conditions or hypoxic conditions. After incubation for 6 h, the cells were incubated in the presence or absence of LPA (10 μM) for 24 h. Invasion ability was determined as relative fluorescence units measured with a

fluorescence plate reader using BD Calcein AM Fluorescent dye, and the results are expressed as the percent changes of fluorescence units compared with those of the control. (b) SNU761 cells were cultured with the indicated concentrations of LPA. 3,4-(5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl) -2H-tetrazolium salt assay was done after 24 h, and data are expressed as the means of the percent change of optical densities as compared with the control (set at 100%).

* $P \leq 0.05$ versus the control; columns represent means; bars represent SDs.

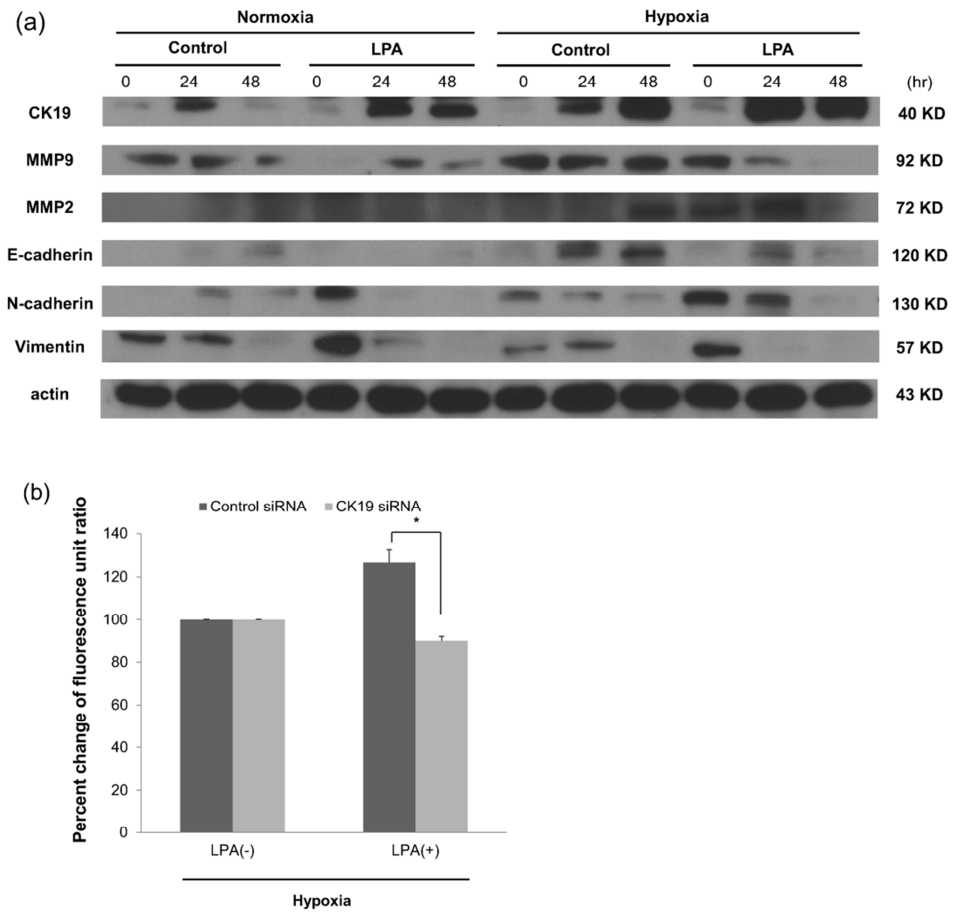

Figure 2. CK19 augmented by LPA treatment, and CK19 siRNA counterbalanced invasion. (a) SNU761 cells were cultured with or without LPA (10 μM) under standard normoxic conditions or hypoxic conditions. The cells were harvested after treatment at the time points indicated, and immunoblot analyses were performed for CK19, MMP9, MMP2, E-cadherin, N-cadherin, and vimentin. Immunoblot analysis for actin was performed as a control for protein loading. (b) SNU761 cells were treated with LPA (10 μM) following transfection with CK19

siRNA or control in hypoxic conditions. Invasion assay was performed, and invasion ability was determined as relative fluorescence units measured with a fluorescence plate reader using BD Calcein AM Fluorescent dye, and the results are expressed as the percent changes of fluorescence units compared with those of the control.

