Original Article

Hypoxia-Induced LXRα Contributes to the Migration and Invasion of Gastric Cancer Cells

(hypoxia / HIF-1α / LXRα / gastric cancer / prognosis)

R. GUO, B. YANG

Department of General Surgery, Shanxi Cancer Hospital, Taiyuan, Shanxi, China

Abstract. Gastric cancer is characterized by the presence of high invasion ability, hypoxia and chemoresistance. Previous studies reported that liver X receptor α (LXRα) was involved in epithelial-mesenchymal transition (EMT) of gastric cancer cells. However, hypoxia-mediated EMT and the role of LXRα in gastric cancer remained elusive. In this study, we demonstrated that *LXRα* mRNA and protein levels were up-regulated by hypoxia treatment and LXRα played an important role in HIF-1 dimer induced-EMT. The putative HIF-1α binding site was identified in the *LXRα* promoter. Expression of LXRα and HIF-1α was significantly up-regulated in gastric cancer tissues compared to that in normal tissues. More importantly, we noticed that the expression of LXRα and HIF-1α was significantly correlated. Taken together, these data suggested that LXRα is regulated by the activity and accumulation of HIF-1α and contributes to EMT of gastric cancer cells. This suggests that targeting LXRα might be a potential approach for improving survival of gastric cancer patients.

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Corresponding author: Bin Yang, Department of General Surgery, Shanxi Cancer Hospital, No.3, Zhigongxincun, Xinghualing District, Taiyuan City, Shanxi, 030013, China. Phone: (+0086) 180 035 165 10; e-mail: yangbin81@tom.com

Abbreviations: ANOVA – one-way analysis of variance, BCA – bicinchoninic acid, BSA – bovine serum albumin, ChIP – chromatin immunoprecipitation, ECL – electrochemiluminescence, EGFR – epidermal growth factor receptor, EMT – epithelial-mesenchymal transition, HBX – hepatitis B virus X protein, HIF-1 – hypoxia-inducible factor 1, HIF-1α – hypoxia-inducible factor 1α, HK2 – hexokinase 2, HRP – horseradish peroxidase, LXRα – liver X receptor α, LXRs – liver X receptors, MMP – matrix metalloproteinase, PVDF – polyvinylidene fluoride, SDS-PAGE – sodium dodecyl sulphate polyacrylamide gel electrophoresis, SEM – standard error of the mean, TFs – transcription factors, TSS – transcriptional start site, VEGF – vascular endothelial growth factor, ZEB1 – zinc-finger E-box binding protein 1.

Introduction

Tumour cells have rapid dividing rate, leading to insufficient nutrients and oxygen shortage that results in hypoxia (Heiden and DeBerardinis, 2017). The hypoxic response is observed in a variety of cancers such as gastric cancer, breast cancer, hepatocellular carcinoma, and colorectal carcinoma (Semenza, 2016; Stoeltzing et al., 2004; Ye et al., 2016; Li et al., 2017). Indeed, expression of hypoxia-inducible factors is a common feature of these cancers, mediating transcription of multiple target genes to regulate cell metabolism, epithelial-mesenchymal transition (EMT), angiogenesis, and tumour microenvironment (Majmundar et al., 2010; Zhu et al., 2014; Chiu et al., 2017). Hypoxia-inducible factor 1α (HIF-1α) can be activated by hypoxia for heterodimer formation (HIF-1 dimer), and thus regulate the target genes at their transcriptional levels. Previous studies have demonstrated that HIF-1α correlates with metastasis, chemoresistance and poor prognosis (Shibaji et al., 2003; Cao et al., 2009; Lu et al., 2018).

Gastric cancer is the fourth most common malignancy worldwide, and the second leading cause of cancer-related death in China (Chen et al., 2016). Despite the fact that surgery and chemotherapy treatments are available, the 5-year survival rate of gastric cancer patients with liver metastases is only about 31.1 % (Kinoshita et al., 2015). Moreover, gastric cancer is predisposed to metastasis, so that novel and targeted therapeutic approaches are extremely needed (Deng and Liang, 2014).

Liver X receptors (LXRs) are nuclear receptors belonging to the superfamily of ligand-activated transcription factors and contain two members, LXRα and LXRβ (Alberti et al., 2000). LXRs play pivotal roles in several cell processes, such as lipid metabolism, cellular cholesterol accumulation, glucose homeostasis, and inflammation (Joseph et al., 2003; Ito et al., 2015). Emerging evidence has suggested that LXRs are critical in the prognosis of breast cancer, ovarian cancer and prostate cancer (Chuu et al., 2007; Casella et al., 2014). LXRα can induce expression of matrix metalloproteinases 2 (MMP2) and MMP9 and promote invasion of gastric cancer cells by modulating NF-κB signalling (Ji et al., 2017). However, its role in hypoxia remains elusive.

In this study, we demonstrated that HIF-1α-dependent regulation of LXRα contributes to hypoxia-induced gastric cancer cell malignancy.

Material and Methods

Cell lines and culture conditions

Human gastric cancer cell lines AGS and MGC-823 were obtained from the Cell Bank at the Chinese Academy of Science (Shanghai, China). Cells were maintained in RPMI1640 medium supplemented with 10% foetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO_2. Hypoxia treatment was performed in an airtight chamber with 1 % O_2 and 5 % CO_2. Briefly, cells were exposed to 5 % CO_2 with 1 % O_2 and 93 % N_2 in an airtight incubator chamber (Billups-Rothenberg Inc., Del Mar, CA). The cells in the airtight chamber were incubated at 37 °C for periods of up to 48 h. AGS cells and MGC-823 cells were authenticated using STR analysis by VivaCell BIOSCIENCES company (Shanghai, China), and all experiments were performed with mycoplasma-free cells.

Transfection and gene knockdown by siRNA

For ectopic expression of HIF-1α, 4 μg cDNA of *HIF1a* (Genecopoeia, Guangzhou, China) was transfected into cells in a 6-well plate using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Proteins and RNAs were harvested 48 h following transfection, and *HIF1a* over-expression was confirmed by Western blot and PCR. To knock down *LXRa* and *HIF1a*, after validation of the knocking-down efficiency, two sets of siRNAs against *LXRa* or *HIF1a* (Genepharma, Shanghai, China) were mixed as one pool at the concentration of 50 nM, respectively, and transfected into cells using Lipofectamine 2000.

