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# Long non-coding RNA LBX2-AS1 activates IL4R to promote glioblastoma metastasis and angiogenesis by binding to the transcription factor NFKB1

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## Abstract

***Introduction:*** *LncRNA LBX2-AS1 drives the development of various cancers, but the exact mechanism whereby LBX2-AS1 affects glioblastoma (GBM) progression is unaddressed. This study intended to delineate the regulatory mechanism of LBX2-AS1 in GBM metastasis and angiogenesis.*

***Material and methods:*** *LBX2-AS1 level in GBM was assessed by bioinformatics methods. The lncRNA-transcription factor (TF)-mRNA trios were predicted using the lncMAP database. Correlation between genes was predicted by Pearson analysis. The binding relationship was predicted by JASPAR. Levels of LBX2-AS1 and its downstream genes were assayed via qRT-PCR. Changes in expressions of VEGF-A, IL4R, and epithelial-mesenchymal transition (EMT)-associated proteins were assessed through western blot. GBM cell proliferation, migration, and invasion were assayed through CCK8, colony formation, and Transwell experiments. In vitro angiogenesis capacity was evaluated via a HUVEC tube formation experiment. The regulatory relationship between various genes was verified through radioimmunoprecipitation (RIP), chromatin immunoprecipitation (ChIP), and dual-luciferase assays.*

***Results:*** *LBX2-AS1 was elevated in GBM, and in vitro experiments demonstrated the stimulatory effect of LBX2-AS1 on GBM cell proliferation, invasion, migration, and angiogenesis. We observed that LBX2-AS1 activated IL4R expression by binding the transcription factor NFKB1, thus promoting the progression of GBM. Rescue experiments illustrated that silencing IL4R or NFKB1 reversed the impact of forced LBX2-AS1 expression on GBM cells.*

***Conclusions:*** *This study revealed the mechanism of the LBX2-AS1/NFKB1/IL4R axis in driving GBM metastasis and angiogenesis, which may help to improve the regulatory network of GBM malignant progression and provide potential targets for GBM treatment.*

***Key words:*** *glioblastoma, long non-coding RNA LBX2-AS1, metastasis, angiogenesis, IL4R, NFKB1.*

## Introduction

Glioblastoma (GBM) as the commonest primary brain tumor represents around 45% of all gliomas, with a very low 5-year survival rate for patients (around 5%) [3,29]. Various treatment modalities have been developed for GBM, such as surgery, radiotherapy, and chemotherapy. In addition to these conventional therapies, new treatments such as immunotherapy and cell ablation have significantly improved the clinical survival of GBM patients [13,36]. Despite progress in the treatment of GBM, there are still problems such as resistance to conventional therapy and a high recurrence risk,

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resulting in a relatively poor prognosis [21]. It is noteworthy that the rapid generation of GBM tumor blood vessels is a main contributor to unfavorable prognosis [14]. Angiogenesis is a significant contributor to tumor development [8]. Angiogenesis in gliomas is tightly linked to high invasive potential of glioma cells, which is an intractable obstacle in glioma treatment [10]. Studies have shown that neovascularization in gliomas can provide nutrients for tumor tissue growth and metabolism, promote tumor cell division and proliferation, and provide an appropriate environment for tumor cell infiltration and migration [25]. Therefore, targeted therapy against angiogenesis is becoming an area of intense interest, and identifying key molecules in angiogenesis may become an effective strategy in the management of GBM.

The development of cancer is aided by lncRNAs – crucial regulators of biological processes in tumor cells [24,28,37]. LBX2-AS1 plays a pro-tumor role in various cancers. Li *et al.* [16] reported that overexpression of LBX2-AS1 drives colorectal cancer proliferation. Cao *et al*. [2] found in ovarian cancer research that LBX2-AS1 can up-regulate the E2F2 oncogene by repressing miR-455-5p and miR-491-5p, thus driving tumor cell phenotypes. In GBM, Li *et al.*'s [17] research revealed that LBX2-AS1 drives tumor progression through the miR-491-5p/LIF axis, and proposed that LBX2-AS1 is a new marker for diagnosis and management, but the team's study of the pro-tumor mechanism of LBX2-AS1 was not comprehensive. Considering that GBM occurrence is closely related to angiogenesis, this team believed that LBX2-AS1 may have a potential association with GBM angiogenesis [25]. Currently, the modulation of LBX2-AS1 in angiogenesis in GBM is not profound. This study explored mechanisms of LBX2-AS1 in manipulating tumor metastasis and angiogenesis to promote GBM progression.

In this study, LBX2-AS1 was significantly upregulated in GBM. As multi-level explorations such as molecular cell experiments revealed, LBX2-AS1 fostered GBM cell malignant behaviors *via* recruitment of the transcription factor (TF) NFKB1 to activate transcription of IL4R. This study revealed the molecular mechanism of the LBX2-AS1/NFKB1/IL4R regulatory axis inducing GBM angiogenesis and metastasis, laying a basis for developing novel strategies for GBM management.

## Material and methods

### Bioinformatics analysis

Two types of expression data (Normal: 5, Tumor: 169) for GBM were downloaded from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/). Differential analysis was completed with the "EdgeR" package (|logFC| > 1, FDR < 0.05). LncRNAs associated with GBM were identified through expression analysis. The downstream TFs of target lncRNA were predicted through lncMAP (http://bio-bigdata.hrbmu.edu.cn/LncMAP/survival.jsp). TFs with a high Pearson correlation coefficient with the target lncRNA were selected for further study. The RPISeq tool (http://pridb.gdcb.iastate.edu/RPISeq/index.html) was employed to assess the possibility of interactions of the lncRNA with TF. JASPAR (http://jaspar.genereg.net/) was employed to forecast binding between TF and its downstream target gene promoter regions.

### Cell culture and transfection

Normal human astrocytes (NHA) (BFN60808805) were accessed from Shanghai Cell Bank (China), while GBM cells A172 (BNCC341782), U251 (BNCC341988), LN229 (BNCC341218), and T98G (BNCC338721) were from BNCC (China). All cells were kept in DMEM with 10% FBS and 1% penicillin-streptomycin (Solarbio, China) at 37°C in a humidified 95% air-5% $CO_2$ atmosphere.

sh-NC, sh-LBX2-AS1, oe-NC, oe-LBX2-AS1 constructed by pcDNA3.1, sh-NFKB1, and sh-IL4R were provided by Shanghai GenePharma (Shanghai, China). The process was completed using Lipofectamine 2000 (Invitrogen) at 37°C with 5% $CO_2$.

