IncRNA \textit{IGKJ2-MALLP2} suppresses LSCC proliferation, migration, invasion, and angiogenesis by sponging \textit{miR-1911-3p/p21}

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Abstract
Because advanced laryngeal squamous cell carcinoma (LSCC) is diagnosed as a malignant tumor with a poor prognosis, the associated mechanisms still need to be further investigated. As key players in the development and progression of LSCC, lncRNAs have attracted increasing attention from many researchers. In this study, a novel lncRNA termed \textit{IGKJ2-MALLP2} was identified and investigated for its effects on the development of LSCC. \textit{IGKJ2-MALLP2} expression was confirmed by RT-qPCR in 78 pairs of tissues and human laryngeal carcinoma cell lines. The results of this study showed that the expression of \textit{IGKJ2-MALLP2} was reduced in LSCC tissues and displayed close relationships with tumor stage, lymph node metastasis, and clinical stage. Using a dual-luciferase reporter assay, the ability of \textit{miR-1911-3p} to bind both \textit{IGKJ2-MALLP2} and \textit{p21} mRNA was demonstrated. \textit{IGKJ2-MALLP2} could upregulate \textit{p21} expression by competitively binding \textit{miR-1911-3p}. Moreover, \textit{IGKJ2-MALLP2} effectively hindered the invasion, migration, and proliferation of AMC-HN-8 and TU212 tumor cells. Furthermore, its high expression could hinder the secretion of VEGF-A and suppress angiogenesis. As revealed by the results of in vitro experiments, \textit{IGKJ2-MALLP2} overexpression could restrict tumor growth and blood vessel formation in a xenograft model of LSCC. As indicated from the mentioned findings, \textit{IGKJ2-MALLP2}, which mediates \textit{p21} expression by targeting \textit{miR-1911-3p}, was capable of regulating LSCC progression and could act as an underlying therapeutic candidate to treat LSCC.

KEYWORDS
angiogenesis, lncRNA, LSCC, p21, progression
1 | INTRODUCTION

Laryngeal carcinoma is one of the most common tumors of the respiratory tract, and LSCC is the most common subtype.\(^1\) In 2019 in the United States, 12,410 new cases of laryngeal carcinoma were reported and 3,760 associated deaths with an incidence rate occurring for men and women of c. 4:1.\(^2\) In particular, nearly 60% of patients are initially diagnosed at an advanced stage (stage III or IV),\(^3\) resulting in laryngeal carcinoma as one of the few tumors with a decrease in the 5-y survival rate, from 66% to 63%, over the past 40 y.\(^4\) Therefore, early diagnosis and therapy are required.

In many tumors, long noncoding RNAs (lncRNAs), which originate from the intergenic regions of mRNAs and are commonly transcribed by RNA polymerase II,\(^5,6\) are longer than 200 nucleotides, and are considered to play crucial roles in tumor development and act as tumor suppressor or oncogenic genes to mediate the biological behaviors of tumor cells.\(^7,8\) According to the results of some studies, lncRNA MIR22HG may participate in the progression of gastric cancer by mediating the Notch2 signaling pathways.\(^9\) IncRNA PCAT1 can facilitate tumor progression in many diseases.\(^10\) Of note, some studies have also shown that lncRNAs are crucial to the development of LSCC.\(^11-14\) These lncRNAs could compete for miRNA binding at shared response elements\(^15\) or can bind to transcription factors to disrupt the targeted mRNA expression.\(^16\) In our previous study, we identified a novel IncRNA, IGKJ2-MALLP2 (ID: NONHSAT072221 in Noncode) from an RNA sequencing assay in which the expression of IGKJ2-MALLP2 in tumor tissues was significantly lower than that observed in the adjacent tissues, and a follow-up analysis also showed that it might have an essential role in the development of laryngeal carcinoma.\(^17\) In addition, Bu et al\(^18\) also reported that the expression of IGKJ2-MALLP2 was significantly altered in some tumors and adjacent tissues. Nevertheless, the role of IGKJ2-MALLP2 in LSCC remains unclear. In the present study, we observed that in 78 patients with laryngeal carcinoma, IGKJ2-MALLP2 expression in laryngeal carcinoma tissues was noticeably lower than that observed in adjacent tissues and was significantly associated with tumor stage, clinical stage and lymph node metastasis. Furthermore, IGKJ2-MALLP2 could bind to miR-1911-3p and regulate the expression of p21, also known as cyclin-dependent kinase inhibitor 1A (CDKN1A), a well known protein associated with cancer. Low \(p21\) expression in laryngeal carcinoma can disrupt normal cell proliferation and differentiation and promote laryngeal carcinoma development.\(^19\) Moreover, the results of in vitro experiments demonstrated that IGKJ2-MALLP2 was involved in cell proliferation, metastasis, invasion, and angiogenesis-related activities. Therefore, the results of this study showed that IGKJ2-MALLP2 may function as a powerful tumor biomarker for the diagnosis and treatment of laryngeal carcinoma.