The sequences of siRNAs targeting *LXRa* were as follows:
5'-AAGTACACAGGAGGCCATCTT-3',
5'-GGATGCTAATGAAACTGGTT-3'.
siRNAs targeting *HIF1a* were as follows:
5'-GTTCACCTGAGCCTAATAGTT-3',
5'-CTGATGACCAGCAACTTGATT-3'.
The negative control siRNA was:
5'-TTCTCCGAACGTGTCACGTTT-3'.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using Trizol (Invitrogen) reagent as described previously (Qian et al., 2016). Two hundred ng RNA was used to synthesize the cDNA by a PrimeScript RT master Kit (Takara, Dalian, China) at 37 °C for 15 min and 85 °C for 10 s. qRT-PCR was performed in 20 μl reaction volume using the SYBR Green method in an ABI7500 real-time PCR system (Applied Biosystems, Foster City, CA) The thermocycling conditions for the qPCR were as follows: (95 °C 30 s, 95 °C 30 s, 60 °C 30 s) for 40 cycles. The expression of each gene was normalized using the $2^{-\Delta\Delta Cq}$ method (Livak et al., 2001). β-Actin was used as internal control.

The primers used were as follows:
LXRa, forward,
5'-TCTGGAGACATCTCGGAGGTA-3',
reverse, 5'-GGCCCTGGAGAACTCGAAG-3';
HIF1a, forward,
5'-GAACGTCGAAAAGAAAAGTCTCG-3',
reverse, 5'-CCTTATCAAGATGCGAACTCACA-3';
Snail, forward,
5'-TCGGAAGCCTAACTACAGCGA-3',
reverse, 5'-AGATGAGCATTGGCAGCGAG-3';
Twist, forward,
5'-GTCCGCAGTCTTACGAGGAG-3',
reverse, 5'-GCTTGAGGGTCTGAATCTTGCT-3';
Vimentin, forward,
5'-AGTCCACTGAGTACCGGAGAC-3',
reverse, 5'-CATTTCACGCATCTGGCGTTC-3';
E-cadherin, forward,
5'-CGAGAGCTACACGTTCACGG-3',
reverse, 5'-GGGTGTCGAGGGAAAAATAGG-3';
N-cadherin, forward,
5'-AGCCAACCTTAACTGAGGAGT-3',
reverse, 5'-GGCAAGTTGATTGGAGGGATG-3';
β-actin, forward, 5'-
CATGTACGTTGCTATCCAGGC-3',
reverse, 5'- CTCCTTAATGTCACGCACGAT-3'.

Western blot

Cells were lysed using RIPA buffer with protease and phosphatase inhibitors. Protein concentration was measured by the bicinchoninic acid (BCA) method. Equal amounts of proteins (60 μg/line) were loaded into each well of 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were then blocked with 5% bovine serum albumin (BSA) for 1 h at room temperature and incubated with primary antibodies at 4 °C overnight. Then, the membrane was incubated with secondary antibodies for 45 min at room temperature. β-Actin was used as internal control. The primary antibodies including anti-LXRα (ab176323, 1 : 1000), anti-HIF-1α (ab2185, 1 : 1000) and anti-E-cadherin (ab1416, 1 : 1000) were purchased from Abcam (Cambridge, MA), and anti-β-actin (66009-1-Ig, 1 : 2000) from Proteintech Group (Wuhan, China). We noted that anti-HIF-1α antibody (ab2185) has been recently discontinued by Abcam, based on specificity issues. Meanwhile, this antibody used for confirming the HIF-1α expression by Western blot has been recently published in many journals (Chen et al., 2021; Vetrovoy et al., 2021; Yu et al., 2021). The secondary antibodies were mouse anti-rabbit IgG-horseradish peroxidase (HRP, sc-2357, 1 : 2000) and m-IgGκ BP-HRP (sc-516102, 1 : 2000) from Santa Cruz Biotechnology (Santa Cruz, CA). The membranes were visualized by the electrochemiluminescence (ECL) method in a ChemiDoc XRS Plus luminescent image analyser (Bio-Rad, Hercules, CA). The densitometry analysis was performed using

ImageJ vision 3.2 (National Institutes of Health, Bethesda, MD).

Wound healing assay

Cells were grown in 6-well plates until a confluence of about 90 %. To create a wound, the cell monolayer was slowly scratched using a 200 µl pipette tip and the cells were let to grow for 24 h. Wound distances were measured by photographing under a microscope. The magnification was 50×.

Transwell assay

The cell invasion assay was performed using Transwell plates (BD Biosciences, San Jose, CA) pre-coated with Matrigel (BD Biosciences). Cells were seeded into the top chamber at a density of 4×10^4 cells per well. After culturing for 48 h, invaded cells were stained with 0.1% crystal violet and the cell number was counted under a microscope. The magnification was 100×.

Luciferase reporter assay

The promoter regions in different lengths of *LXRa* were amplified from genomic DNA and cloned into pGL3-basic plasmid (Promega, Madison, WI). Cells in a 24-well plate were co-transfected with 400 ng pGL3-basic-LXRα and 40 ng Renilla plasmid (Promega) and cultured in normoxia and hypoxia conditions, together with 400 ng *HIF1a* cDNA or empty vector transfection. We tested the putative binding of HIF1α in the *LXRa* promoter. Cells were maintained for 48 h and then lysed for further measurement. The luciferase activity was measured by a Dual Luciferase reporter assay kit (Promega) using GloMax® Microplate Luminometer (Promega). The Renilla luciferase luminescence activity was used as internal control. We recorded the ratio of Firefly/Renilla as normalized value, and the normoxia with empty vector group was indicated as control group.