### Cell proliferation assay

Viability was assayed with the Cell Counting Kit-8 (CCK-8) kit (Dojindo, Japan). Cells were plated in 96-well plates ($3 \times 10^3$ cells/well) for 0, 24, 48, 72, and 96 hours of culture. After supplementing CCK-8 (10 µl/well), cells were cultivated for 2 hours and OD values at 450 nm were assessed.

Cell proliferation ability was measured by colony formation. Cells were plated in six-well plates ($8.0 \times 10^2$ cells/well) and maintained for 11 days. When there were obvious clonal cell clusters, colonies were cleaned twice with PBS, fixed for 10 minutes with 4% paraformaldehyde, and stained for 30 minutes with 0.1% crystal violet.

### Transwell assay

For determination of migration ability, $2.5 \times 10^4$ GBM cells were supplemented to the upper chamber of a Transwell system and cultured in medium without FBS, while 700 µl of DMEM with 10% FBS was supplemented to the lower chamber. Cells were incubated under routine conditions with saturated humidity for 24 hours. Cells that could not cross the membrane were removed. After being preserved for 30 minutes with methanol and stained for 20 minutes with 0.1% crystal

violet, migrating cells were counted under an inverted microscope. Cell invasion ability assay required coating the Transwell upper chamber with Matrigel (Corning, USA), and other steps mirrored the cell migration assay.

## Formation of tubular structure in human umbilical vein endothelial cells (HUVECs)

Thawed extracellular Matrigel was added to a pre-cooled 96-well plate and maintained at 37°C for 60 minutes. The HUVEC suspension ($2.5 \times 10^4$ cells/well) was seeded and incubated until cells adhered to the wall. The medium was then replaced with supernatant of GBM cells that had been successfully transfected and incubated for 4-6 hours. Suitable fields were chosen, and photography was done under a microscope [35].

## qRT-PCR

Trizol reagent (Invitrogen, USA) was recommended for total RNA isolation from cells, and RNA concentration was detected with a spectrophotometer. cDNA synthesis was completed with a PrimeScript RT kit (Takara, Japan). qRT-PCR was done with SYBR Green Premix Ex Taq (Takara, Japan). Relative RNA expression was determined on the ABI 7500 real-time PCR system (Applied Biosystems, USA) with GAPDH as the control gene. The formula used for calculation was $2^{-\Delta\Delta CT}$. Primer sequence information is provided in Table I.

## Western blot

Total protein isolation from cells was completed with cell lysis buffer, and concentration was assessed with the BCA kit (TIANGEN, China). A sample of 20 µg of protein was loaded per lane, subjected to SDS-PAGE, and transferred to a PVDF membrane (Millipore, USA). The membrane was sealed for one hour using 5% skim milk from Beyotime in China, at room temperature. PVDF membrane was maintained at 4°C with diluted primary rabbit antibodies against IL4R (ab271041, 1 : 1000), E-cadherin (ab40772, 1 : 1000), N-cadherin (ab18203, 1 : 1000), MMP-2 (ab92536, 1 : 1000), VEGF-A (ab46154, 1 : 1000), and GAPDH (ab9485, 1 : 2500) overnight. Next, goat anti-rabbit IgG H&L (HRP) secondary antibody (ab97051, 1 : 2000) was added for 1-hour incubation. Antibodies were provided by Abcam (UK). An ECL reagent kit (BB-3501, Amersham, USA) was utilized for visualization, and images were captured by the Bio-Rad image analysis system (BIO-RAD, USA), with analyses performed using Quantity One v4.6.2 software. Protein levels were displayed by grayscale values of the corresponding protein bands and the GAPDH protein band.

**Table I.** Primer sequences

| Gene | Primer sequence (5′→3′) |
|---|---|
| *NFKB1* | Forward: 5′-GCAGCACTACTTCTTGACCACC-3′ |
| | Reverse: 5′- TCTGCTCCTGAGCATTGACGTC-3′ |
| *GAPDH* | Forward: 5′-CCACATCGCTCAGACACCAT-3′ |
| | Reverse: 5′-TGACAAGCTTCCCGTTCTCA-3′ |
| *LBX2-AS1* | Forward: 5′-TTCCCAAGAAGGTTCGATTG-3′ |
| | Reverse: 5′-TGCAGACTCCTGCTGTTGTT-3′ |
| *IL4R* | Forward: 5′-TGTGCCAAACGTCCTCACAGCA-3′ |
| | Reverse: 5′-TCCAGGCATCGAAAAGCCCGAA-3′ |

## Dual-luciferase reporter assay

The pGL3-Basic vector was utilized to establish wild-type or mutant IL4R promoter region luciferase reporter vectors. The vectors were co-transfected with sh-LBX2-AS1, oe-NFKB1, and negative controls into A172 cells with Lipofectamine 3000 (Invitrogen, USA). Luciferase activity was tested with a luciferase assay kit (K801-200, BioVision, USA).

## Radioimmunoprecipitation (RIP) assay

An RIP kit (Millipore, USA) was utilized to assay binding of lncRNA LBX2-AS1 and TF NFKB1 protein. Cells were lysed on ice, and supernatant was harvested by centrifugation. The cell extract was coprecipitated with antibody, washed with magnetic beads, and resuspended in RIP washing buffer. The antibody was added, and the magnetic bead-antibody complex was rinsed and collected. Following PCR detection, the sample was digested with proteinase K for RNA extraction [35].

## Chromatin immunoprecipitation (ChIP) assay

Chromatin immunoprecipitation analysis was completed with an EZ-Magna ChIP kit (Millipore, USA). Cross-linking was induced by applying 1% formaldehyde solution to cells, and it was quenched with 140 mM glycine. The resulting nucleoprotein complex was lysed to obtain DNA fragments of 200-500 bp, and immunoprecipitation was performed with experimental antibodies or IgG. After incubation at 4°C overnight, cross-linking of DNA was discarded, and qRT-PCR analysis was performed. Information on primers is provided in Table II.