* $P \leq 0.05$ versus the control; columns represent means; bars represent SDs.

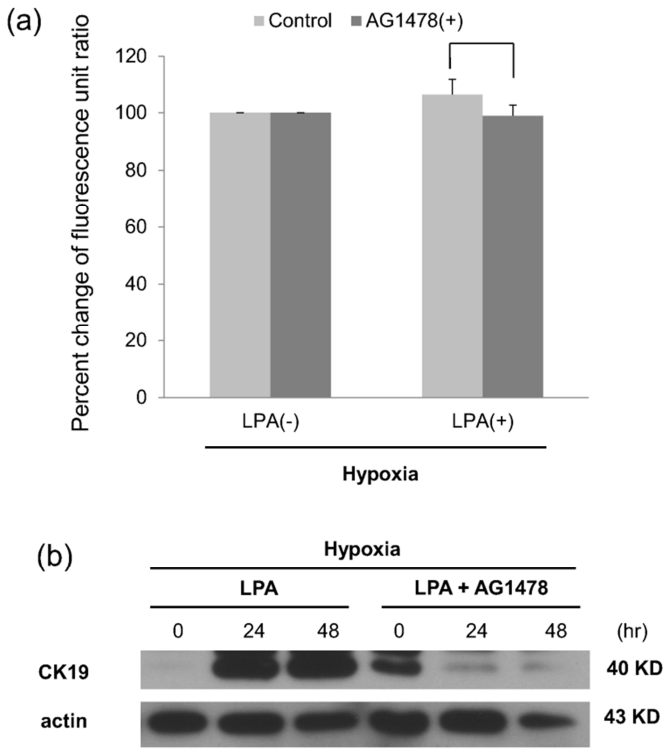

Figure 3. EGFR inhibition reduced LPA induced cell invasion at hypoxic conditions. (a) SNU761 cells were treated with or without LPA (10 μM) and with or without EGFR inhibitor, AG 1478 (10 μM) for 24h in hypoxic conditions. Invasion assay was performed, and invasion ability was determined as relative fluorescence units measured with a fluorescence plate reader using BD Calcein AM Fluorescent dye, and the results are expressed as the percent changes of fluorescence units compared with those of the control. (b) SNU761 cells were treated with or without LPA (10 μM) and with or without AG 1478 (10 μM) for 24h

in hypoxic conditions. The cells were harvested after treatment at the time points indicated, and immunoblot analyses were performed for CK19. AG 1478 reduced CK19 expression induced by LPA.

Columns represent means; bars represent SDs.