Chromatin immunoprecipitation (ChIP)

Cells were cultured at 21% O_2 (normoxia) and 1% O_2 (hypoxia) for 6 h, respectively. Then, the cells were fixed with 1% formaldehyde for 15 min. Genomic DNA was sheared to about 500 bp fragments by sonication (30 s on, 30 s off, 10 min at 4 °C). ChIP was performed using primary antibodies against HIF-1α and control IgG, incubated with protein A agarose beads and DNA fragments overnight at 4 °C and then eluted following the manufacturer's protocol (No. 17-371, EZ-Magna ChIP™ A/G Chromatin Immunoprecipitation Kit, Millipore, Burlington, MA).

Data availability

The expression of LXRα and HIF-1α was obtained from the Gene Expression Omnibus (GSE: GSE27342, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE27342) data set containing 80 paired gastric cancer tissues and normal tissues (Cui et al., 2011b). We divided the tumour group and normal group and explored the expression level of LXRα (probe ID: 3329685) and HIF-1α (probe ID: 3539070). The prognostic value of LXRα in gastric cancer patients was explored by the Kaplan-Meier Plotter database (http://kmplot.com/analysis/index.php?p=service&cancer=gastric). The Affymetrix probe for LXRα (NR1H3) is 203920 in that database.

Statistical analysis

Data were present as mean ± standard error of the mean (SEM) with at least three independent experiments. Statistical analysis was performed by SPSS21.0. The difference between each group was measured by Student's *t*-test for two groups and one-way analysis of variance (ANOVA) test for multiple groups. The correlation between LXRα and HIF-1α expression was measured by Pearson's correlation test. The expression of LXRα and HIF-1α between normal and tumour tissues was measured by Wilcoxon matched-pairs rank test. A P value less than 0.05 was considered statistically significant.

Results

LXRα regulates EMT under hypoxia

To determine the effect of LXRα, AGS and MGC-823 cells were treated in hypoxic conditions for different times. We noticed that there was a robust elevation of HIF-1α mRNA and LXRα mRNA levels in gastric cancer cells at 2 h exposure to hypoxia and then, these two mRNAs gradually decreased (Fig. 1A and C). However, the expression of epithelial marker E-cadherin decreased with time in both cell types (Fig. 1A and C). In addition, Western blot results showed that HIF-1α in both cell types was up-regulated at 2 h and LXRα was up-regulated at 4 h and 2 h in AGS cells and MGC-823 cells, respectively, while E-cadherin decreased with time (Fig. 1B, D, and Fig. 2). It is known that HIF-1 dimer (HIF-1α and HIF-1β) could induce EMT, evidenced by an increase in mesenchymal markers, N-cadherin, vimentin, Snail, and Twist (Yang et al., 2008; Li et al., 2019). Indeed, these markers were significantly increased, while E-cadherin was significantly decreased by hypoxia treatment both at mRNA and protein levels (Fig. 1E-G), which was due to the hypoxia-induced HIF-1α over-expression. However, the *LXRa* knockdown inhibited hypoxia-induced HIF-1α at both mRNA and protein levels (Fig. 1E and G), and therefore the decreased amount of HIF-1α significantly inhibited the mRNA and protein levels of these mesenchymal markers while restored hypoxia-reduced E-cadherin expression in AGS cells (Fig. 1E-G). The blockage of hypoxia-induced HIF-1α expression by *LXRa* knockdown was also reported in other cell types (Na et al., 2011; Ménégaut et al., 2020). Na et al. (2011) demonstrated the binding of HIF-1α to the *LXRa* promoter in mouse and human macrophages, suggesting that a cross-talk exists between hypoxia and LXRα signalling. Further study to