## Data analysis

Statistical data analysis was performed using GraphPad Prism 7 (GraphPad Software, USA). Values were shown as mean ±SD from at least 3 independent repetitions. Two-group comparisons were made by *t*-test and multiple comparisons by one-way ANOVA. For cor-

**Table II.** Primer sequences

| Primer sets | Primer sequence (5′→3′) |
|---|---|
| Primer pair 1 | Forward: GGCCCTCCTGACCTGAAATAC |
| | Reverse: CGAACACAGGCCCCATAGAC |

relation analysis, the Pearson correlation coefficient was used. $P < 0.05$ was considered statistically significant.

## Results

### LBX2-AS1 is substantially elevated in GBM

Modulatory function of LBX2-AS1 in angiogenesis in GBM was not profound, so LBX2-AS1 was studied as a target gene. Differential expression analysis of TCGA data revealed that LBX2-AS1 was noticeably elevated in GBM tissues (Fig. 1A). LBX2-AS1 level in human astrocytes NHA and four GBM cell lines (U251, T98G, LN229, and A172) was assessed through qRT-PCR. LBX2-AS1 was notably enhanced in GBM cells (Fig. 1B). Since LBX2-AS1 in A172 and LN229 cells was relatively high, these two cell lines were subsequently utilized for silence treatment in functional experiments. LBX2-AS1 was elevated in GBM, and its abnormality may be implicated in GBM malignant progression.

### Silencing LBX2-AS1 inhibits malignant behaviors of GBM cells

To elucidate the influence of LBX2-AS1 on GBM cell biologic features, we transfected sh-NC and sh-LBX2-AS1 into LN229 and A172 cells, respectively. LBX2-AS1 was markedly decreased in sh-LBX2-AS1 group, and efficiency of LBX2-AS1 silencing in cells was sufficient for further experiments (Fig. 2A). Subsequently, *in vitro* cellular functional experiments were conducted to evaluate the influence of LBX2-AS1 silencing on GBM cell behaviors. As depicted in Figure 2B, C, with the si-NC group as the control, proliferation of LN229 and A172 cells was substantially repressed with sh-LBX2-AS1. Silencing of LBX2-AS1 notably repressed migration and invasion of LN229 and A172 cells compared to the control (Fig. 2D, E). In addition, through HUVECs for angiogenesis assays, compared with the control, branch length of blood vessels in the sh-LBX2-AS1 group was found to decrease, suggesting that silencing of LBX2-AS1 hindered angiogenesis (Fig. 2F). Furthermore, LBX2-AS1 regulates the epithelial-mesenchymal transition (EMT) signaling pathway [15], so EMT-related proteins were assayed by western blot. Silencing of LBX2-AS1 enhanced E-cadherin and inhibited N-cadherin, MMP-2, and VEGF-A [6] (Fig. 2G). We concluded that silencing LBX2-AS1 significantly repressed GBM cell malignant behaviors.

### LBX2-AS1 activates IL4R expression by binding to TF NFKB1

To investigate the downstream modulatory mechanism of LBX2-AS1 in GBM progression, we employed the lncMAP database to predict the trimeric complex of lncRNA LBX2-AS1 and discovered the LBX2-AS1/NFKB1/IL4R regulatory axis. RPISeq database scoring showed that SVM classifier and RF classifier values were both > 0.5, indicating that the interaction between LBX2-AS1 and NFKB1 was highly reliable (Fig. 3A). Meanwhile, the mechanism of NFKB1 in GBM is unclear [19], so NFKB1 was selected as the downregulated target. RIP detection was utilized to verify whether LBX2-AS1 binds the

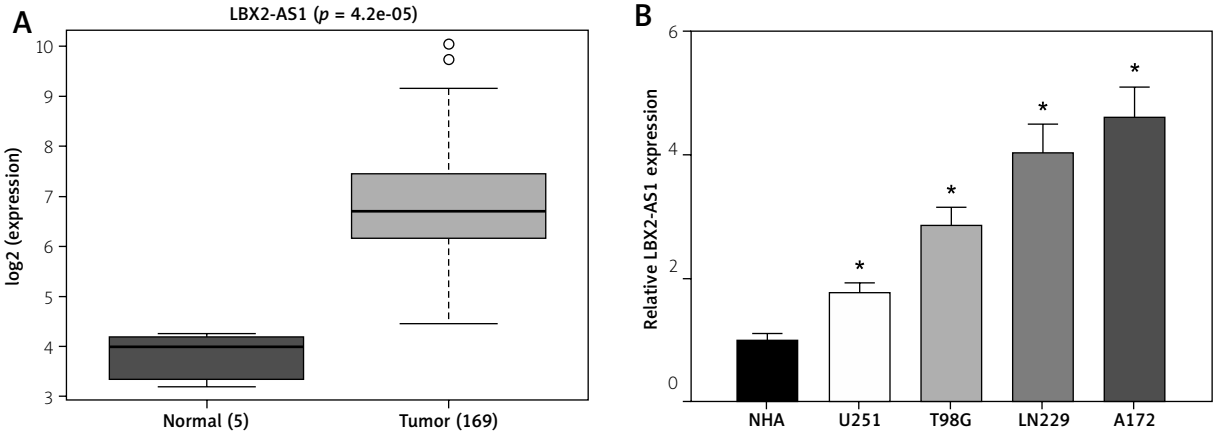


**Fig. 1.** LBX2-AS1 is highly expressed in glioblastoma (GBM). **A**) Box plot showing the expression levels of LBX2-AS1 in adjacent normal tissues and GBM tissues; **B**) Relative expression levels of LBX2-AS1 in normal human astrocytes (NHA) and four GBM cell lines (U251, T98G, LN229, and A172); *$p < 0.05$.