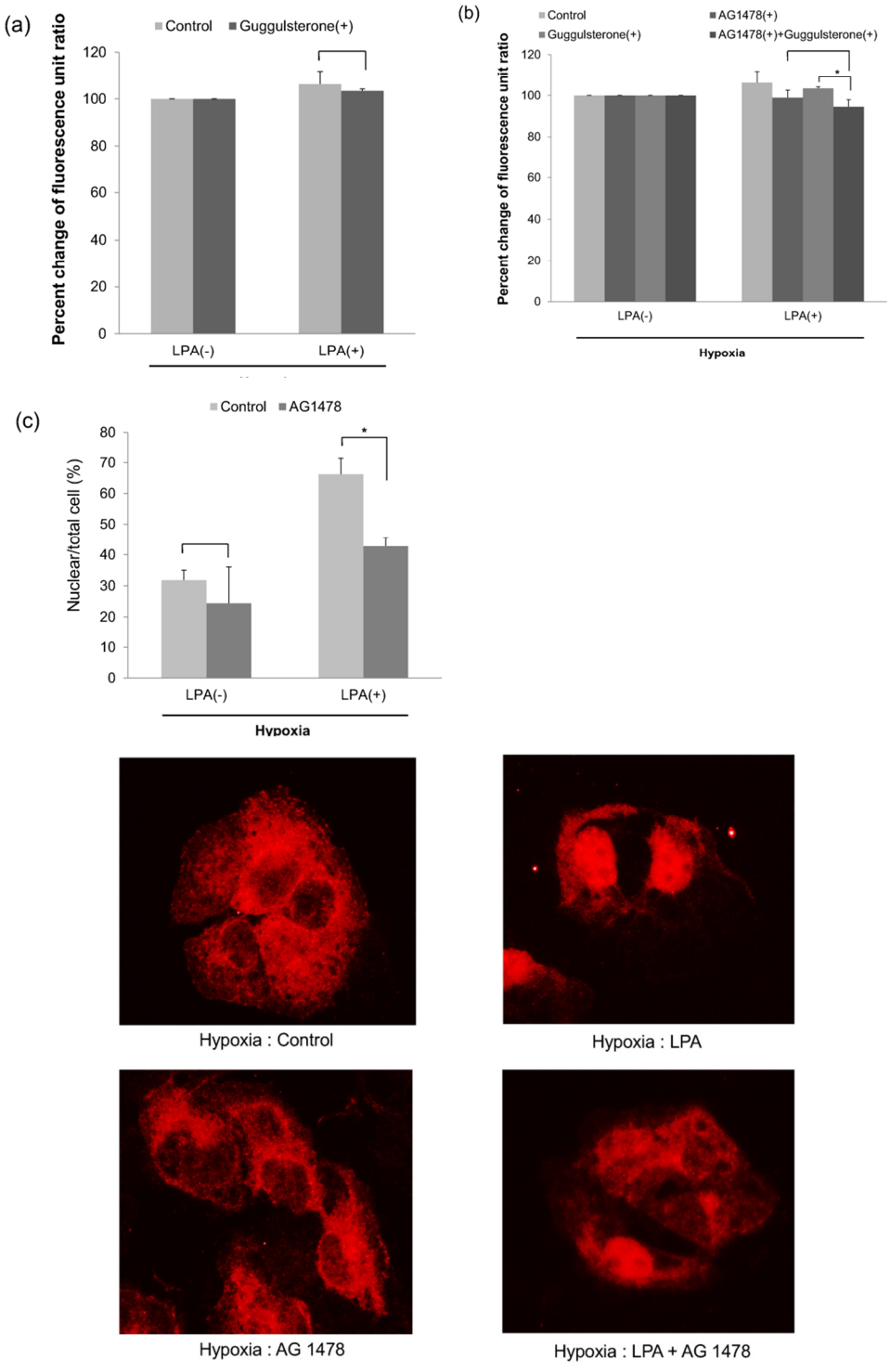
(a)
Control
Guggulsterone(+)
Percent change of fluorescence unit ratio
120
100
80
60
40
20
0
LPA(-)
LPA(+)
(b)
Control
AG1478(+)
Guggulsterone(+)
AG1478(+)+Guggulsterone(+)
Percent change of fluorescence unit ratio
120
100
80
60
40
20
0
*
LPA(-)
LPA(+)
Hypoxia
(c)
Control
AG1478
Nuclear/total cell (%)
80
70
60
50
40
30
20
10
0
*
LPA(-)
LPA(+)
Hypoxia
Hypoxia : Control
Hypoxia : LPA
Hypoxia : AG 1478
Hypoxia : LPA + AG 1478

Figure 4. EGFR inhibition reduced NF-κB activation. (a) SNU761 cells were treated with or without lysophosphatidic acid (LPA) (10 μM) and with or without NF-κB inhibitor, gugglsterone (20 μM) for 24h in hypoxic conditions. Invasion assay was performed, and the results are expressed as the percent changes of fluorescence units compared with those of the control. (b) SNU761 cells were treated with or without LPA (10 μM), with or without AG 1478 (10 μM) and with or without gugglsterone (20 μM) for 24h in hypoxic conditions. Invasion assay was performed, and the results are expressed as the percent changes of fluorescence units compared with those of the control. (c) SNU761 cells were pretreated with or without LPA (10 μM) and treated with or without EGFR inhibitor, AG 1478 (10 μM) for 18 h. Cells were subsequently stained with anti-NF-κB (p65) antibodies. Immunofluorescent staining was quantified by the percentage of cells with nuclear-translocated NF-κB (p65) out of the total cells.

