Overexpression of KIAA1199 is an independent prognostic marker in laryngeal squamous cell carcinoma

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Background: KIAA1199 is a recently identified novel gene that is up regulated in various human cancers with poor survival, but its role and the underlying mechanisms in laryngeal squamous cell carcinoma remain unknown. Here, we collected tissues from 105 cases of laryngeal squamous cell carcinoma (LSCC) to investigate the relationships between KIAA1199 protein expression and clinical factors.

Methods: Western blotting and quantitative real-time PCR (qPCR) were utilized to detect the protein and mRNA expression of KIAA1199 in LSCC tissue. Immunohistochemistry (IHC) staining was used to detect the expression of KIAA1199. Patient clinical information, for instance sex, age, pathological differentiation, clinical region, T stage, N stage, clinical stage, operation type, neck lymph dissection, smoking status, and drinking status, were recorded. Kaplan-Meier survival analysis and Cox analysis were applied to find out the relationship between KIAA1199 and LSCC.

Results: Western blotting results showed KIAA1199 protein was significantly higher in tumor tissues vs adjacent non cancerous tissues (0.9385±0.1363 vs1.838±0.3209, P=0.04). It revealed that KIAA1199 mRNA expression was considerably higher in tumor tissues (P<0.001) than in adjacent non cancerous tissues by qPCR. IHC results showed , the up-regulated KIAA1199 expression, which was related with some severe clinicopathological parameters: pathologic differentiation (P, 0.002), T stage (P<0.001), N stage (P<0.001), clinical stage (P<0.001), survival time (P, 0.008) and survival status (P<0.001). Kaplan-Meier survival analysis revealed that patients with high KIAA1199 protein expression had poor overall survival(OS) (P<0.05). Cox analysis suggested that the KIAA1199 protein expression was an independent prognostic marker for LSCC patients (P<0.001).

Conclusion: Our findings revealed that KIAA1199 protein expression may be used to predict LSCC patient outcome.

1 **Overexpression of KIAA1199 is an independent**

2 **prognostic marker in laryngeal squamous cell**

3 **carcinoma**

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Abstract

Background: KIAA1199 is a recently identified novel gene that is upregulated in various human cancers with poor survival, but its role and the underlying mechanisms in laryngeal squamous cell carcinoma remain unknown. Here, we collected tissues from 105 cases of laryngeal squamous cell carcinoma (LSCC) to investigate the relationships between KIAA1199 protein expression and clinical factors.

Methods: Western blotting and real time quantitative PCR (RT- PCR) were used for detect the protein and mRNA expression of KIAA1199 in LSCC tissue. Immunohistochemistry (IHC) staining was used to detect the expression of KIAA1199. Patient clinical information, for instance sex, age, pathological differentiation, clinical region, T stage, N stage, clinical stage, operation type, neck lymph dissection, smoking status, and drinking status, were recorded. Kaplan-Meier survival analysis and Cox analysis were applied to identify the relationship between KIAA1199 and LSCC.

Results: Western blotting results showed KIAA1199 protein was significantly higher in tumor tissues vs adjacent non cancerous tissues (0.9385 ± 0.1363 vs 1.838 ± 0.3209, P=0.04). The KIAA1199 mRNA expression was considerably higher in tumor tissues (P<0.001) than in adjacent non cancerous tissues by RT-PCR. IHC results showed up-regulated KIAA1199 expression was related with some severe clinicopathological parameters: pathologic differentiation (P, 0.002), T stage (P<0.001), N stage (P<0.001), clinical stage (P<0.001), survival time (P, 0.008) and survival status (P<0.001). Kaplan-Meier survival analysis showed that patients with high KIAA1199 protein expression had poor overall survival(OS)(P<0.05). Cox analysis suggested that the KIAA1199 protein expression constituted an independent prognostic marker for LSCC patients (P<0.001).

Conclusion: Our findings revealed that KIAA1199 protein expression may be used to predict LSCC patient outcome.