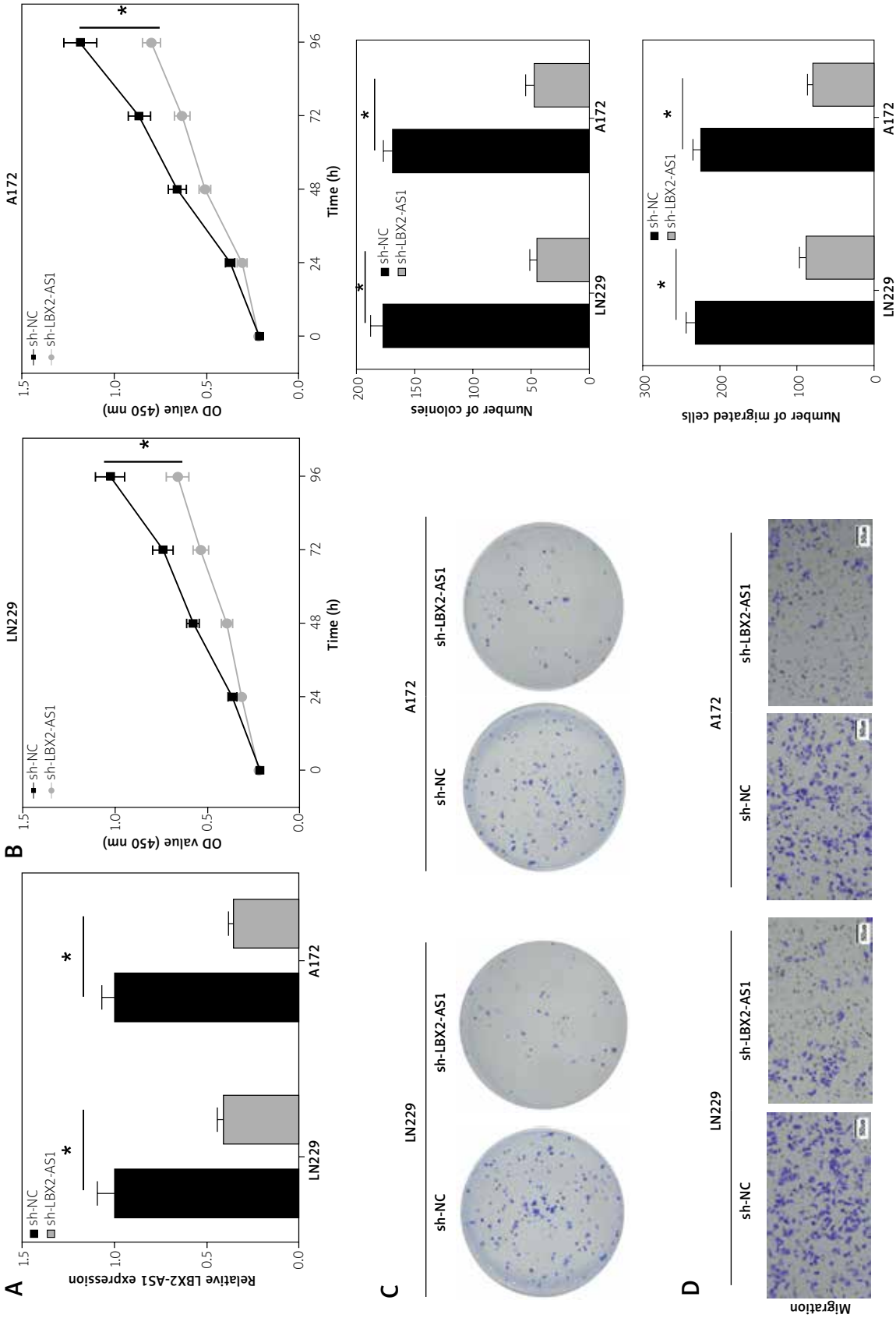


**Fig. 2.** Silencing of LBX2-AS1 represses malignant behaviors of glioblastoma (GBM) cells. **A**) qRT-PCR assayed transfection efficiency of sh-NC and sh-LBX2-AS1 treatment in LN229 and A172 cells; **B**, **C**) CCK-8 and colony formation assayed proliferation capacity after transfection; **D**) Transwell assayed migration and invasion after transfection.