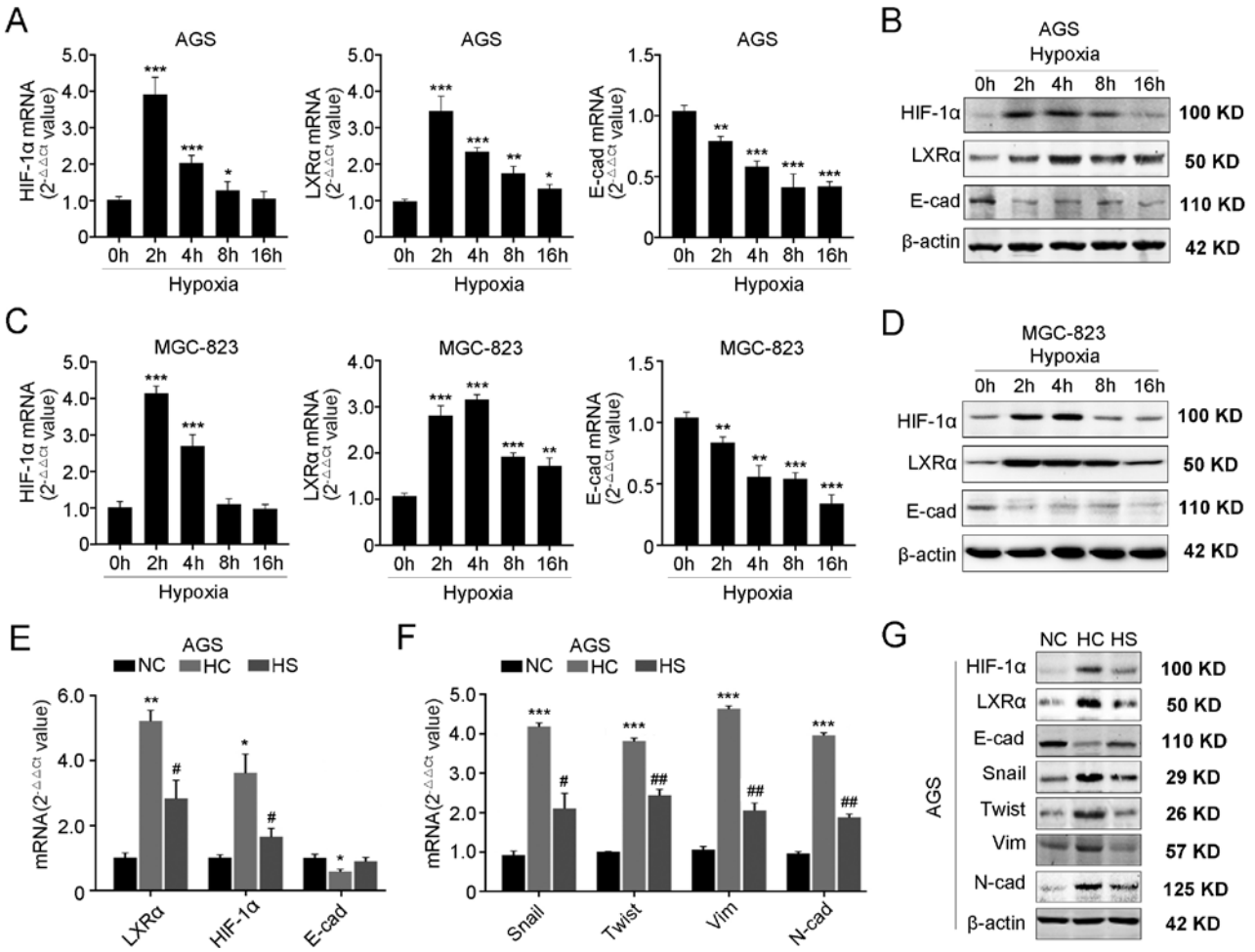

Fig. 1. LXRα is up-regulated by hypoxia and critical for hypoxia-induced EMT genes in gastric cancer cell lines AGS and MGC-823. HIF-1α and LXRα mRNA levels in AGS cells (**A**) and MGC-823 cells (**C**) showed rapid up-regulation by hypoxia in 2 h. E-cadherin mRNA in AGS cells (**A**) and MGC-823 cells (**C**) was decreased after exposure to hypoxia. Western blot showed that hypoxia enhanced HIF-1α and LXRα expression, but reduced E-cadherin in AGS cells (**B**) and MGC-823 cells (**D**). The expression of HIF-1α was normalized and calculated according to the density of each band by ImageJ. (**E-F**) AGS cells and MGC-823 cells transfected with LXRα siRNAs and control siRNAs were exposed to hypoxia and the mRNAs of EMT genes were examined. E-cad, E-cadherin; Vim, vimentin; NC, normoxia+negative control; HC, hypoxia+negative control; HS, hypoxia+siLXRα. (**G**) Expression of indicated EMT marker proteins was examined by Western blot. * – $P < 0.05$, ** – $P < 0.01$, and *** – $P < 0.001$ represent a significant difference between the indicated groups and 0 h group (one-way ANOVA in Fig. 1A, C) or between the NC group and HC group (Student's *t*-test in Fig. 1E-F); $^{\#}$ – $P < 0.05$ and $^{\#\#}$ – $P < 0.01$ represent a significant difference between the HS group and HC group (Student's *t*-test).

elucidate the detailed mechanism of this effect will be required in the future. Collectively, the data suggested that LXRα may regulate EMT under hypoxia.

LXRα contributes to migration and invasion of gastric cancer cells under hypoxia

To further explore the role of LXRα in aggressiveness of gastric cancer cells under hypoxia, AGS and MGC-823 cell migration and invasion were assessed upon *LXRa* knockdown. The validation of *LXRa* knockdown was performed by qRT-PCR and Western blot (Fig. 3). Knockdown of *LXRa* slightly suppressed cell migration and invasion in normoxia conditions; however, under hypoxia conditions, *LXRa* knockdown significantly inhibited cell migration and invasion in AGS and MGC-823 cells (Fig. 4).

HIF-1α directly regulates LXRα

Based on the time-dependent regulation of LXRα by hypoxia and its critical role in inducing EMT, we investigated whether LXRα could be regulated directly by HIF-1α. Ectopic over-expression of HIF-1α up-regulated LXRα at both mRNA and protein levels (Fig. 5A). On the other hand, *HIF1a* knockdown inhibited LXRα expression in hypoxia (Fig. 5B). Since the HIF-1 dimer