Keywords: KIAA1199, CEMIP, Laryngeal squamous carcinoma, Prognostic marker

Introduction

68   Laryngeal squamous cell carcinoma is the most common laryngeal cancer , and it is the second
69   highest incidence among head and neck cancer(Ling Gao, et al., 2018). In 2016, Laryngeal
70   malignancies accounted for approximately 13,400 cancer cases in U.S.A, of which an estimated
71   3600 patients succumbed to the disease(Rebecca L. Siegel, et al., 2019). The number of laryngeal
72   cancer has radically changed in the last 20 years in USA(S. Michael Rothenberg, et al., 2012). In
73   the available treatments, there have been improvements, but the patients still need identified novel
74   gene that is up-regulated in human cancer suffer from poor prognosis. So, in the pathogenesis of
75   LSCC, identification of the key molecules is urgently needed to improve the treatment of LSCC.
76   The KIAA1199 gene, which was first discovered in association with non-syndromic hearing
77   loss(Satoko Abe, et al., 2003). Nowadays, we have known that the KIAA1199 gene is expressed
78   in a wide range of normal human tissues(Yongsheng Zhang, et al., 2014). Over-expression of
79   KIAA1199 contributes to resistance to cell immortalization and cancerization in normal human
80   cells and is associated with cell death(Eriko Michishita, et al., 2006). Several researches have
81   illuminated that KIAA1199 is over-expressed in different cancers, including oral squamous cell
82   carcinoma(Pitak Chanthammachat, et al., 2013), breast cancer(Nikki A. Evensen, et al., 2013),
83   gastric cancer(Shinji Matsuzaki, et al., 2009), colorectal tumors((K Birkenkamp-Demtroder, et al.,
84   2011; Lawrence C. LaPointe, et al., 2012), prostate cancer(Eriko Michishita, et al., 2006), ovarian
85   cancer(Fan Shena, et al., 2019) and hepatocellular carcinoma(Zhengchen Jiang, et al., 2018). The
86   workings proved, that KIAA1199, which regulates the proliferation, migration, and invasion of
87   tumors. So far, in LSCC remain an enigma, we have no report about   KIAA1199 expression in
88   LSCC, and the clinical value and biological role of KIAA1199.

89   We implemented immunohistochemical detection of KIAA1199 protein expression to
90   investigate the clinical significance of KIAA1199 and to detect whether it plays a key role in the
91   progression of LSCC in 105 paired formalin-fixed and paraffin-embedded cancer and adjacent
92   noncancer tissues obtained from patients with LSCC. In the end, we illuminated the
93   clinicopathologic characteristics of LSCC patients was related to KIAA1199, which were
94   statistically evaluated.

95

96   **Materials & Methods**

97   **Patient enrolment and arrange follow up**
98   The research was implemented in the Department of ENT, the Third Xiangya Hospital, Central
99   South University. We collected 10 pairs of fresh specimens and their matched adjacent non
100 cancerous specimens, which were from patients diagnosed with human laryngeal squamous cell
101 carcinoma by pathological examination in February 2018. A total of 105 patients who had been

102 performed curative resection for LSCC were registered from 2009 to 2014. Patients with
103 recurrence of laryngeal cancer or multiple cancers were excluded. No anticancer therapy was given
104 before surgery. Postoperative pathological examination of patients diagnosed with laryngeal
105 squamous cell cancer. Patient clinical data such as sex, age, pathological differentiation, clinical
106 region, T stage, N stage, clinical stage, operation type, neck lymph dissection, smoking status, and
107 drinking status were collected. To investigate the prognostic value of KIAA1199 in postoperative
108 patients, we examined the overall survival rate (OS) of the LSCC patients. The average arrange
109 follow up cycle was 54 months (5 months extent to 10 years). Prior to the start of the study, we
110 obtained the written informed consent of all patients and the approval of the hospital's Human
111 Research Ethics Committee in accordance with the Helsinki Declaration Guidelines(NO. 2018-
112 S084). All tissue samples were treated and anonymous in accordance with ethical and legal
113 standards. The tumor stage was determined according to the TNM(tumor, lymph node, metastasis)
114 grading of the International Union Against Cancer (UICC, 2002).

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116 **RNA extraction and real-time RT-PCR**

117   According to the manufacturer's protocol, the total RNA was isolated from LSCC and matched
118 adjacent tissues by using TRIzol Reagent (Invitrogen). Nanodroplet spectrophotometer (Thermo
119 Scientific, Waltham, MA, USA) was used to measure the concentration and purity of total RNA.
120 According to the manufacturer's instructions, the total RNA was converted to cDNA using a
121 quantitative PCR (qPCR) reverse transcription kit (TOYOBO Life Science, Shanghai, P.R. China),
122 fresh tissues were used to synthesize cDNA. Real-time RT-PCR was applied three times using a
123 KOD SYBR qPCR Mix Fluorescent Quantitative PCR kit (TOYOBO Life Science, Shanghai, P.R.
124 China). PCR and data collection were conducted by using an EP Real-time PCR System
125 (Eppendorf Inc., Hauppauge, NY, USA). For standardization, we used GAPDH as an endogenous
126 control. The primers used in our study were purchased from Sangon Biotech (Shanghai, P.R.
127 China), and the following primer sequences were used: KIAA1199, F primer 5′-
128 CCAGTAACCTGCGAATGAAGA-3′ and R primer 5′-TGGTCCCAGTGGATGGTGTAG-3′.
129 GAPDH, F primer 5′-TTGGTATCGTGGAAGGACTCA-3′ and R primer 5′-
130 TGTCATCATATTTGGCAGGTT-3′. The reaction conditions were 95°C for 5 min, followed by
131 40 cycles at 95°C for 15 sec and 58°C for 30 sec. The relative expression level was determined by
132 the 2-ΔΔCt method.