2 | MATERIALS AND METHODS

2.1 | Clinical sample collection

From July 2016 to June 2018, 78 LSCC and matched adjacent noncancerous tissue (standard samples) specimens were harvested from patients at the Second Affiliated Hospital of Harbin Medical University and rapidly frozen in liquid nitrogen. All patients were first diagnosed with LSCC and then received total or partial laryngectomy with or without cervical lymph node dissection. No preoperative chemotherapy or neoadjuvant therapy was performed. All procedures complied strictly with the laryngeal carcinoma staging criteria of the American Joint Committee on Cancer (AJCC) staging system (8th version) in 2017. All informed consent forms were obtained from the patients, and the project was approved by the Ethics Committee of the Second Hospital of Harbin Medical University.

2.2 | Cell culture

Human bronchial epithelioid cells (16-HBE) and human laryngeal cancer cells (TU212 and AMC-HN-8) were obtained from the BeNa Culture Collection (BNCC), all of which were cultured routinely in high glucose Dulbecco’s modified Eagle’s medium (H-DMEM, HyClone) containing 10% fetal bovine serum (Biological Industries). Human umbilical vein endothelial cells (HUVECs) were purchased from the American Type Culture Collection (ATCC) and were cultured in endothelial cell medium (ECM; ScienCell). When the cells reached 90% confluency, they were passaged with a 0.25% trypsin-EDTA solution (Beyotime Biotechnology). The cells were cultured in a humidified incubator at 37°C under an atmosphere with 5% \(\text{CO}_2\) in air (Thermo Fisher).

2.3 | Cell transfection

Using the IncRNA IGKJ2-MALLP2 and \(\text{miR}-1911-3p\) sequences in the NCBI database (https://www.ncbi.nlm.nih.gov/gene/), lentiviruses expressing negative control (NC) RNA and IncRNA IGKJ2-MALLP2 were developed by GeneChem Biotechnology in China, and a \(\text{miR}-1911-3p\) mimic and mimic-NC were designed by RiboBio in China. All of these sequences were transfected into cells in the logarithmic growth phase using Lipofectamine 2000 transfection reagent (Invitrogen) in accordance with the manufacturer’s protocol. When transfection and verification were completed, the cells were used in subsequent experiments.

2.4 | Quantitative real-time PCR (RT-qPCR)

Cells used for RT-qPCR were cleaned with cold phosphate-buffered saline (PBS). Subsequently, total RNA was obtained using TRIzol Reagent (Invitrogen), and cDNA was generated from 2 µg of total RNA using a Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics). RNA expression was quantitatively assessed by RT-qPCR using a FastStart Universal SYBR Green Master kit (Roche Diagnostics). The relative expression levels of IncRNA IGKJ2-MALLP2, \(p21\) and VEGF-A were normalized to that of GAPDH, while those of \(\text{miR}-1911-3p\) were normalized to that of U6, with expression
levels calculated using the $2^{-\Delta\Delta CT}$ method. The sequences of primers used for RT-qPCR are presented in Table 1.

### 2.5 Western blot (WB) analysis

For WB analysis, cells were lysed in RIPA buffer (Beyotime Biotechnology, China) supplemented with 1 mmol/L phenyl-methanesulfonyl fluoride (Beyotime Biotechnology) and 1x protease inhibitor (Beyotime Biotechnology). The cell lysates were centrifuged, and the pelleted debris was discarded. Total protein was then mixed with 5x SDS-PAGE loading buffer and heated to 100°C for 5 min to prevent protein degradation. Protein samples (30 μg) were run on an SDS-PAGE gel and then transferred to a PVDF membrane. Subsequently, the membrane was blocked with 5% milk for 1 h and then incubated with the indicated primary antibody overnight. After being washed with TBS Tween (TBST) solution, the membrane was incubated with a secondary antibody and detected with enhanced chemiluminescence (ECL) reagents. Relative protein expression was analyzed using ImageJ software.