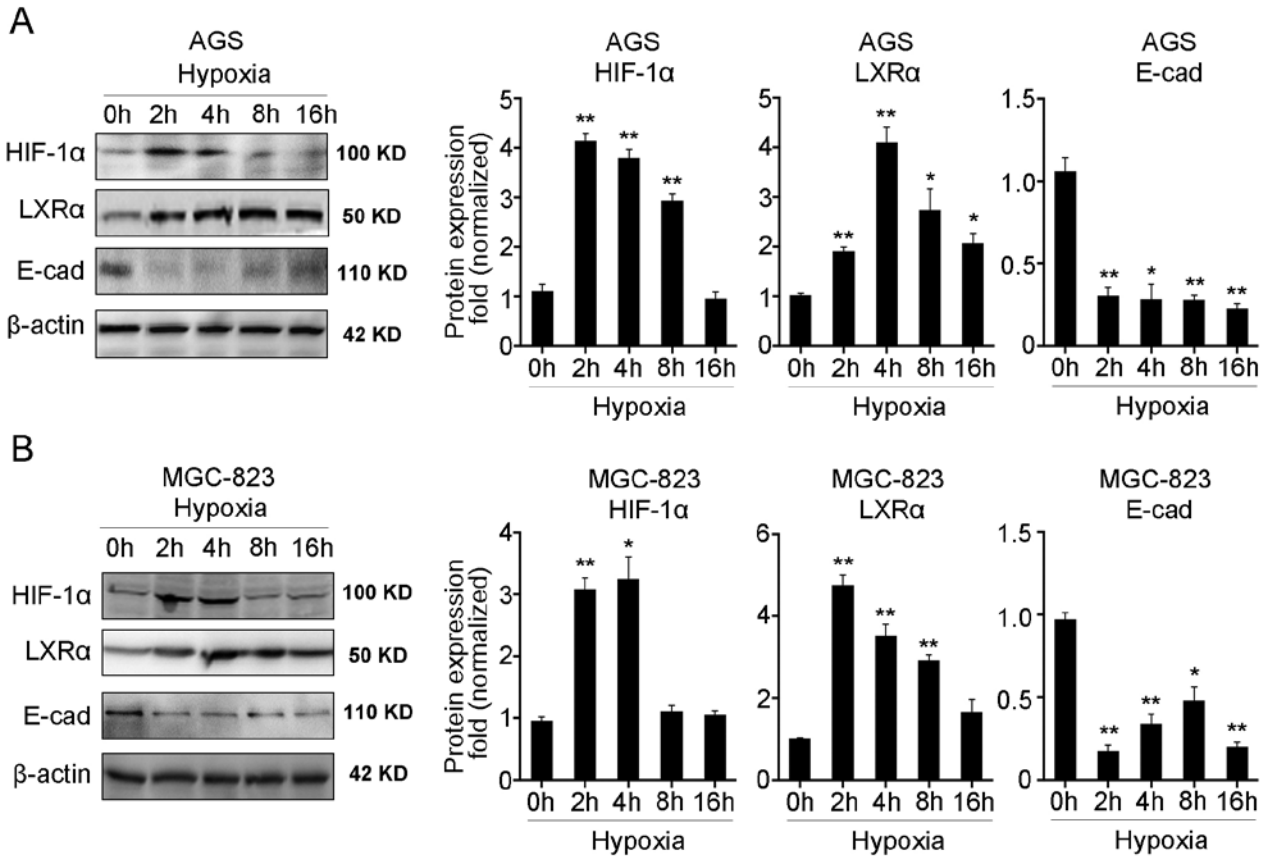

Fig. 2. Western blot showing HIF-1α, LXRα and E-cadherin expression in gastric cancer cell lines after exposure to hypoxia. Hypoxia enhanced HIF-1α and LXRα expression, but reduced E-cadherin in AGS cells (**A**) and MGC-823 cells (**B**). The expression level of protein was calculated according to the density of each band by ImageJ. * – $P < 0.05$, ** – $P < 0.01$ (Student's *t*-test).

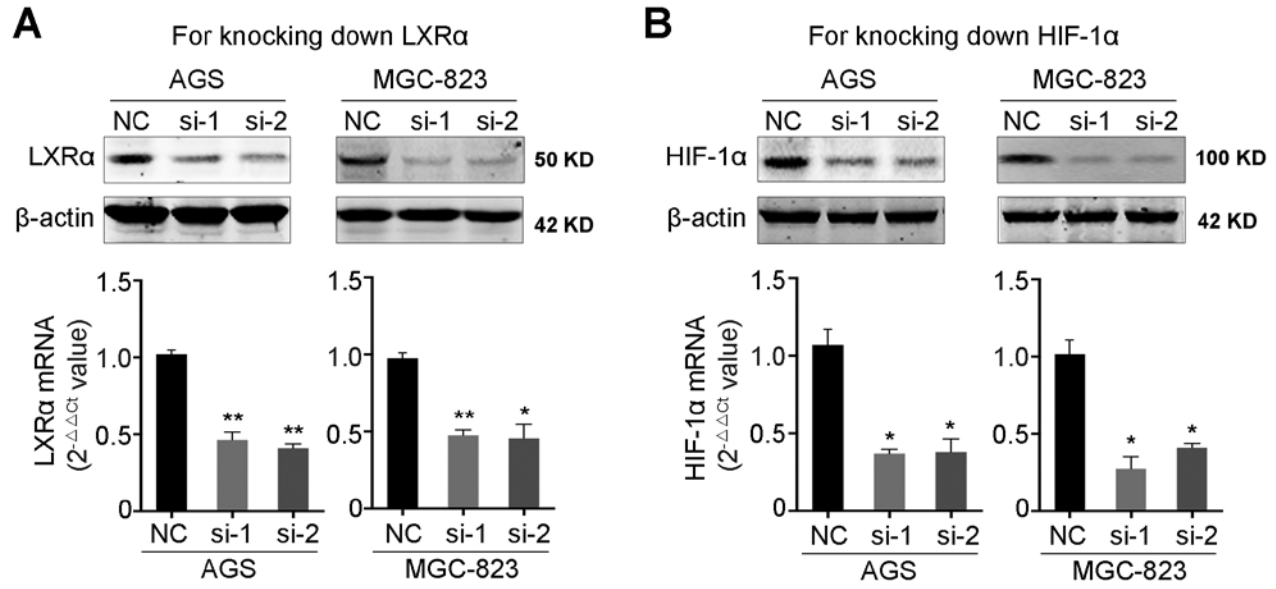

Fig. 3. Verification of LXRα and HIF-1α protein and mRNA expression in AGS and MGC-823 cells via RNA interference. The expression levels of protein and mRNA of LXRα (**A**) and HIF-1α (**B**) in cells after knockdown were measured by Western blot and qRT-PCR. * – $P < 0.05$, ** – $P < 0.01$ (Student's *t* test).