133

134 **Western Blotting Analysis**

135   Proteins were extracted from LSCC fresh tissue samples and adjacent non cancerous   fresh
136 tissue samples. The Western blotting analysis was carried out according to our previous

137   article(Wei Li, et al., 2012).Primary antibodies were used as follows: polyclonal rabbit anti-
138   KIAA1199 antibody (diluted 1:1,000), anti-GAPDH antibody (diluted 1:5000), and horseradish
139   peroxidase-conjugated secondary antibody (1:10,000).

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141   **Immunohistochemistry**

142   One hundred and five formalin-fixed, paraffin-embedded LSCC tissues were used for the
143   immunohistochemistry (IHC) studies. Briefly, the tissue was sliced continuously into
144   approximately 4 μm section, paraffin was removed from the sections using a graded alcohol series
145   of 100% and 95% in xylene, rehydrated in 75%, and finally washed with PBS. Subsequently, the
146   antigen was prepared with sodium citrate buffer PBS and incubated in 3% H_2O_2 deionized water
147   for 15 minutes to inactivate endogenous peroxidase. The sections were washed 3 times with PBS,
148   incubated with calf serum to block non-specific antigen for 10 min, incubated with polyclonal
149   rabbit anti-KIAA1199 antibody (1:70) at room temperature for 1 hour, washed with PBS three
150   times, and then incubated with secondary antibody at room temperature for 30 minutes. Sections
151   were washed with PBS 3 times, stained with diaminobenzidine (DAB) for 4 minutes, washed 3
152   more times with PBS, restained with haematoxylin for 30 seconds, washed with flowing water,
153   dried and sealed. Dried sections were observed with an optical microscope. The positive control
154   was gastric cancer tissue confirmed by pathological examination, and the adjacent normal tissues
155   from patients with LSCC were used as the negative control.

156   The positive expression of KIAA1199 was patchy with aggregates of brown granules in the
157   cytoplasm. Semiquantitative analysis was used to determine the percentage of positive cells under
158   the microscope and score the staining intensity. Two senior pathologists of the Department of
159   Pathology were assigned to read the slides in a double-blinded manner, and 3-5 different fields
160   were randomly selected from each IHC staining section for observation. The staining results were
161   semiquantitatively analysed in terms of staining intensity and percentage of cells with positive
162   expression. Evaluation of dyeing intensity: range, 0-3; colourless (negative) = 0, weak (pale
163   yellow) = 1, medium (brown-yellow) = 2, strong (tan) = 3. Percentage of stained cells: range, 0-3;
164   percent positive cells < 5% = 0, 0.5%-10% = 1, 10%-50% = 2, ≥50% = 3. The score of the two
165   was multiplied to show the positive grade: 0 is negative (-), ≤3 is low expression, and >3 is higher
166   expression.
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169   **Statistical analysis**

170   Our results were interpreted with GraphPad Prism version 7.0 (GraphPad Software, Inc., La
171   Jolla, CA, USA) and SPSS 23.0 software package (SPSS, 112 Y.-H. HAO ET AL. Chicago, IL,
172   USA). Chi-square test was used to analyze the associate with KIAA1199 protein expression and
173   clinicopathological characteristics in LSCC patients. Cox regression analysis estimated the risk of

174 death associated with KIAA1199 protein expression. Kaplan-meier method was used to analyze
175 the total survival curve. Other data were analysed using Student's t-test, and ANOVA was
176 conducted to determine the differences in two or more groups. All data are presented as the mean
177 ± SD with P < 0.05 (*P<0.05, **P<0.01, ***P<0.005, ****P<0.001).

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179

Results

181 **1. Clinical data**
182 To understand the clinical features of patients, the detailed data of the patients, such as sex, age,
183 pathological differentiation, clinical region, T stage, N stage, clinical stages, operation type, neck
184 lymph dissection, smoking status, and drinking status, were collected from their medical records,
185 and these data are summarized in Table 1 for the 105 patients in this study; 103 (98.1%) were men,
186 and 2 (1.9%) were women, ranging in age from 37 to 82 years. T1-T2 stage was detected in 70
187 patients (66.6%), N1-N3 stage was detected in 20 patients (19%), and the overall survival (OS)
188 time ranged from 6 to 108 months.

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191 **2. Increased Expression of KIAA1199 in Human LSCC tissues**
192 To uncover the role of KIAA1199 expression in LSCC, we first detected KIAA1199 protein
193 and mRNA expression in 10 pairs of fresh human LSCC specimens and their matched adjacent
194 noncancerous specimens using Western blotting (Fig. 1A, 1B), IHC (Fig. 2) and RT-PCR (Fig.
195 1C). As shown in, KIAA1199 protein levels were significantly higher in LSCC tissues (1.838 ±
196 0.3209 vs 0.9385 ± 0.1363, P=0.04) (Fig.1B) than in adjacent noncancerous tissues. RT-PCR
197 revealed that KIAA1199 mRNA expression was considerably lower in adjacent noncancerous
198 tissues (P<0.001) than in cancer tissues (Fig. 1C). Then, we compared the expression of
199 KIAA1199 in 105 LSCC tissues and their adjacent noncancerous tissues through
200 immunohistochemistry. There was weak or negative expression of KIAA1199 in adjacent
201 noncancerous tissue but high expression in the cytoplasm of LSCC tissue cells. The positive
202 staining and negative staining rates in LSCC tissues were 52.4% (55/105, Table 1) and 47.6%
203 (50/105), respectively. Semiquantitative analysis showed that KIAA1199 was significantly
204 increased in LSCC tissues. Representative photographs of the immunostaining are shown in figure
205 3 A-F. RT-PCR results for KIAA1199 mRNA levels were agree with the Western blotting and
206 IHC results, showing that KIAA1199 is increased in LSCC tissues.