### 2.6 Migration and invasion assay

Cells were seeded into the upper chamber of a 24-well transwell plate (Corning), while the lower chamber was filled with 0.6 mL of H-DMEM supplemented with 10% FBS. At the indicated times, the cells on the upper surface of the filter were scratched, while cells on the lower surface were fixed with 4% paraformaldehyde and stained with crystal violet. To identify the invasion ability of the cells, the upper chamber was coated with a layer of 20% Matrigel (Corning), and the subsequent procedures were performed as described above.

### 2.7 Cell counting kit-8 (CCK-8) assay

To observe cell proliferation, a CCK-8 assay (Dojindo) was performed. Cells were seeded in 96-well plates (Corning) and, at the indicated times, 10 μL of CCK-8 solution was added to each well, after which the cells were incubated at 37°C for 2 h. The absorbance value of each well was measured at 450 nm and was reported as the cell viability.

### 2.8 Colony formation assay

Cells were suspended in 2 mL of culture medium and then cultured in a 35-mm diameter Petri dish for 2 wk. The culture medium was replaced every other day and, after 2 wk, the cells were fixed with 4% paraformaldehyde and stained with crystal violet. Plates with positive colony formation were imaged.

### 2.9 Cell-cycle analysis

Cells were seeded into 6-well plates and cultured overnight until reaching c. 60%-70% confluency. To perform cell-cycle analyses, cells were collected and resuspended in 500 μL of propidium iodide (PI)/RNase staining buffer for 30 min at 37°C in the dark. Subsequently, the cell cycle was analyzed by flow cytometry.

### 2.10 Enzyme-linked immunosorbent assay (ELISA)

Transfected cells were cultured in complete medium until cells reached c. 90% confluency. Then, the culture supernatant was harvested and centrifuged to remove the cell debris, and the conditioned medium (CM) supernatant was aliquoted and stored at −80°C. Subsequently, the VEGF-A concentration in CM was measured using a commercial ELISA kit (R&D Systems).

### 2.11 Angiogenesis assays

For tube formation assays, a 48-well plate was covered with Matrigel, and HUVECs were seeded and cultured in a mixed medium (CM/ECM, 50:50, v/v). At 6 h later, tube formation was observed under a microscope. For the aortic ring sprouting assay, thoracic aortas were acquired from a 6-wk-old Sprague-Dawley rat. Next, 2 ml-thick aortic rings were placed in a 48-well plate (Corning) and covered by Matrigel. Then, 50 μL of H-DMEM supplemented with 10% FBS and

### TABLE 1 The sequences of specific primers

| Primer       | Forward primer (5' to 3')                  | Reverse primer (5' to 3')                  |
|--------------|--------------------------------------------|--------------------------------------------|
| IGKJ2-MALLP2 | GACATATCTTTCAGGTTCCC                      | GTGAGACATACATCACCACCATCC                   |
| p21 (for qRT-PCR) | TCCAGCGACCTTTCTCTCTACAC | TCCATAGCTCTCTACGCACCACATC                  |
| miR-1911-3p (for qPCR) | GCGCACCAGGCCATTGTG | AGTGCAGGGTCCGGAGTTAT                    |
| VEGF-A (for qPCR) | GTGACAAGCCAAGGCGGTAG | GATGTTGGTGTGTTGTTGAGCATG                  |
| U6 (for qPCR) | CTCGCTTCGGCAGCACA                      | AACGCTTCAGAATTTGCGT                       |
| GAPDH (for qPCR) | ACCCACTCTCTACCTTTGAC | TGTGTCTGTACCCAAATTCGTT                   |
50 µL of CM was added to each well. Finally, 6 d later, the resulting mesh structures were imaged.