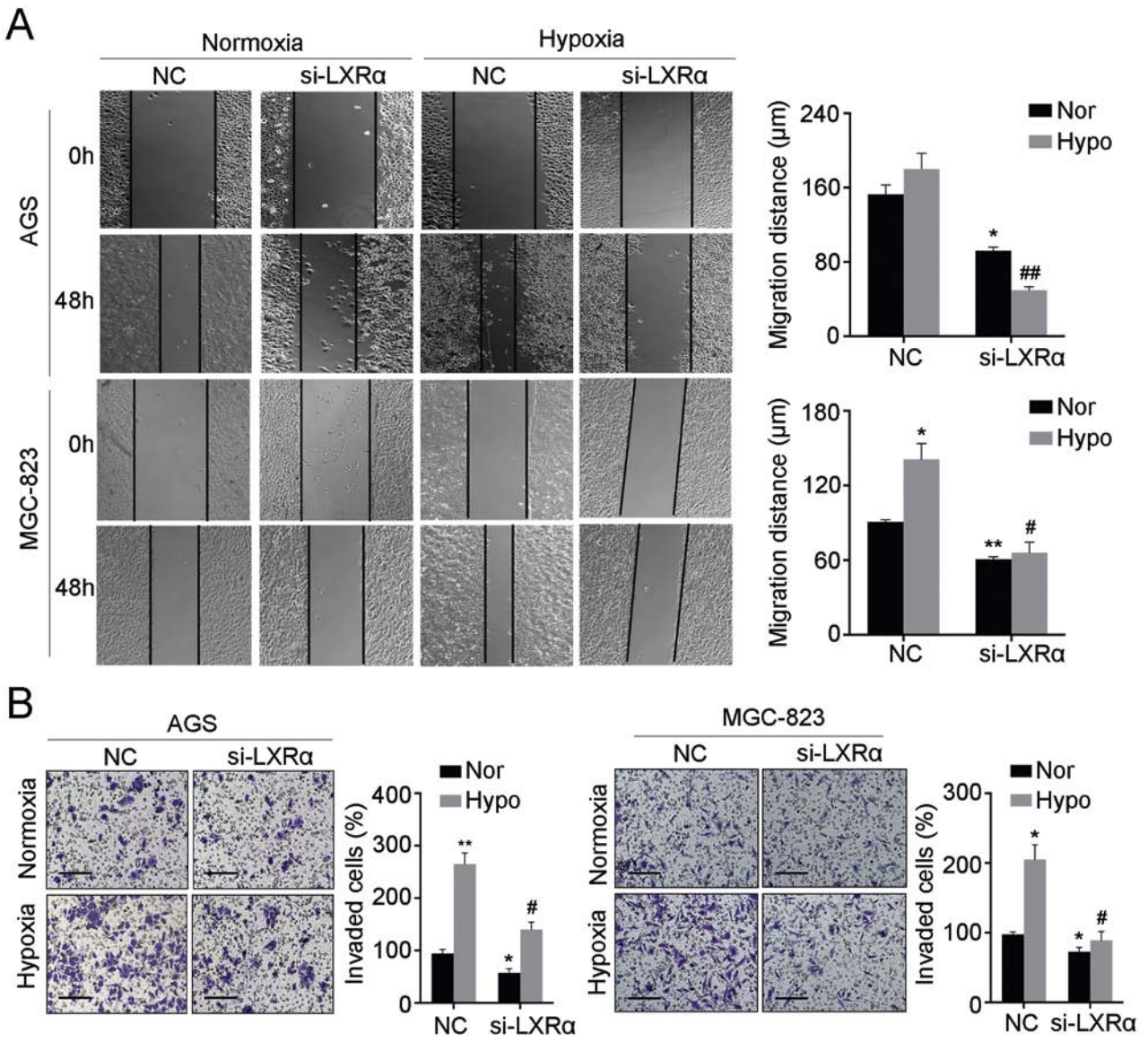

Fig. 4. Cell migration and invasion analysis upon *LXRa* knocking down under normoxia and hypoxia. (**A**) Wound healing assay and (**B**) transwell assay of gastric cancer cells under normoxia and hypoxia after *LXRa* knocking down. The invaded cells were calculated in five random fields of each group and the control group was normalized to 100 %. Scale bar: 100 μm. Nor, normoxia; Hypo, hypoxia. * – $P < 0.05$, ** – $P < 0.01$ represent a significant difference between the normoxia groups (Student's *t* test); $^{\#}$ – $P < 0.05$, $^{\#\#}$ – $P < 0.01$ represent a significant difference between the hypoxia groups (Student's *t* test).

is a pivotal transcriptional activator, we performed a luciferase reporter assay to study whether LXRα could be transcriptionally activated. Fragments of the *LXRa* promoter were constructed to explore the putative binding sites of HIF-1α (Fig. 5C). The full-length *LXRa* promoter was cloned ranging from −1320 ahead of transcriptional start site (TSS) to +185 downstream of TSS. The luciferase activity of the full-length promoter was increased by about 3.5 fold by hypoxia treatment. When HIF-1α was over-expressed, this luciferase activity was further elevated both in normoxia and hypoxia conditions. Similar results were obtained for the F1 and F2 promoter fragments. These observations indicated that HIF-1α might bind to the region from −520 to −120 upstream of *LXRa* TSS (Fig. 5D). Meanwhile, no other hypoxia response elements were found in this region in the current study. To further verify this putative binding site, chromatin immunoprecipitation assay was performed under normoxia and hypoxia conditions. HIF-1α could bind to the *LXRa* promoter under normoxia, and this binding was increased by up to two folds by hypoxia treatment (Fig. 5E and F). Taken together, these data suggested that the increased amount of HIF-1α could up-regulate the HIF-1 dimer, and thus activate *LXRa* at the transcriptional level. However, the effects of HIF-1α over-expression on the binding to the *LXRa*