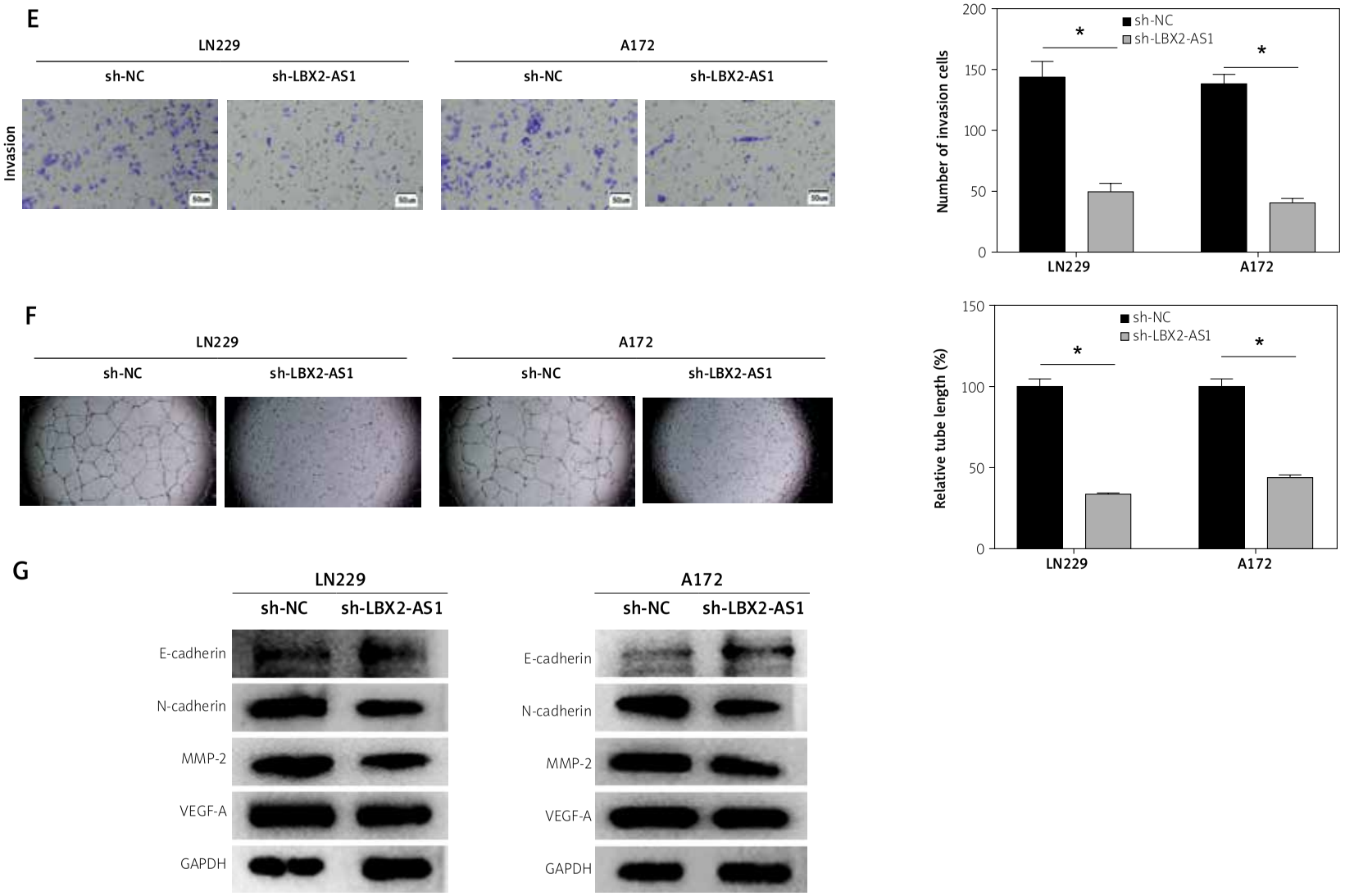


**Fig. 2.** Cont. **E**) Transwell assayed migration and invasion after transfection; **F**) Angiogenesis assay tested influence of LBX2-AS1 on angiogenesis after transfection; **G**) Western blot assessed protein expression of VEGF-A, N-cadherin, E-cadherin, and MMP-2 in LN229 and A172 cells after transfection; *$p < 0.05$.

TF NFKB1, and the results showed that compared with IgG, the addition of NFKB1 antibody significantly elevated enrichment of LBX2-AS1 in cells, implying that the NFKB1 protein specifically bound LBX2-AS1 (Fig. 3B). IL4R, as a downstream gene of TF NFKB1, can promote tumor dedifferentiation and metastasis, so IL4R was selected as the target for the study [5,9,27]. Bioinformatics analysis revealed that IL4R was remarkably elevated in GBM tumor tissues (Fig. 3C), and IL4R was significantly positively correlated with LBX2-AS1 and NFKB1 expression (Fig. 3D). Further qRT-PCR detection found that IL4R and NFKB1 expression in GBM cells was remarkably elevated (Fig. 3E). Based on the JASPAR database prediction of the IL4R promoter region and the binding sequence of NFKB1 (Fig. 3F), ChIP validated binding of NFKB1 to the IL4R promoter region. Compared with IgG, the enrichment of IL4R was dramatically elevated with NFKB1 (Fig. 3G). Furthermore, the regulatory relationship between NFKB1 and IL4R was detected by dual luciferase assay. Co-transfection of oe-NFKB1 and IL4R-WT markedly enhanced luciferase intensity, while luciferase activity of IL4R-MUT was not significantly influenced, suggesting that NFKB1 directly bound to IL4R. At the same time, the co-transfection of sh-LBX2-AS1 and IL4R-WT notably lowered luciferase intensity, while luciferase activity of IL4R-MUT was not remarkably influenced, indicating that IL4R could bind with LBX2-AS1 and NFKB1 (Fig. 3H). Meanwhile, increased LBX2-AS1 markedly enhanced mRNA and protein levels of IL4R (Fig. 3I, J). In summary, LBX2-AS1 can upregulate IL4R by binding to TF NFKB1 in GBM.

### LBX2-AS1 drives malignant phenotypes of GBM cells by increasing transcription of IL4R through binding to TF NFKB1

To investigate the impact of the LBX2-AS1/NFKB1/IL4R axis on biological features of GBM cells, rescue experiments were carried out by transfecting oe-NC + sh-NC, oe-LBX2-AS1 + sh-NC, oe-NC + sh-IL4R/sh-NFKB1, and oe-LBX2-AS1 + sh-IL4R/sh-NFKB1 into A172 cells. Compared with the oe-NC + sh-NC group, IL4R was substantially elevated in the oe-LBX2-AS1 + sh-NC group, decreased in the oe-NC + sh-IL4R group and oe-NC + sh-NFKB1 group, and restored to the oe-NC + sh-NC group level in the oe-LBX2-AS1 + sh-IL4R and oe-LBX2-AS1 + sh-NFKB1 groups. This indicated that silencing IL4R and NFKB1 reversed the promoting influence of LBX2-AS1 overexpression on IL4R expression (Fig. 4A, B, Fig. 5A, B). Enforced expression of LBX2-AS1 noticeably facilitated proliferation while silencing IL4R or NFKB1-repressed cell proliferation. Furthermore, silencing IL4R or NFKB1 reversed the stimulatory impact of LBX2-AS1 overexpression on GBM cell proliferation (Fig. 4C, D, Fig. 5C, D). Transwell results illustrated that migration and invasion ability were elevated by increased LBX2-AS1, decreased by silencing IL4R or NFKB1, and silencing IL4R or NFKB1 reversed the influence of forced LBX2-AS1 expression on GBM cells (Fig. 4E, Fig. 5E). The angiogenesis assay detected the angiogenesis in HUVECs. LBX2-AS1 overexpression could promote angiogenesis while silencing IL4R or NFKB1 could inhibit angiogenesis and reverse the stimulatory impact of LBX2-AS1 on angiogenesis (Fig. 4F, Fig. 5F). Western blot showed that LBX2-AS1 overexpression decreased E-cadherin protein and enhanced MMP-2, N-cadherin, and VEGF-A protein expression. Silencing IL4R or NFKB1 showed the opposite results, and silencing IL4R or NFKB1 rescued the impact of forced LBX2-AS1 expression on these proteins [6] (Fig. 4G, Fig. 5G). In summary, LBX2-AS1 drove the GBM cell malignant phenotype by activating transcription of IL4R through binding to the TF NFKB1.