* $P \leq 0.05$ versus the control; columns represent means; bars represent SDs; Representative immunofluorescent images are also shown (×400).

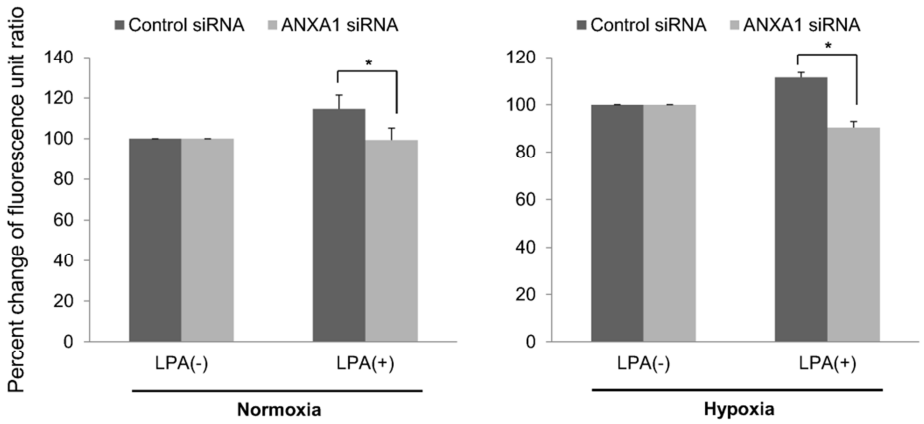

Figure 5. ANXA1 siRNA counterbalanced invasion promoted by LPA. SNU761 cells were treated with LPA (10 μM) following transfection with ANXA1 siRNA or control in hypoxic conditions. Invasion assay was performed, and invasion ability was determined as relative fluorescence units measured with a fluorescence plate reader using BD Calcein AM Fluorescent dye, and the results are expressed as the percent changes of fluorescence units compared with those of the control.

* P ≤0.05 versus the control; Columns represent means; bars represent SDs.