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209 **3. KIAA1199 expression is associated with pathologic differentiation, T, N, clinical stage,**
210 **survival status and survival times of LSCC**

211   In order to further reveal the character of KIAA1199 in LSCC, we evaluated the relationship
212 between its expression and the clinicopathological characteristics of LSCC. As shown in Table 2,
213 upregulation of KIAA1199 expression was associated with some clinicopathological parameters:
214 pathologic differentiation (P, 0.002), T stage (P<0.001), N stage (P<0.001), clinical stage
215 (P<0.001), survival time (P, 0.008) and survival status (P<0.001). However, KIAA1199 expression
216 was not correlated with age, sex, clinical region, smoking status, or drinking status.

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219 **4. Survival assessment: A high level of KIAA1199 is predictive of poor prognosis in LSCC**
220 **patients**
221   The survival curve was plotted by kaplan-meier method, and the survival time was tested by
222 log-rank test. The results showed that LSCC patients with high KIAA1199 expression had a lower
223 prognosis, and low KIAA1199 expression in LSCC patients (P<0.001, for OS) was related with
224 considerably longer OS compared with high KIAA1199 expression. The median OS for high
225 KIAA1199 expression was 60±4.113 months and that for low KIAA1199 expression was
226 96±7.928 months (Fig. 3 G). Then analyzed independent prognostic factors for survival in patients
227 with LSCC by using univariate and multivariate Cox proportional hazards analysis. The univariate
228 analysis results (Table 3) showed that age (HR =1.032, 95% CI: 1.001 - 1.063; P, 0.04), pathologic
229 differentiation (HR =0.643, 95% CI: 0.524 - 0.789; P<0.001), T stage (HR =1.402, 95% CI: 1.139
230 - 1.724; P<0.001), N stage (HR =1.679, 95% CI: 1.148 - 2.4577; P, 0.008), clinical stage (HR
231 =1.445, 95% CI: 1.180 - 1.769; P<0.001), operation type (HR =0.380, 95% CI: 0.222 - 0.650;
232 P<0.001) and KIAA1199 expression (HR =12.165, 95% CI: 5.434 - 27.233; P<0.001) were
233 significantly associated with the overall survival of LSCC patients. Multivariate survival analysis
234 (table 4) showed that KIAA1199 expression was statistically significant predictor of OS(HR
235 =27.937, 95% CI: 10.600–73.632; P<0.0001) and that age (HR =1.039, 95% CI: 1.003 - 1.077; P,
236 0.0354), clinical stage (HR =0.704, 95% CI: 0.581–0.960; P, 0.023), operation type (HR =0.285,
237 95% CI: 0.093-0.870; P, 0.027), T stage (HR =0.68, 95% CI: 0.529-0.874; P, 0.003) and smoking
238 status (HR =0.19, 95% CI: 0.057–0.630; P, 0.007) were independent predictive factors for OS.

239

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Discussion

242   In order to identify novel gene that is up-regulated in human cancer with poor prognosis, a
243 deeply cognition to the molecular biology profiles of LCSS is a vital work. To outcomes remain
244 elusive, the molecular pathways involved in LSCC incidence, progression and clinical yet.
245 Espeially the endoplasmic reticulum, KIAA1199, which is a glycosylated protein which located
246 in the cytoplasm and membrane(Amit Tiwari, et al., 2013; Eriko Michishita, et al., 2006; Nikki A.
247 Evensen, et al., 2013). The relationship between cancer and KIAA1199 have been studied in many
248 research directions. KIAA1199 is a recently identified novel gene that can regulate cell growth

249 and invasion and could be a new therapeutic target in breast cancer(Mohammad-Saeid Jami, et al.,
250 2014). A similar analysis reported, KIAA1199 overexpression can predict poor survival in patients
251 with colon cancer(Jian Xu, et al., 2015). By several mechanisms, KIAA1199 protein can accelerate
252 cancer progression. Simultaneously, other research have shown that the KIAA1199 protein
253 expression level is elevated upon p53 activation(Shinji Matsuzaki, et al., 2009). KIAA1199 is also
254 related to angiogenesis in rheumatoid arthritis(Xinyu Yang, et al., 2015). However, the mechanism
255 of KIAA1199 tumor-promoting effects in LSCC is little known.