## Discussion

Mounting evidence suggests that lncRNAs are pivotal in malignancy progression by affecting key events, including GBM [33,37]. As a newly discovered type of lncRNA in recent years, LBX2-AS1 is useful for predicting the prognosis of malignant glioma [18]. Recent studies have investigated LBX2-AS1 function in tumor biology, revealing LBX2-AS1 as a pivotal oncogene in various human solid tumors such as colorectal cancer [20], ovarian cancer [4], liver cancer [30], and gastric cancer [34], participating in manipulating proliferation, apoptosis, migration, and invasion behaviors. Additionally, Wen *et al.* [31] reported that silencing LBX2-AS1 in GBM could significantly repress the cell malignant phenotype through the Akt/GSK3β pathway. In line with these studies, we observed that LBX2-AS1 drove GBM cell malignant phenotypes. Furthermore, LBX2-AS1 promoted EMT-related protein (N-cadherin and MMP-2) expression. Importantly, Li *et al.* [17] also found that LBX2-AS1 promoted EMT in gliomas. Thus, the LBX2-AS1-mediated EMT process is crucial in the migration of GBM cells.

Tumor growth, invasion, and metastasis are dependent on angiogenesis, supplementing tumor cells with sufficient oxygen and nutrients [7]. Some studies have shown that amongst all clinical and pathological features of GBM, angiogenesis has strong prognostic significance, and extensive angiogenesis is often associated with poor prognosis [32]. Therefore, exploring new mechanisms that affect GBM angiogenesis is crucial for the development of new anti-angiogenic agents. We found that LBX2-AS1 enhanced the ability of HUVECs to form blood vessels *in vitro* and upregulated the expres-

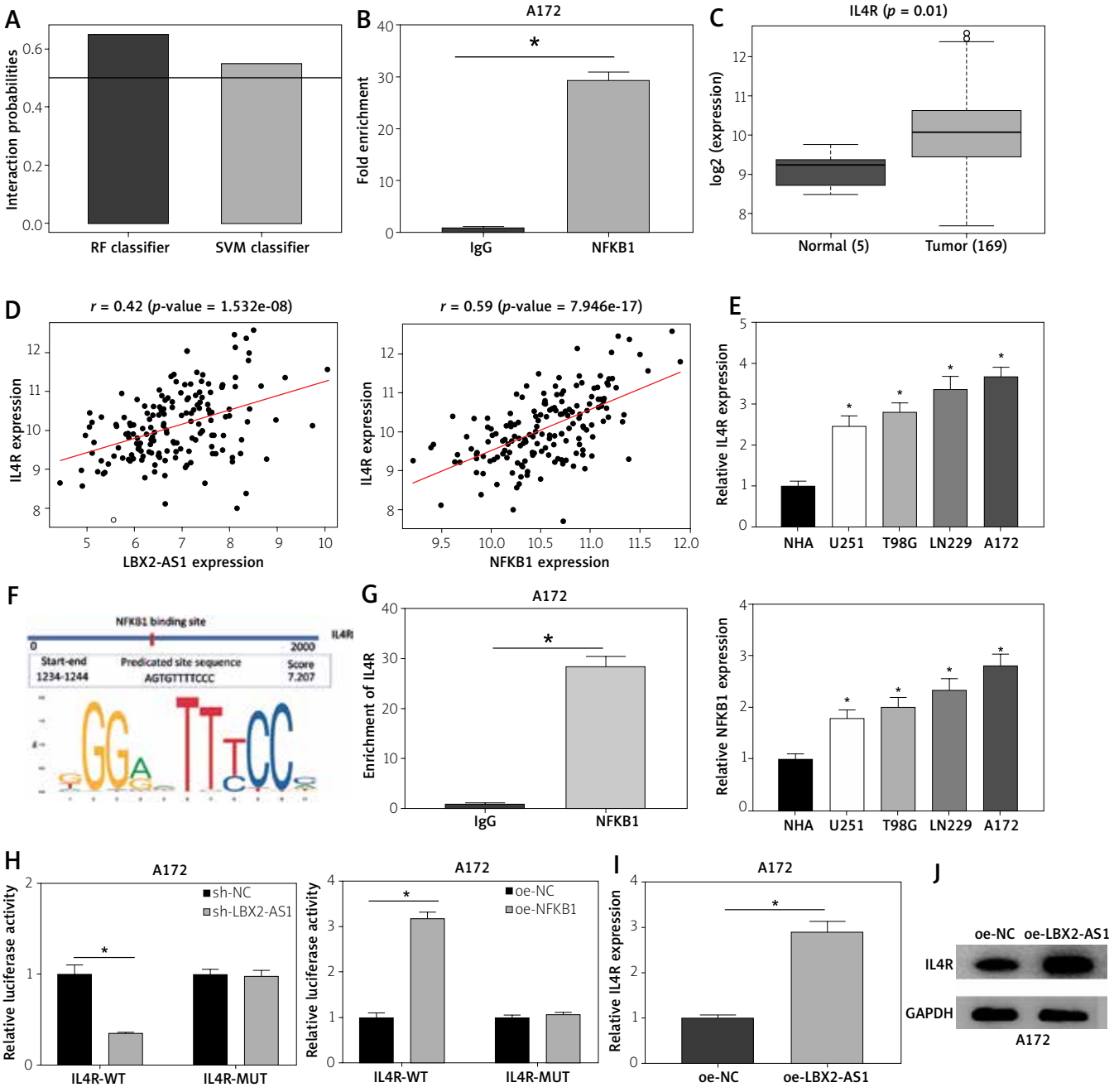


**Fig. 3.** LBX2-AS1 activates IL4R expression by binding to the TF NFKB1. **A**) RPISeq database predicted interaction score of LBX2-AS1 with NFKB1 protein; **B**) RIP detected the binding of LBX2-AS1 and NFKB1; **C**) Expression of IL4R in GBM tissue and adjacent tissues; **D**) Correlation between IL4R expression and LBX2-AS1/NFKB1 expression; **E**) Relative expression of IL4R and NFKB1 in normal astrocytes and four GBM cell lines; **F**) JASPAR database forecasted binding site of NFKB1 in promoter region of IL4R; **G**) ChIP experiment detected the binding of NFKB1 and IL4R in the promoter; **H**) Dual-luciferase assay confirmed binding of LBX2-AS1, NFKB1, with IL4R. Control (sh-NC or oe-NC); **I, J**) qRT-PCR and western blot assayed mRNA and protein levels of IL4R after LBX2-AS1 overexpression. *$p < 0.05$.