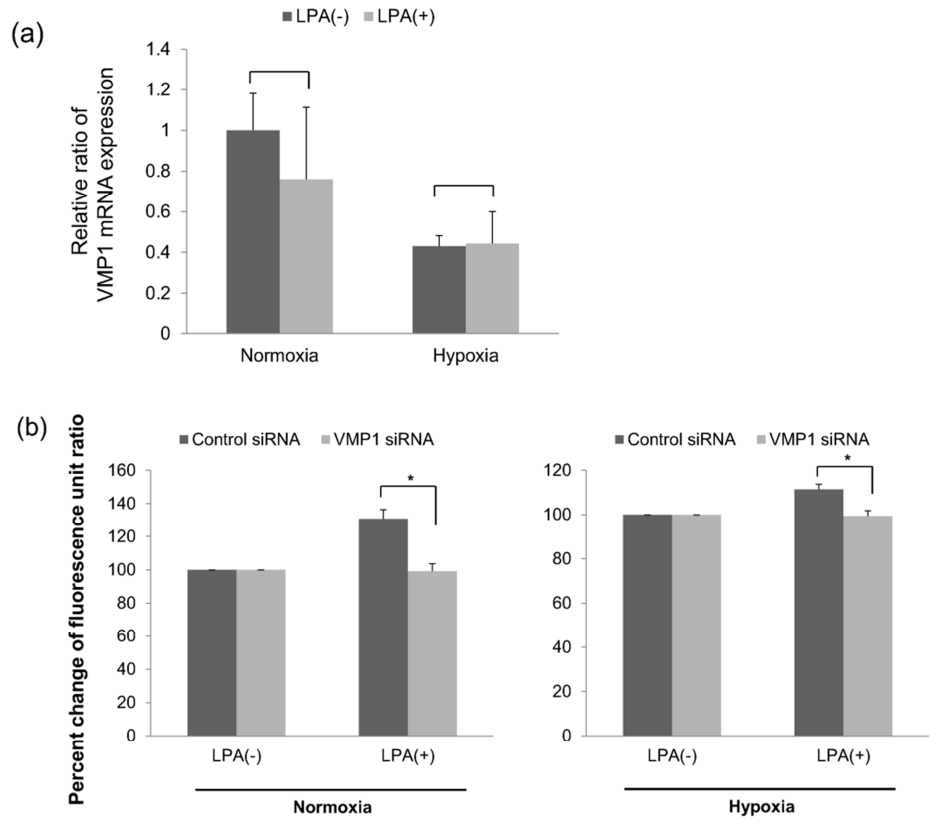

Figure 6. LPA downregulates VMP1, and VMP1 siRNA counterbalanced invasion. (a) VMP levels were measured by real-time PCR. After serum starvation for 24h, SNU761 cells were cultured with or without LPA (10 μM). Quantitative real-time PCR for VMP1 was carried out after 24h of each treatment. Results are expressed as the ratio of VMP1 mRNA to the mRNA of a housekeeping gene in the same RNA sample. (b) SNU761 cells were treated with LPA (10 μM) following transfection with VMP siRNA or control in hypoxic conditions. Invasion assay was performed, and invasion ability was determined as relative fluorescence units

measured with a fluorescence plate reader using BD Calcein AM Fluorescent dye, and the results are expressed as the percent changes of fluorescence units compared with those of the control.

* $P \leq 0.05$ versus the control; Columns represent means; bars represent SDs.

Discussion

In this study, we demonstrated that LPA promoted invasion of HCC cells, especially under hypoxic condition. Moreover, the mechanism related to this process was further evaluated. First, we showed that CK19 expression was augmented by LPA treatment, and EGFR signaling pathway is required for CK19 expression. We also showed that NF-κB signaling pathway is involved in cellular invasion promoted by LPA, and this pathway may have interaction with EGFR signal pathway.

LPA is involved in tumor progression, invasion and metastasis, and a few recent studies have shown that LPA can promote cell invasion in HCC (4, 5). Matrix metalloproteinase-9 (MMP-9) is a key mediator of remodeling of extracellular matrix and angiogenesis, and previous studies showed that MMP-9 expression is increased by LPA induction in HCC (4, 5). Our study confirms that LPA promotes HCC invasion, especially under hypoxic condition, but contrary to previous studies, MMP-9 expression was not enhanced by LPA under both normoxic and hypoxic conditions (Fig. 2a).

This study demonstrated that LPA can induce CK19 expression and mediate cellular invasion through this pathway. CK19 expression in HCC is known to be associated with metastasis and invasion, therefore CK19-positive HCCs showed

poor prognosis (7, 19). CK19 expression is also thought to have correlation with high recurrence rates after curative treatments (8). The mechanisms of the aggressiveness of CK19-positive HCC is unclear yet. A recent study found CK19-positive HCC showed high expression of molecules which are related to epithelio-mesenchymal transition (EMT) and invasion as a possible mechanism of cellular invasion induced by LPA (20). CK19 expression may be associated with phenotypic trans-differentiation from hepatic to biliary phenotype, but it is beyond the scope of this study to find this association (11).

EGFR is frequently overexpressed in HCC (21), and recently EGFR inhibitors are suggested as a new promising target for HCC prevention in cirrhotic patients (22). Previous studies showed that EGFR signaling pathway is associated with HCC cell proliferation (23) and development of CK19-positive HCCs (11). In ovarian cancer, LPA-induced signal transduction requires EGFR transactivation (24). To our knowledge, the effect of EGFR signaling pathway on LPA-mediated cellular invasion in HCC has not been studied yet. In this study, we showed that inhibition of EGFR reduced HCC invasion augmented by LPA under hypoxic condition, although we failed to show statistically significant differences. Furthermore, inhibition of EGFR markedly reduced CK19 expression induced by LPA, suggesting that EGFR signaling pathway is involved in LPA=mediated cellular invasion as an upstream signal of CK19.