2.12 | Luciferase reporter assays

IncRNA-IGKJ2-MALLP2-WT, IncRNA-IGKJ2-MALLP2-MUT, p21-3’UTR-WT, and p21-3’UTR-MUT were obtained by PCR. After the correct sequences were confirmed by sequencing, NotI/XbaI-digested pcFluc-Check2 fragments were ligated into a NotI/XbaI-digested plasmid. Luciferase assays were performed using 293T cells. Cells were seeded into 96-well plates, and, after reaching 50%-70% confluency, the cells were transfected with pSi-Check2-IncIGKJ2-MALLP2-WT (or pSi-Check2-IncIGKJ2-MALLP2-MUT) or pSi-Check2-P21-Wt (or pSi-Check2-P21-MUT) and the miR-1911-3p mimic or mimic-NC with Lipofectamine 3000 (Invitrogen).

The luciferase activity in cell lysates was measured 48 h after cells were transfected with a Dual-Luciferase System (Luciferase Assay Reagent Promega).

2.13 | Animal xenograft tumor model

All animal experiments were approved by the Ethics Committee of Harbin Medical University, and the animal experiments were performed in the Second Affiliated Hospital of the Harbin Medical University. Here, 4-wk-old male nude BALB/c mice were provided by the Beijing Charles River Laboratory Animal Center and were fed and housed in pathogen-free cages. To observe tumor formation, TU212 cells stably expressing IGKJ2-MALLP2 and NC under puromycin selection were identified. In total, 1 x 10^7 TU212 cells stably expressing of NC/IGKJ2-MALLP2 were suspended in 200 µL of PBS
and injected hypodermically into the right side of BALB/c nude mice \( (n = 6) \). The mice were anesthetized with 3% isoflurane and euthanized by \( \text{CO}_2 \) inhalation. Subsequently, the tumors were removed, weighed, and fixed in 4% paraformaldehyde.

### 2.14 Hematoxylin and eosin (H&E) staining

Tumor samples were embedded in paraffin and cut into 5 μm-thick sections before being dewaxed, hydrated, and stained with H&E.

### 2.15 Immunohistochemistry (IHC)

The paraffin-embedded tumor tissues were removed from the paraffin and rehydrated for IHC, after which antigen recovery was performed under high pressure in an EDTA buffer solution. After incubation with primary and secondary antibodies, the slices were incubated with dianobenzidine and counterstained with hematoxylin (Solarbio). Subsequently, the samples were imaged under a microscope (Olympus).

### 2.16 Statistical analysis

All experiments were performed at least 3 times, and the data are presented as the mean values ± SEMs. One-way ANOVA and two-tailed Student t test were conducted to analyze differences between samples. The associations between miR-1911-3p and IGKJ2-MALLP2 expression were determined by Spearman rank correlation. The correlations between IGKJ2-MALLP2 expression and clinicopathological characteristics were assessed using a chi-squared \( (\chi^2) \) test. Differences were considered significant at \( P < .05 \).

### 3 RESULTS

#### 3.1 IGKJ2-MALLP2 expression in laryngeal carcinoma tissue and laryngeal carcinoma cell lines

To assess the expression of IGKJ2-MALLP2 in laryngeal carcinoma, 78 pairs of LSCC tissues and adjacent specimens were analyzed by RT-qPCR. The results showed that IGKJ2-MALLP2 expression was considerably lower in laryngeal carcinoma tissues than that observed in adjacent normal tissues (Figure 1A). Specifically, IGKJ2-MALLP2 expression was decreased in 84.6% \((66/78)\) of the test samples. Moreover, the results suggested that the expression of IGKJ2-MALLP2 was negatively associated with advanced clinical stage, lymph node metastasis and tumor stage in patients with laryngeal carcinoma (Figure 1B-D). To further evaluate the association between IGKJ2-MALLP2 expression and clinical pathological characteristics, 78 human laryngeal carcinoma tissue samples were divided into 2 groups, the high IGKJ2-MALLP2-expressing group \((n = 39)\) and the low IGKJ2-MALLP2-expressing group \((n = 39)\), according to the median ratio of relative IGKJ2-MALLP2 expression. The correlation analysis revealed that low IGKJ2-MALLP2 expression was tightly associated with tumor stage \( (P = .0225) \), lymph node metastasis \( (P = .0015) \) and clinical stage \( (P = .0012) \). In contrast, drinking history, smoking history, age, and gender displayed no correlations with IGKJ2-MALLP2 expression (Table 2). Furthermore, low IGKJ2-MALLP2 expression was also observed in 16-HBE, AMC-HN8, and TU212 cells (Figure 1E).