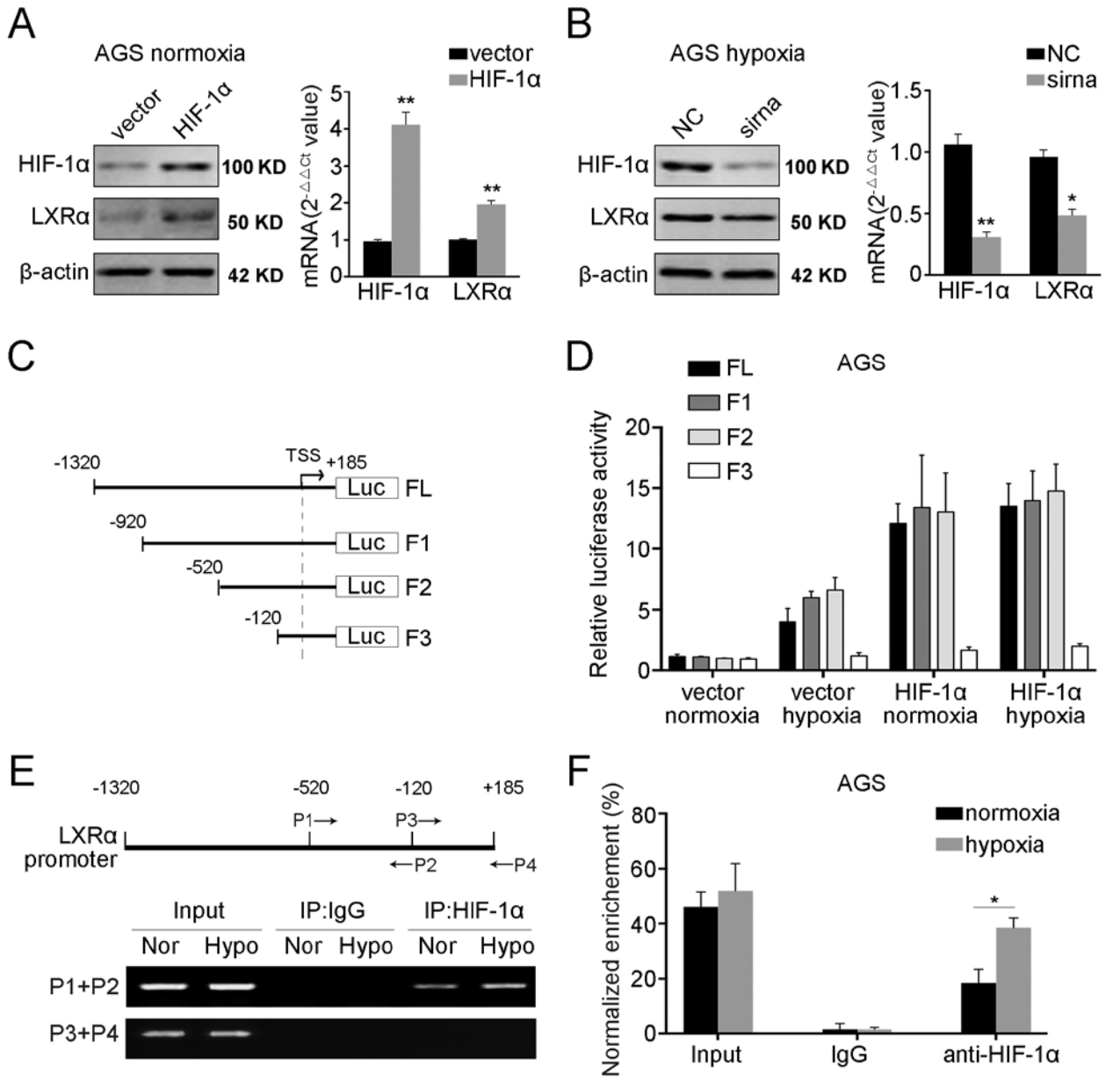

Fig. 5. LXRα is a direct target of HIF-1α. (**A**) Cells with HIF-1α over-expression were examined for LXRα expression at the protein and mRNA levels. (**B**) Cells under hypoxia with *HIF1a* knockdown were examined for LXRα expression. (**C**) Schematic representation of *LXRa* full-length promoter and different types of truncates. TSS, transcriptional start site. (**D**) Luciferase reporter assay was used to compare the relative luciferase activity between cells transfected with different types of promoter under normoxia and hypoxia conditions. (**E**) Scheme of the *LXRa* promoter and primers used (upper). PCR was performed with ChIP-isolated DNA fragments. Input DNA (non-IP enriched) was used as control. Nor, normoxia, Hypo, hypoxia (lower). (**F**) Relative enrichment of each group normalized to the Normoxia Input group. * – $P < 0.05$, ** – $P < 0.01$ (Student's *t*-test).

promoter should be further investigated as to reinforce the current findings.

LXRα is correlated with HIF-1α in gastric cancer tissues

We next investigated whether the correlation between LXRα and HIF-1α could indicate any clinical significance in gastric cancer. We analysed mRNA expression levels of LXRα and HIF-1α in the GEO database (GSE27342), which included 80 pairs of gastric cancer tissues and matched non-cancer tissues (Cui et al., 2011a). As shown in Fig. 6A and 6B, the levels of LXRα and HIF-1α were significantly higher in cancer tissues than those in non-cancer tissues, respectively. Moreover, the correlation between LXRα and HIF-1α was examined