256   In our study, we first verified KIAA1199 protein and mRNA expression in 10 pairs of fresh
257 surgically resected LSCC samples by Western blotting, IHC and real-time RT-PCR. Our results
258 have drawn a conclusion that KIAA1199 was highly expressed in LSCC cancerous in contrast to
259 adjacent non cancerous tissue. In view of our data, we also can censor the hidden expression of
260 KIAA1199 by immunohistochemistry in 105 paraffin-embedded sections (2009-2014) to further
261 explore the relationship between KIAA1199 and clinicopathological characteristics. By our data
262 analysis, showed that KIAA1199 expression was not kenspeckle related with clinical parameters
263 which as age, sex, clinical region, smoking, or drinking. Interestingly, for some severe
264 clinicopathological parameters: pathologic differentiation (P, 0.002), T stage (P<0.001), N stage
265 (P<0.001), clinical stage (P<0.001), survival time (P, 0.008) and survival status (P<0.001), the
266 significant correlations were observed. Through our experiments, we obtained many data, which
267 provides a new evidence that KIAA1199 is highly expressed in primary LSCC tissues and its
268 immunoreactivity is higher in cancerous than adjacent noncancerous tissues, revealing that
269 KIAA1199 might help distinguish benign from malignant larynx tumors. Moreover, our results
270 and analysis illuminated that the expression of KIAA1199 was elevated in LSCC tissues with
271 aggressive clinicopathological characteristics, suggesting its potential as a marker of cancer
272 invasionality.

273   The abnormal expression of KIAA1199 has also been found in other cancer studies, such as oral
274 squamous cell carcinoma(Pitak Chanthammachat, et al., 2013), breast cancer(Nikki A. Evensen,
275 et al., 2013), gastric cancer(Shinji Matsuzaki, et al., 2009), colorectal tumors(Amit Tiwari, et al.,
276 2013; K Birkenkamp-Demtroder, et al., 2011; Lawrence C. LaPointe, et al., 2012), prostate
277 cancer(Eriko Michishita, et al., 2006), ovarian cancer(Fan Shena, et al., 2019) and hepatocellular
278 carcinoma(Zhengchen Jiang, et al., 2018). It was reported(Xuehua Jiao, et al., 2019) that
279 KIAA1199 was abnormaly increased in the papillary thyroid tumor compared with normal
280 specimens tissues and that upregulation of KIAA1199 was positively correlated with more
281 advanced clinical variables. There was analysis showed that the cell invasion and migration were
282 related with KIAA1199. KIAA1199 silencing inhibited the invasive ability of papillary thyroid
283 cancer cells by affecting epithelial-mesenchymal transition (EMT) in vitro and in vivo.
284 Additionally, the same as our study, In clone cancer study(Jian Xu, et al., 2015) proved the

285   expression of KIAA1199 was also observably associated with tumor invasion, metastasis and
286   TNM staging. Increased mortality risks associated with overexpression of KIAA1199 in primary
287   hepatocellular cancer patient. Previous researches have demonstrated that up-regulation of
288   KIAA1199 motivates carcinogenesis, motility and apoptosis. Metastasis, invasion, and cell
289   movement of a variety of cell types are associated with KIAA1199 expression(Yongsheng Zhang,
290   et al., 2014). By the Wnt/β-catenin signalling pathway, EMT is one of the important processes
291   mediated, which plays a key role in cancer invasion and metastasis(Yanyuan Wu, et al., 2012).
292   Interestingly, the KIAA1199 signalling pathway also induces the development and progression of
293   tumor. Other researches showed that the cell proliferation and mobility of colorectal cancer cells
294   were inhibited by knocking down the expression of CEMIP in vitro, and the EMT process of
295   colorectal cancer cells is suppressed by shRNA-CEMIP via inactivation of the Wnt/β-catenin/Snail
296   pathway(Guodong Liang, et al., 2018). Collectively, our results demonstrated that the
297   overexpression of KIAA1199 mRNA may affect tumor spread, lymph node metastasis, tumor
298   differentiation and prognosis(Shinji Matsuzaki, et al., 2009). In a report, it was defined KIAA1199
299   as an carcinogenic protein induced by HPV infection and compositive NF-kB activity that
300   transmits pro-survival and aggressive signals via EGFR signalling(Kateryna Shostak, et al., 2014).
301   Research has suggested that KIAA1199 may promote the development of ovarian cancer by
302   regulating PI3K/AKT signalling(Fan Shena, et al., 2019). One study insisted, AMPK/GSK3β/β-
303   catenin cascade triggered KIAA1199 over-expression may promote migration and invasion in
304   anoikis-resistant prostate cancer cells by increasing PDK4-associated metabolic reprogramming,
305   which may provide a novel therapeutic target for the prostate cancer(Peng Zhang, et al., 2018).
306   Therefore, in the light of the upper research about the KIAA1199-related signalling pathway, we
307   can draw a conclusion that KIAA1199 can influence the occurrence and development of laryngeal
308   cancer, which may also be related to the Wnt/β-catenin, EGFR, PI3K/AKT, and AMPK/GSK3
309   signalling pathways and other pathways. Thence, we will carry out a molecular mechanism
310   research of KIAA1199 in LSCC cells and animal models in our future study.