NF-κB signaling pathway was shown to mediate LPA signaling in bronchial epithelial cells (25) and ovarian cancer (14, 24). The present study is the first study to elucidate the role of NF-κB signaling pathway in LPA-mediated cellular invasion in HCC. A previous study in ovarian cancer demonstrated that NF-κB and EGFR pathway promotes invasion in an independent way (24), while another study in ovarian cancer showed that NF-κB and EGFR pathway might share common path of signal transduction (14). This subject is still in debate. In our study, NF-κB inhibition reduced cellular invasion induced by LPA under hypoxic condition, although the difference was not significant. Inhibition of EGFR and NF-κB showed additive effect in prevention of invasion. Moreover, we also found that EGFR inhibition reduced intracellular NF-κB activation induced by LPA. Together, these results suggest that NF-κB and EGFR pathway might share a common path of signal transduction in LPA-induced cellular invasion.

Vacuole membrane protein 1 (VMP1) is a membrane protein which is recently studied, and it is thought to be an important component of cell to cell interaction (26). In HCC there are several evidences that down-regulation of VMP1 increases invasion (17, 18). Annexin A1 (ANXA1) is a Ca dependent membrane protein, and in HCC ANXA1 plays an important role in malignant transformation and tumor metastasis (16, 27). We found that blocking VMP1 and ANXA1 signal counterbalanced LPA induced cellular invasion, and LPA down-regulated VMP1 mRNA translation. Since there are limited data in our study, solid conclusion cannot be made, but we showed the possibility that VMP1 and ANXA1 may be have roles

in LPA signaling pathways. A recent study analyzed ANXA1 expression in HCC and cholangiocarcinoma, showing that ANXA1 is highly expressed in cholangiocarcinoma only (28). And our current study found that a biliary marker CK19 is overexpressed in LPA induced cellular invasion. These two results suggest that transformation from hepatic to biliary phenotype may occur in the LPA induced cellular invasion pathway, and further study needs to be performed on this subject.

In summary, LPA promotes invasiveness of HCC cells by augmenting EGFR transactivation, and then CK 19 expression and NF-κB activation, especially under hypoxic condition. The results of our study suggest that EGFR transactivation and NF-κB activation pathways may share common pathways. Therefore, these pathways may be therapeutic targets in HCCs, particularly in the tumors exposed to hypoxic environments.

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요약 (국문 초록)

배경: Lysophosphatidic acid (LPA)는 세포의 증식, 이동과 생존 등 다양한 생물학적인 활동에 관여하는 지질 매개 물질이다. LPA 는 종양세포의 증식, 확산과 생존과 연관되어 있는 것으로 밝혀졌다. 본 연구에서는 LPA 가 간세포암의 침습에 미치는 영향과 LPA 에 의해 조절되는 신호 전달 체계를 밝혀내고자 한다.

방법과 결과: 이 연구에서는 인간 간세포암 세포주인 SNU761 세포주를 이용하여 실험을 시행하였다. 간세포의 침습은 invasion assay kit 를 사용해서 평가하였고, LPA 처리 이후의 혈질변화는 면역블롯법을 이용하여 알아보았다. Epidermal growth factor receptor (EGFR) 억제제인 AG 1478 과 nuclear factor kappa B (NF-κB) 억제제인 guggulsterone 을 처리해서 간세포 침습의 변화와 면역블롯의 변화를 알아보았다. LPA 처리 후 혐기성 환경에서 CK19 의 활성이 증가하였으며 siRNA 로 CK19 활성을 억제한 후 LPA 에 의한 간세포 침습의 증가가 상쇄되었다. AG1478 처리 후 LPA 에 의한 간세포 침습의 증가와 CK19 활성이 억제되었으며, AG1478 과 guggulsterone 은 LPA 에 의한 간세포 침습의 증가에 상가적 효과를 보였다.

결론: 본 연구에서는 LPA 가 EGFR 활성, CK19 발현과 NF-κB 활성을 통해서 혐기성 환경에서 간세포암 세포의 침습을 증가시킨다는 것을 알 수 있었다. 따라서 LPA 신호경로를 차단하는 것은 특히 혐기성 환경에 노출된 간세포암에서 향후 중요한 암 치료의 표적이 될 수 있을 것이다.

주요어: 간세포암, 침습, lysophosphatidic acid, EGFR, NF-κB

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