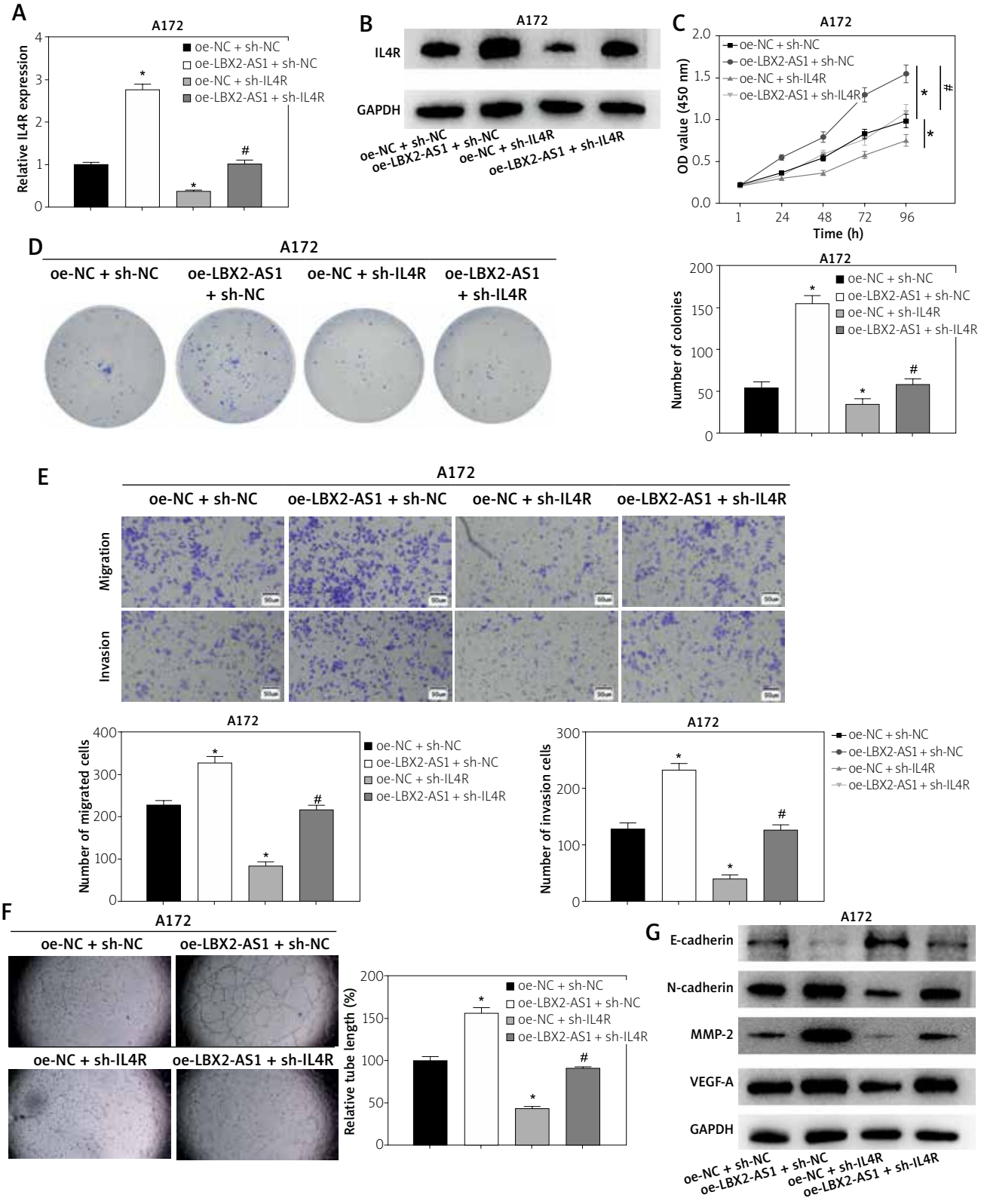


**Fig. 4.** LBX2-AS1 promotes glioblastoma (GBM) cell proliferation, migration, invasion, and angiogenesis by upregulating IL4R expression. **A, B**) qRT-PCR and western blot experiments assayed IL4R levels after transfection; **C, D**) CCK-8 and colony formation assayed cell proliferation ability; **E**) Transwell assayed cell migration and invasion; **F**) Tube formation assay tested angiogenesis after transfection; **G**) Western blot assayed protein expression of N-cadherin, E-cadherin, VEGF-A, and MMP-2 after transfection; *vs. oe-NC + sh-NC group, #vs. oe-LBX2-AS1 + sh-NC group, * and # $p < 0.05$.