311   Some limitations exist in our research. First, the sample size of this study was a little small. As
312   a retrospective study design that the selection bias might not be ignored. Second, our study did not
313   explore the effect of other treatments for LSCC on the prognosis of patients, such as radiotherapy
314   and chemotherapy. So, in the future studies, we will carry out cell biology experiments to verify
315   our findings, such as gene transfection and cell migration assays.

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317

318   **Conclusions**

319   In conclusion, our results revealed significant associations of KIAA1199 protein expression
320   with various clinicopathologic characteristics and the prognosis of LSCC patients. Moreover,
321   survival analysis illuminated KIAA1199 was an independent prognostic factor for overall survival
322   in LSCC. All of these findings indicate that the KIAA1199 protein might be used as a pathological
323   marker to identify individuals with poor outcomes and to provide a reference for clinical therapy
324   in the future. Further studies are required to investigate its rationality as a marker and the potential
325   pathways involved in KIAA1199-mediated cell invasion and metastasis.
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Acknowledgements

328 This study was supported by the Hunan Provincial Science Foundation of China (No
329 2016JJ4104) and the National natural science foundation of China (NO.81702706).

330   We thank Dr. Sheng Xiao for assistance with the IHC experiments.

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References

334 **Eriko Michishita GGs, J. Carl Barrett, Izumi Horikawa. 2006.** Upregulation of the KIAA1199
335   gene is associated with cellular mortality. *Cancer Lett* **239:**71-77. DOI
336   10.1016/j.canlet.2005.07.028.

337 **Fan Shena Z-hZ, Yao Liuc, Shuo Chena, Xiu-jie Shenga, Yang Zhao. 2019.** CEMIP promotes
338   ovarian cancer development and progression via the PI3K/AKT signaling pathway.
339   *Biomed Pharmacother* **114:**108787. DOI 10.1016/j.biopha.2019.108787.

340 **Gu Changjiang NQ, Ni Kan, Zhang Shu, Qian Haixin. 2018.** Expression and clilical significance
341   of KIAA1199 in primary hepatocellular carcinoma. *Natl Med J China* **98:**1609-1613. DOI
342   10. 3760/cma. j. issn. 0376-2491. 2018. 20. 017.

343 **Guodong Liang XF, Yubo Yang, Yan Song. 2018.** Silencing of CEMIP suppresses Wnt/β-
344   catenin/Snail signaling transduction and inhibits EMT program of colorectal cancer cells.
345   *Acta Histochemica* **120:**56–63. DOI 10.1016/j.acthis.2017.11.002.

346 **Jian Xu YL, Xudong Wang, Jianfei Huang, Huijun Zhu, Zhiqian Hu, Defeng Wang. 2015.**
347   Association between KIAA1199 overexpression and tumor invasion, TNM stage, and poor
348   prognosis in colorectal cancer. *Int J Clin Exp Pathol* **8:**2909-2918.

349 **K Birkenkamp-Demtroder AM, F Mansilla, K Thorsen, CL Andersen, B Øster, S Hahn, TF**
350   **Ørntoft. 2011.** Repression of KIAA1199 attenuates Wnt-signalling and decreases the
351   proliferation of colon cancer cells. *Br J Cancer* **105:**552-561. DOI 10.1038/bjc.2011.268.

352 **Kateryna Shostak XZ, Pascale Hubert, Serkan Ismail Go¨ktuna, Zheshen Jiang, Iva**
353   **Klevernic, Julien Hildebrand, Patrick Roncarati, Benoit Hennuy, Aure´lie Ladang,**
354   **Joan Somja, Andre´ Gothot, Pierre Close, Philippe Delvenne, Alain Chariot. 2014.**
355   NF-kappaB-induced KIAA1199 promotes survival through EGFR signalling. *Nat Commun*
356   **5:**5232. DOI 10.1038/ncomms6232.

357 **Lawrence C. LaPointe SKP, Robert Dunne, Glenn S. Brown, Letitia Pimlott,Snigdha Gaur,**
358   **Aidan McEvoy, Melissa Thomas, David Wattchow, Peter L. Molloy, Graeme P.**
359   **Young. 2012.** Discovery and validation of molecular biomarkers for colorectal adenomas
360   and   cancer   with   application   to   blood   testing.   *PLoS   One*   **7:**e29059.
361   10.1371/journal.pone.0029059.

362 **Mohammad-Saeid Jami JH, Miao Liu, Michelle L Varney, Hesham Hassan, Jixin**
363   **Dong,Liying Geng, Jing Wang, Fang Yu, Xin Huang, Hong Peng, Kai Fu1, Yan Li,**
364   **Rakesh K Singhand Shi-Jian Ding. 2014.** Functional proteomic analysis reveals the
365   involvement of KIAA1199 in breast cancer growth, motility and invasiveness. *BMC Cancer*
366   **14:**194. DOI 10.1186/1471-2407-14-194.