#### 3.2 IGKJ2-MALLP2 regulates cell proliferation, migration and invasion

After transfecting cells with the lentivirus, IGKJ2-MALLP2 expression was increased in both IGKJ2-MALLP2 groups of AMC-HN-8 and TU212 cells (Figure 2A). In the CCK-8 assay, the proliferation of cells was decreased after the overexpression of IGKJ2-MALLP2 in both AMC-HN-8 and TU212 cells (Figure 2B). In the colony formation assay, the results showed that IGKJ2-MALLP2 overexpression inhibited the formation of both AMC-HN-8 and TU212 tumor cell colonies (Figure 2C). To
elucidate the effects of IGKJ2-MALLP2 on cell proliferation, cell-cycle assays were performed. The results showed that IGKJ2-MALLP2 overexpression caused a notable increase in cell-cycle arrest at the G1-G0 phase in both AMC-HN-8 and TU212 cells (Figure 2D).

The migration and invasion abilities of tumor cells are crucial in tumor development. In the migration assay, the number of cells passing through the filter in the IGKJ2-MALLP2 overexpression group was considerably decreased in both AMC-HN-8 and TU212 cells (Figure 2E). In the invasion assay, the number of AMC-HN-8 and TU212 cells that passed through the Matrigel was also reduced when they were transfected with the IGKJ2-MALLP2 lentivirus (Figure 2F).

3.3 IGKJ2-MALLP2 suppresses VEGF-A expression and angiogenesis

Blood vessels are extremely important for tumor development. To elucidate the effects of IGKJ2-MALLP2 on angiogenesis, tube
FIGURE 3 IncRNA IGKJ2-MALLP2 suppresses angiogenic activities in vitro. A, The tube structures formed by HUVECs treated with CM from AMC-HN-8 and TU212 cell cultures. B, The mesh structures sprouting from aortic rings treated with CM from AMC-HN-8 and TU212 cell cultures. C, Quantitative analysis of tube structures. D, Quantitative analysis of mesh structures. E, Relative VEGF-A mRNA expression in AMC-HN-8 and TU212 cells transfected with NC or IGKJ2-MALLP2 lentivirus. F, The concentration of VEGF-A in the culture supernatants of AMC-HN-8 and TU212 cells transfected with NC or IGKJ2-MALLP2 lentivirus. The data are presented as the means ± SD, n = 3; *, P < .05; **, P < .01; ***, P < .001.
FIGURE 4 IncRNA IGKJ2-MALLP2 binds miR-1911-3p to regulate p21 in LSCC cells. A, The subcellular position of IGKJ2-MALLP2 was ascertained in the cytoplasm or nucleus using GAPDH and U6 as cytoplasmic and nuclear controls, respectively (P < .05). B, Illustration showing the vector structure of IGKJ2-MALLP2 and p21-3'UTR. C, The potential binding sites of miR-1911-3p and IGKJ2-MALLP2. D, Luciferase reporter assay results demonstrated the interaction between miR-1911-3p and IGKJ2-MALLP2. E, The potential binding sites of miR-1911-3p and p21-3'UTR. F, Luciferase reporter assay results demonstrated the interaction between miR-1911-3p and p21-3'UTR. G, The level of miR-1911-3p expression in laryngeal cancer and noncancerous tissues (n = 78). H, Spearman’s correlation analysis between miR-1911-3p and IGKJ2-MALLP2 levels in LSCC tissues (n = 78, P < .001). I, The level of mRNA p21 expression in laryngeal cancer and noncancerous tissues (n = 78). J, Spearman’s correlation analysis between miR-1911-3p and p21 levels in LSCC tissues (n = 78, P < .01). The data are presented as the means ± SD, n = 3 **; P < .01; *** P < .001.
formation and aortic ring sprouting assays were performed. In contrast with that observed using CM from the NC group, the number of tube structures formed by HUVECs decreased when they were incubated in CM from both the AMC-HN-8 and TU212 IGKJ2-MALLP2-overexpressing groups (Figure 3A, C). In the aortic ring sprouting assay, fewer mesh structures were also observed in CM from the IGKJ2-MALLP2 overexpression group than that observed in CM from NC group for both AMC-HN-8 and TU212 cells (Figure 3B, D). Due to its role as one of the most important cytokines to promote angiogenesis, we measured the levels of VEGF-A in CM from AMC-HN-8 and TU212 cells. The RT-qPCR results showed that IGKJ2-MALLP2 overexpression inhibited the mRNA expression of VEGF-A in both AMC-HN-8 and TU212 cells (Figure 3E). In addition, the concentration of VEGF-A in the culture supernatant was also decreased when AMC-HN-8 and TU212 cells were transfected with the IGKJ2-MALLP2 lentivirus (Figure 3F).