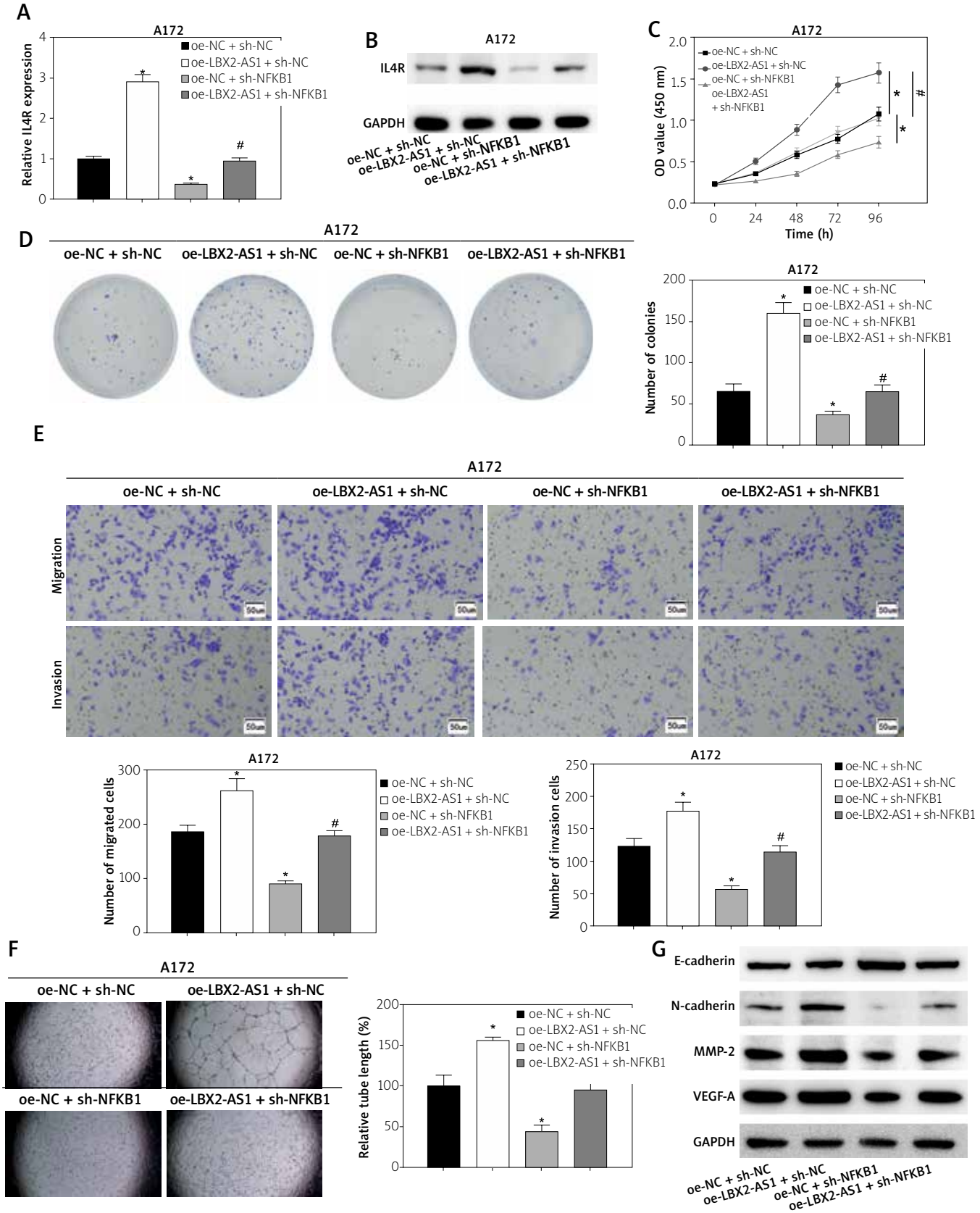


**Fig. 5.** LBX2-AS1 promotes glioblastoma (GBM) cell malignant behaviors by recruiting TF NFKB1. **A, B**) qRT-PCR and western blot experiments assayed IL4R levels after transfection; **C, D**) CCK-8 and colony formation assayed cell proliferation ability; **E**) Transwell assayed cell migration and invasion; **F**) Tube formation assay tested angiogenesis after transfection; **G**) Western blot assayed protein expression of N-cadherin, E-cadherin, VEGF-A, and MMP-2 after transfection; *vs. oe-NC + sh-NC group, #vs. oe-LBX2-AS1 + sh-NC group, * and # $p < 0.05$

sion of VEGFA, indicating that LBX2-AS1 had a role in inducing tumor angiogenesis in GBM. This study is the first to report the relation of LBX2-AS1 with tumor angiogenesis, suggesting that it may become a novel target for inhibiting angiogenesis in GBM.

Exploring LBX2-AS1 function in GBM angiogenesis and metastasis, we further found that LBX2-AS1 could upregulate IL4R expression by binding to the TF NFKB1. IL4R is overexpressed in many malignant tumors [1]. For example, IL4Rα (a component of IL4R) facilitates metastasis of breast cancer [26]; IL4R induces dedifferentiation, mitosis, and metastasis in rhabdomyosarcoma [9]. Clinical trial results have shown that patients with gliomas and astrocytoma are more likely to die if they have the IL4R rs1801275 GG genotype [11]. Similarly, heterozygous and homozygous mutations of the IL4R gene SNP S503P are significantly associated with glioma occurrence [23]. Therefore, the polymorphism of IL4R may have a role in survival of glioma patients. However, the regulatory role and related mechanisms of IL4R in GBM progression are uncharacterized. It was noted that IL4R was substantially upregulated in GBM, confirming an earlier study that found overexpression of IL4R in brain tumors *in situ* [12]. IL4R is involved in the process of angiogenesis, and epistatic interaction of IL4R SNP rs1801275 with VEGF-associated SNP rs6921438 is associated with a marked elevation in VEGF plasma levels [22]. Importantly, activation of the IL4/IL4R axis promotes tumor growth, angiogenesis, and metastasis, and inhibits anti-tumor immune responses [1]. However, little is known about whether IL4R is involved in GBM angiogenesis, so elucidating the key role of IL4R in angiogenesis may offer new evidence for the pathogenesis of GBM. This study found that silencing IL4R expression significantly repressed GBM cell malignant potential, and partially reversed the impact of LBX2-AS1 on GBM cells, which confirmed that IL4R may be an attractive GBM anti-angiogenesis therapy target.

In summary, this study found that *in vitro*, LBX2-AS1 facilitated GBM metastasis and angiogenesis. Its potential mechanism was that LBX2-AS1 induced IL4R expression by recruiting NFKB1, thereby further driving GBM cell proliferation, migration, invasion, and angiogenesis. Targeting the LBX2-AS1/NFKB1/IL4R regulatory axis may provide better strategies for anti-metastasis and anti-angiogenesis in GBM treatment. Based on these findings, identifying the exact mechanisms involved in the regulatory axis in GBM progression is necessary, and more research is needed to improve the understanding of this regulatory network.

## Disclosure

The authors report no conflict of interest.

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