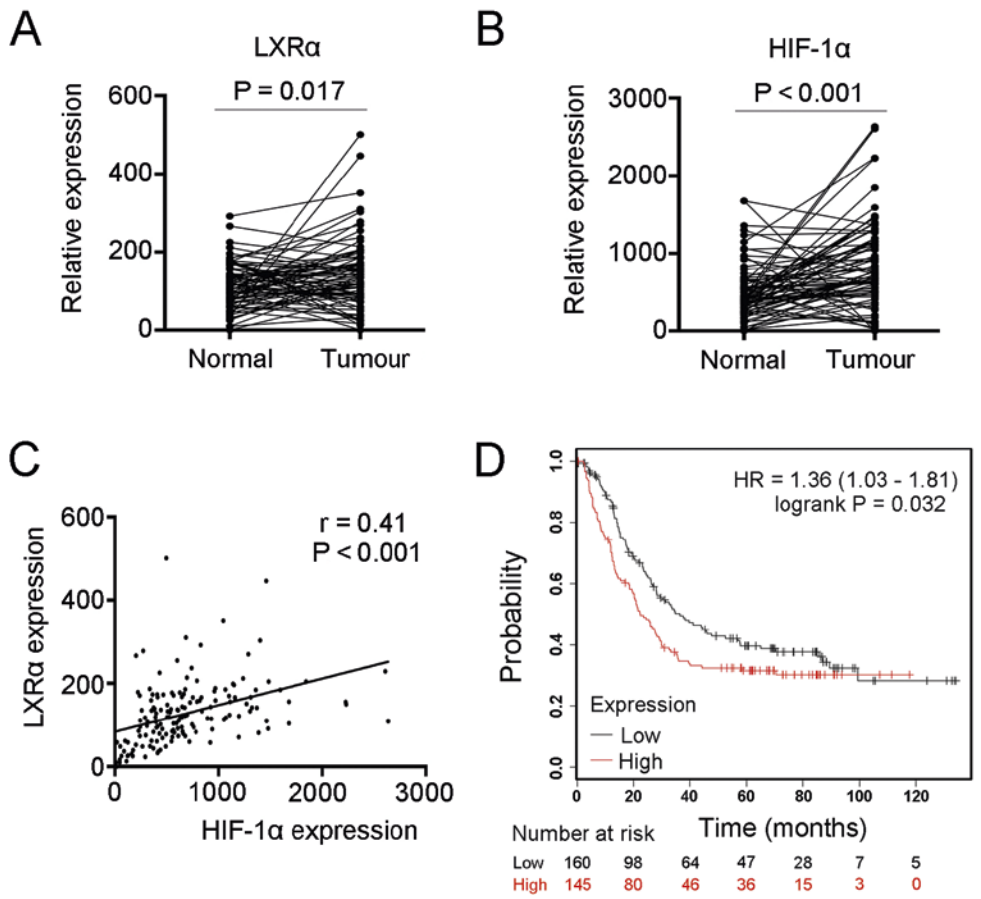

Fig. 6. LXRα is correlated with HIF-1α in gastric cancer and indicates poor prognosis. LXRα expression (**A**) and HIF-1α expression (**B**) levels were measured in GSE27342. (**C**) The expression association of LXRα and HIF-1α was examined. (**D**) Kaplan-Meier plotter of LXRα expression in overall survival of 305 cases of gastric cancer patients in stage III.

by Pearson's correlation test and LXRα expression was closely correlated with HIF-1α ($r = 0.41$, $P < 0.001$, Fig. 6C). To confirm the relationship between LXRα expression and the prognosis in gastric cancer patients, the Kaplan-Meier plotter database (Affymetrix probe number: 203920) was further analysed. It showed that there was no significant association between LXRα expression and prognosis in gastric cancer patients (overall survival, $P = 0.25$; progression-free survival, $P = 0.17$, data not shown). However, we found that high expression of LXRα was associated with short survival in gastric cancer patients in stage III ($P = 0.032$, Fig. 6D). Collectively, these data suggested that LXRα is correlated with HIF-1α expression and could be a potential target for gastric cancer.

Discussion

The hypoxic microenvironment is found in a wide range of tumours such as gastric cancer, breast cancer, prostate cancer, and colorectal cancer and contributes to tumour chemoresistance, metastasis and relapse. Hypoxia-induced EMT is involved in all stages of cancer progression, and the precise mechanisms underlying the changes in phenotypes remain elusive. It is well recognized that EMT is initiated by activation of EMT-activating transcription factors (TFs), mainly of Snail, Twist and Zinc-finger E-box binding protein 1 (ZEB1), leading to the suppression of E-cadherin and up-regulation of N-cadherin (Brabletz et al., 2018). Emerging evidence also suggested that these transcriptional factors also induced pro-inflammatory and immunosuppressive cytokines, thereby triggering immuno-evasion of cancer cells (Wu et al., 2009; Noman et al., 2017). Thus, better understanding of hypoxia-induced EMT is critical for the approach to cancer treatment.

LXRα has been demonstrated in several types of cancers such as lung cancer, brain cancer, breast cancer, and oral cancer. However, its diverse roles in cancer need to be further elucidated. Melloni et al. reported that LXRα seemed to be an independent prognostic factor indicating better survival in resected stage II and III lung cancer patients (Melloni et al., 2018). Epidermal growth factor receptor (EGFR) signalling opposed LXR effects on cholesterol homeostasis, and LXRα agonist induced apoptosis in cancer cells (Gabitova et al., 2015; Villa et al., 2016). In contrast, LXRα could be induced by hepatitis B virus X protein (HBx) and modulate hepatic lipo-

genesis in HBV-associated hepatic carcinogenesis (Na et al., 2009). LXRα ligand increased ER-dependent growth and LXR-dependent metastasis in mouse models of breast cancer (Nelson et al., 2013). In our present study, LXRα was up-regulated in gastric cancer tissues, and high expression of LXRα showed a significantly shorter survival in gastric cancer patients in stage III, implying its oncogenic roles in gastric cancer. Furthermore, we noticed that *LXRa* was a hypoxia-inducible gene and had a pivotal role in EMT.

Previous study has demonstrated that LXRα is significantly over-expressed in gastric cancer tissues and cell lines, and knocking down *LXRa* decreased secretion of matrix metalloproteinases (MMP)-2/9, as well as N-cadherin and vimentin, thereby inhibiting the cell invasion ability (Ji et al., 2017). Likewise, we found that depletion of LXRα reduced EMT-activating TFs both under normoxia and hypoxia conditions. Moreover, knocking down *LXRa* significantly reduced the cell EMT under hypoxia. As we showed, E-cadherin mRNA and protein levels were markedly decreased under hypoxia, while HIF-1α and LXRα were significantly up-regulated, suggesting that LXRα could be induced by hypoxia in a short time and might be involved in hypoxia-facilitating EMT. Further investigation indicated that LXRα was responsible for the EMT phenotype of gastric cancer cells under hypoxia, which was supported by the observation that hypoxia-induced EMT markers (Snail, Twist, vimentin and N-cadherin) were significantly decreased with *LXRa* knocking down. The results of *in vitro* wound healing assay and transwell assay corroborated the findings that LXRα was critical for hypoxia-induced EMT.

The HIF-1 dimer is a critical transcriptional activator, as evidenced by directly regulating numerous genes such as vascular endothelial growth factor (VEGF), CD47 and hexokinase 2 (HK2), facilitating cancer cell metabolism, proliferation and immunosuppression (Kim et al., 2007; Ghosh et al., 2009; Zhang et al., 2015). Indeed, our results identified a new target of HIF-1α, LXRα, that could also be directly activated by HIF-1α, as demonstrated by luciferase reporter assay and ChIP assay. To support the association between HIF-1α and LXRα in gastric cancer cells, we explored in parallel the expression of HIF-1α and LXRα in the GEO database, and found that LXRα and HIF-1α were significantly correlated, indicating the direct regulation of LXRα. Furthermore, we were the first to demonstrate that the LXRα expression level could predict prognosis in patients with stage III gastric cancer.

In conclusion, the present study provided evidence that LXRα is one of the pivotal factors for maintaining the EMT properties of cancer cells under hypoxia and may be a target of a therapeutic approach in gastric cancer.

Acknowledgments

Not applicable.

Availability of data and materials

The analysed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

R. G. designed the study; R. G. and B. Y. performed the experiments and collected the data, did the data analysis; R. G. wrote the manuscript; All authors approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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