3.4 | IGKJ2-MALLP2 regulates LSCC cells by sponging miR-1911-3p/p21

To elucidate how IncRNA IGKJ2-MALLP2 functions, in this study, the IGKJ2-MALLP2 contents in nuclear and cytoplasmic cell fractions were determined separately. The results showed that IGKJ2-MALLP2 primarily occurs in the cytoplasm (Figure 4A). As it has been reported that lncRNA in cytoplasm could realize its function by ceRNA, we wanted to assess the sponging activity of IGKJ2-MALLP2. After creating the vector structure of IGKJ2-MALLP2 and p21-3’UTR (Figure 4B), miR-1911-3p was predicted to interact with IncRNA IGKJ2-MALLP2 and p21 using DIANA-LncBase v.2.0, TargetScan 7.2 and MiRTarBase 7.0 software. We identified the corresponding targets for IncRNA IGKJ2-MALLP2, miR-1911-3p and p21-3’UTR (Figure 4C, E), after which the associated mechanism was verified in cells using dual-luciferase reporter assays. The present study showed that in contrast with cells cotransfected with mimic-NC and IGKJ2-MALLP2-WT, the luciferase activity of cells cotransfected with the miR-1911-3p mimic and IGKJ2-MALLP2-WT was considerably decreased, whereas no change in activity was observed in cells cotransfected with the mimic and IGKJ2-MALLP2-MUT (Figure 4D). Similarly, in contrast with that observed in cells cotransfected with mimic-NC and p21-WT, the luciferase activity of cells cotransfected with the mimic and p21-WT was considerably decreased, whereas no change in activity was observed in cells cotransfected with the mimic and p21-MUT (Figure 4F). The expression of miR-1911-3p and p21 mRNA in LSCC tissue was assessed, and the results revealed that miR-1911-3p exhibited high expression (Figure 4G) that was inversely correlated with IGKJ2-MALLP2 expression (Figure 4H), whereas p21 exhibited low expression (Figure 4I) that was inversely correlated with miR-1911-3p expression (Figure 4J).

To further investigate the IGKJ2-MALLP2/miR-1911-3p/p21 axis, AMC-HN-8 and TU212 cells were transfected with the NC or IGKJ2-MALLP2 lentivirus. Compared with that observed in cells transfected with the NC lentivirus, the expression of miR-1911-3p was decreased and the mRNA/protein expression of p21 was increased in cells transfected with the IGKJ2-MALLP2 lentivirus (Figure 5A, B).

3.5 | IGKJ2-MALLP2 binds miR-1911-3p to regulated p21 in TU212 cells

TU212 cells were transfected with the miR-1911-3p mimic or mimic-NC and the IGKJ2-MALLP2 or NC lentivirus. In contrast with that observed in the NC + mimic-NC group, the expression of miR-1911-3p was increased in the NC + mimic group (Figure 6A). The expression of miR-1911-3p was lower in the NC + mimic group than that observed in the NC + mimic group (Figure 6A). To further demonstrate the relationships between IGKJ2-MALLP2 and miR-1911-3p/p21, the expression of p21 in each group was assessed. The results showed that the p21 mRNA and protein levels in the NC + mimic group were the lowest among the 3 groups (Figure 6A, B). The CCK-8 assay results confirmed that cell proliferation in the
NC + mimic group was greater than that observed in the NC + mimic-NC and IGKJ2-MALLP2 + mimic groups (Figure 6C). In contrast with that observed in the NC + mimic-NC and IGKJ2-MALLP2 + mimic groups, the number of tumor colonies in the NC + mimic group was increased (Figure 6D, G). Furthermore, the migration and invasion abilities of TU212 cells in the NC + mimic group was also increased compared to that observed in the NC + mimic-NC and IGKJ2-MALLP2 + mimic groups (Figure 6E, F, H, I).