367 **Nikki A. Evensen CK, Hoang-Lan Nguyen, Kevin Zarrabi, Antoine Dufour, Pournima**
368   **Kadam, You-jun Hu, Ashleigh Pulkoski-Gross, Wadie F. Bahou, Stanley Zucker, Jian**
369   **Cao. 2013.** Unraveling the role of KIAA1199, a novel endoplasmic reticulum protein, in
370   cancer cell migration. *J Natl Cancer Inst* **105:**1402-1416. DOI 10.1093/jnci/djt224.

371 **Peng Zhang YS, Yadong Sun, Xuechao Li, Lifeng Chen, Likun Yang, Yifei Xing. 2018.**
372   AMPK/GSK3beta/beta-catenin cascade-triggered overexpression of CEMIP promotes
373   migration and invasion in anoikis-resistant prostate cancer cells by enhancing metabolic
374   reprogramming. *FASEB J***:**fj201701078R. DOI 10.1096/fj.201701078R.

375 **Pitak   Chanthammachat WP, Kowit Pruegsanusak, Sittiruk Roytrakul, Chantragan**
376   **Srisomsap,   Daranee   Chokchaichamnankit,   Jisnuson   Svasti,   Pleumjit**
377   **Boonyaphiphat,   Singkhamanan K, Paramee Thongsuksai. 2013.** Comparative
378   proteomic analysis of oral squamous cell carcinoma and adjacent non-tumour tissue from
379   Thailand. *Arch Oral Biol* **58:**1677-1685. DOI 10.1016/j.archoralbio.2013.08.002.

380   **Rebecca L. Siegel KDM, Ahmedin Jemal, DVM. 2019.** Cancer statistics, 2019. *CA Cancer J*
381   *Clin* **69:**7-34. DOI 10.3322/caac.21551.

382   **S. Michael Rothenberg LWE. 2012.** The molecular pathogenesis of head and neck squamous
383   cell carcinoma. *J Clin Invest* **122:**1951-1957. DOI 10.1172/jci59889.

384   **Satoko Abe S-iU, Yusuke Nakamura. 2003.** Mutations in the gene encoding KIAA1199 protein,
385   an inner-ear protein expressed in Deiters' cells and the fibrocytes, as the cause of
386   nonsyndromic hearing loss. *J Hum Genet* **48:**564-570. DOI 10.1007/s10038-003-0079-2.

387   **Shinji Matsuzaki FT, Koshi Mimori, Kouichirou Tahara, Hiroshi Inoue, Masaki Mori. 2009.**
388   Clinicopathologic significance of KIAA1199 overexpression in human gastric cancer. *Ann*
389   *Surg Oncol* **16:**2042-2051. DOI 10.1245/s10434-009-0469-6.

390   **Xinyu Yang PQ, Bingbing Chen, Yaoyao Lin, Zhonghao Zhou, Renshan Ge, Hai Zou,**
391   **Jianmin Wang, Jianguang Wang. 2015.** KIAA1199 as a potential diagnostic biomarker
392   of rheumatoid arthritis related to angiogenesis. *Arthritis Res Ther* **17:**140. DOI
393   10.1186/s13075-015-0637-y.

394   **Xuehua Jiao JY, Xiujie Wang, Xueyan Yin, Guodong Zhang, Xingbo Cheng. 2019.** KIAA1199,
395   a Target of MicoRNA-486-5p, Promotes Papillary Thyroid Cancer Invasion by Influencing
396   Epithelial-Mesenchymal Transition (EMT). *Med Sci Monit* **25:**6788-6796. DOI
397   10.12659/MSM.918682.

398   **Yanyuan Wu CG, Juri Kim, Nicole Mosher, Seyung Chung, Dennis Slamon, Jaydutt V.**
399   **Vadgama. 2012.** Expression of Wnt3 activates Wnt/beta-catenin pathway and promotes
400   EMT-like phenotype in trastuzumab-resistant HER2-overexpressing breast cancer cells.
401   *Mol Cancer Res* **10:**1597-1606. DOI 10.1158/1541-7786.MCR-12-0155-T.

402   **Yongsheng Zhang SJ, WEN G. JIANG. 2014.** KIAA1199 and its biological role in human cancer
403   and cancer cells (review). *Oncol Rep* **31:**1503-1508. DOI 10.3892/or.2014.3038.

404   **Zhengchen Jiang XZ, Binyao shi, Dan luo, Bin Jin. 2018.** KIAA1199 overexpression is
405   associated with abnormal expression of EMT markers and is a novel independent
406   prognostic biomarker for hepatocellular carcinoma. *Onco Targets Ther* **11:**8341-8348.
407   DOI 10.2147/OTT.S187389.