3.6 | Effect of IGKJ2-MALLP2 in vivo

The tumor formation of TU212 cells overexpressing IGKJ2-MALLP2 in mice was smaller and lighter than that of TU212 cells transfected with the NC lentivirus in vivo (Figure 7A-C). The IHC staining results showed increased p21 expression in the IGKJ2-MALLP2 overexpression group (Figure 7D, E), while H&E staining results showed the presence of fewer blood vessels in the IGKJ2-MALLP2 overexpression group (Figure 7F, G).

4 | DISCUSSION

As one of the most common head and neck squamous cell carcinomas, LSCC causes substantial economic and health burdens to society worldwide. Because the incidence of LSCC grows every year, it is urgent to understand the pathogenesis of LSCC and discover new diagnostic biomarkers for better treatments. The results of the

**FIGURE 6** Verification of the relationships between IGKJ2-MALLP2 and miR-1911-3p/p21 in TU212 cells. A, The relative expression of miR-1911-3p and p21 mRNA in TU212 cells with different transfections. B, The protein levels of p21 in each group were ascertained by WB and normalized to β-actin. C, The proliferation of TU212 cells in different groups was examined by CCK-8 assay. D, Colony formation assays to ascertain the proliferation ability of TU212 cells. E, Migration assays to ascertain the cell migration ability of TU212 cells with different treatments. F, Invasion assays to analyze the invasive ability of cells. G, Quantitative analysis of colonies in the different groups. H, Quantitative analysis of the number of TU212 cells migrating through the filter. I, Quantitative analysis of the number of TU212 cells passing through the filter and Matrigel. The data are presented as the means ± SD; n = 3; *, P < .05; **, P < .01; ***, P < .001
present study demonstrated that IGKJ2-MALLP2 had a clear regulatory role in LSCC in vitro and in vivo and may be a new therapeutic target.

IncRNAs are the largest and most diverse category of noncoding transcripts, and up to 60,000 IncRNA genes are present in the human genome.6 These genes can interact with protein molecules, RNA or DNA, regulating gene expressions and affecting cellular processes through different mechanisms.21 Although a considerable number of IncRNAs have been identified in the human genome, only a few genes have been experimentally verified and annotated in carcinomas. For instance, in non-small-cell lung cancer, IncRNA NEAT1 has been shown to regulate tumor progression and apoptosis and to induce CSC-like phenotypic variations by interacting with RNA or protein.22-24 IncRNA NEAT1 has been also shown to be highly expressed in nasopharyngeal cancer and LSCC and can regulate radio-resistance, proliferation, invasion and apoptosis.25,26 In addition, some other important IncRNAs (eg, H19,27 NOTCH1,28 MALAT129 and HOXAT30) were proven to be expressed in both tumors and adjacent tissues. Furthermore, some IncRNAs expressed in laryngeal carcinomas have been studied. For instance, IncRNA FOXD2-AS1 was shown to enhance chemotherapy resistance in LSCC by activating STAT3.31 And, in the present study, the novel IncRNA IGKJ2-MALLP2 was shown to have low expression in laryngeal carcinoma tissues and high expression in the normal adjacent tissues. When IGKJ2-MALLP2 expression was increased in laryngeal carcinomas, the expression of p21 was down-regulated in vitro, and the xenograft tumor formation and proliferation were inhibited in vivo. Therefore, IGKJ2-MALLP2 was shown to have a regulatory role in LSCC. Meanwhile, the expression of IGKJ2-MALLP2 was verified to be decreased in the tumor tissues from 18 patients of LSCC. Therefore, we presume that low expression of IGKJ2-MALLP2 may be a novel therapeutic target in LSCC.

**FIGURE 7** Effects of IncRNA IGKJ2-MALLP2 on tumor growth in vivo. A, B, Representative images of xenograft tumors formed by TU212 cells transfected with NC or IGKJ2-MALLP2 lentivirus. C, Tumor weights from NC and IGKJ2-MALLP2 groups. D, IHC staining showed the differences in p21 expression between the NC and IGKJ2-MALLP2 groups, with the associated bar chart (E). F, H&E staining showing the different number of blood vessels in the 2 groups, with the associated bar chart (G). The data are presented as the means ± SD of 6 independent experiments. **, *P < .01; ***, *P < .001
carcinoma cells with an IGKJ2-MALLP2 lentivirus, it inhibited the proliferation, colony formation, migration and invasion of these cells.

To determine how IGKJ2-MALLP2 functions, the level of IGKJ2-MALLP2 expression in the cytoplasm and nucleus of cells was assessed. The results of the present study showed that IGKJ2-MALLP2 was primarily localized in the cytoplasm. Over recent years, studies have shown that ceRNAs represent an extremely important post-transcriptional regulatory system in which lncRNAs sponge miRNAs and sometimes degrade the targeted miRNA, to alter the expression of corresponding transcribed genes thereby affecting the occurrence and development of tumors. For instance, IncRNA JPX can upregulate Twist1 expression via competitive sponging of miR-33a-5p and downregulating the expression of miR-33a-5p, and participate in the progression and lung metastasis of endometrial carcinoma through the activation of Wnt/β-catenin signaling. IncRNA Gm10451 can regulate the expression of pTIP mRNA by binding miR-338-3p, which facilitates the differentiation of Nkx6.1+ pancreatic progenitor cells and increases the differentiation efficiency and cell function of mature β-like cells. There are also other lncRNAs that can participate in ceRNA networks and play roles in the development of carcinoma. The results of the present study showed that the interaction of lncRNA IGKJ2-MALLP2, miRNA-1911-3p and p21 formed a ceRNA network regulating laryngeal carcinoma growth and metastasis. However, further studies are required to ascertain the abundance of these 3 components and to verify the functional activity of the ceRNA.

Persistent angiogenesis plays an essential role in tumor growth and metastasis. Therefore, identifying promising anti-angiogenesis targets is considered to be an approach to treat cancer. Previous studies have reported that in colorectal cancer, the novel primate-specific lncRNA FLANC not only affects tumor growth but also participates in angiogenesis by regulating the STAT3/VEGF-A axis. In breast cancer IncRNA RAB11B-AS1 was reported to be induced under hypoxia and facilitate the expression of angiogenic factors (eg, VEGF-A and ANGPTL4) in breast cancer cells by facilitating recruitment of RNA polymerase II, leading to angiogenesis and metastasis in breast cancer. Furthermore, regarding the relationship between p21 and vascular endothelial growth factor A (VEGF-A), it was observed that when p21 levels increased, VEGF-A expression decreased. The identical phenomenon was also confirmed in this study. When p21 expression was upregulated by the overexpression of IGKJ2-MALLP2, VEGF-A expression was downregulated, and angiogenic activities were also reduced. Furthermore, in the xenograft tumor model, we also observed that with the increased expression of p21, the number of blood vessels decreased. The aforementioned results indicate that low IGKJ2-MALLP2 expression could facilitate angiogenesis by regulating p21 expression via the mediator VEGF-A. Based on these results, we demonstrate that IGKJ2-MALLP2 can not only regulate the function of TU212 laryngeal carcinoma cells but also the associated angiogenesis in the surrounding area.

In summary, the results of our study show that lncRNA IGKJ2-MALLP2 can regulate p21 expression as a ceRNA by sponging miR-1911-3p. IGKJ2-MALLP2 regulates LSCC cell proliferation, colony formation, migration, and invasion. Moreover, it can also regulate angiogenesis in the area surrounding laryngeal carcinoma. A better understanding of the IGKJ2-MALLP2/miR-1911-3p/p21 axis may provide new strategies and targets for the further treatment of laryngeal carcinoma.

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DISCLOSURE
The authors have no conflicts of interest.

ETHICAL CONSIDERATIONS
The present study was approved by the Ethics Committee of The Second Affiliated Hospital of Harbin Medical University. The research has been carried out in accordance with the World Medical Association Declaration of Helsinki. All patients and healthy volunteers provided written informed consent prior to their inclusion within the study. All animal procedures were performed following approval from the Animal Care and Use Committee of The Second Affiliated Hospital of Harbin Medical University.

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