408   **Wei Li, Guolin Tan, Yanhong Ma, Heqing Li and Guangxiang He. 2012.** Inhibition of alpha
409   folate receptor resulting in a reversal of taxol resistance in nasopharyngeal carcinoma.
410   *Otolaryngol Head Neck Surg* **146:**250-258. 10.1177/0194599811426260.

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Figure 1

Figure 1 The protein of KIAA1199 was overexpression in LSCC tissue specimens.

(A) (B) The protien expression of KIAA1199 in adjacent non cancerous tissue and LSCC tissue by Western bloting.****P<0.001. (C) The mRNA expression of KIAA1199 in adjacent non cancerous tissue and LSCC tissue by RT-PCR. ****P<0.001.

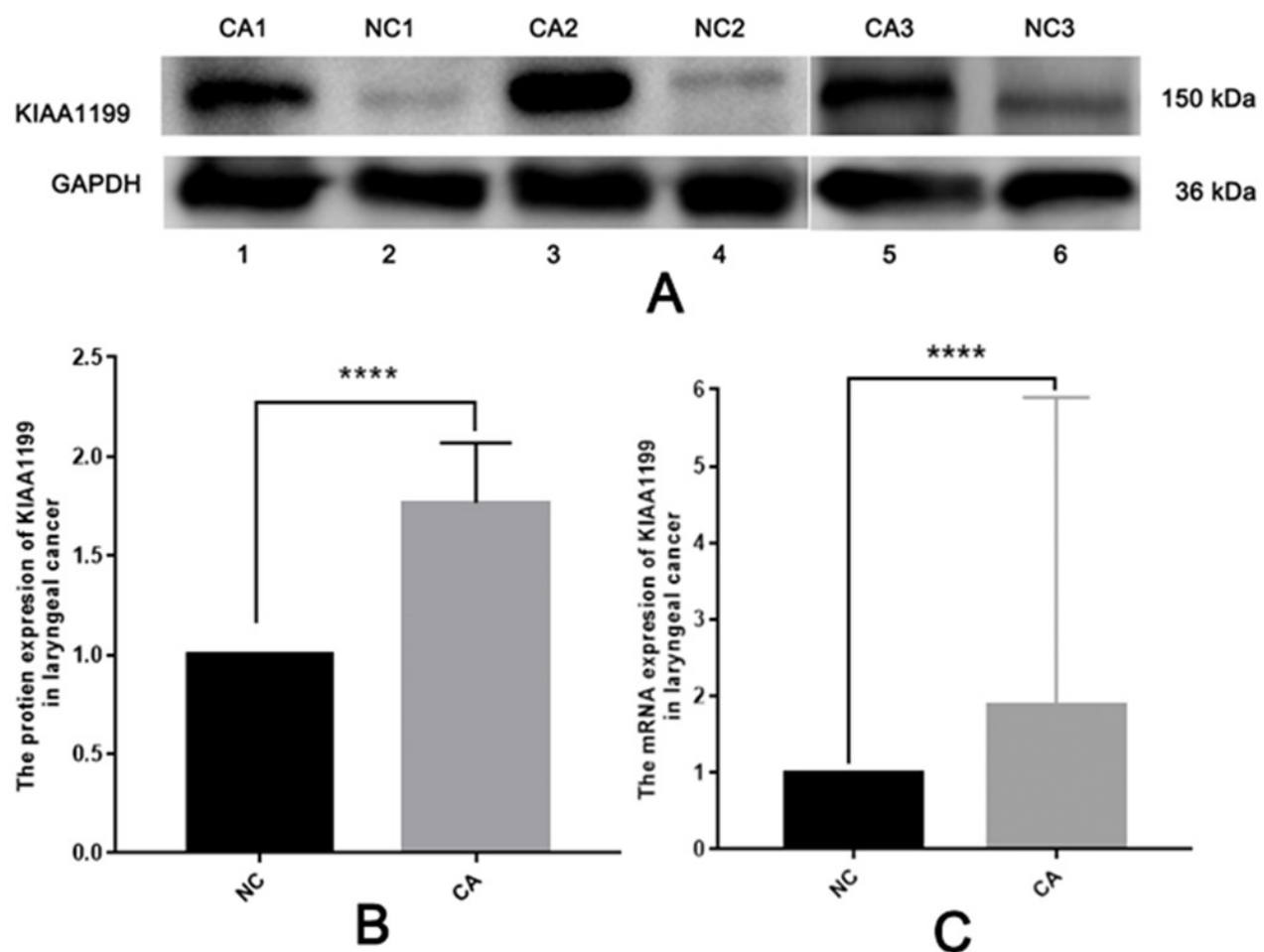



Figure 2

Figure 2 Representative images of immunohistochemical staining for KIAA1199 expression in larynx specimens.

(A) Negative expression of KIAA1199 in adjacent non cancerous specimens. (B) Low expression of KIAA1199 in LSCC pecimens. (C) High expression of KIAA1199 in LSCC specimens. Original magnification: 40X; scale bars: 20um.

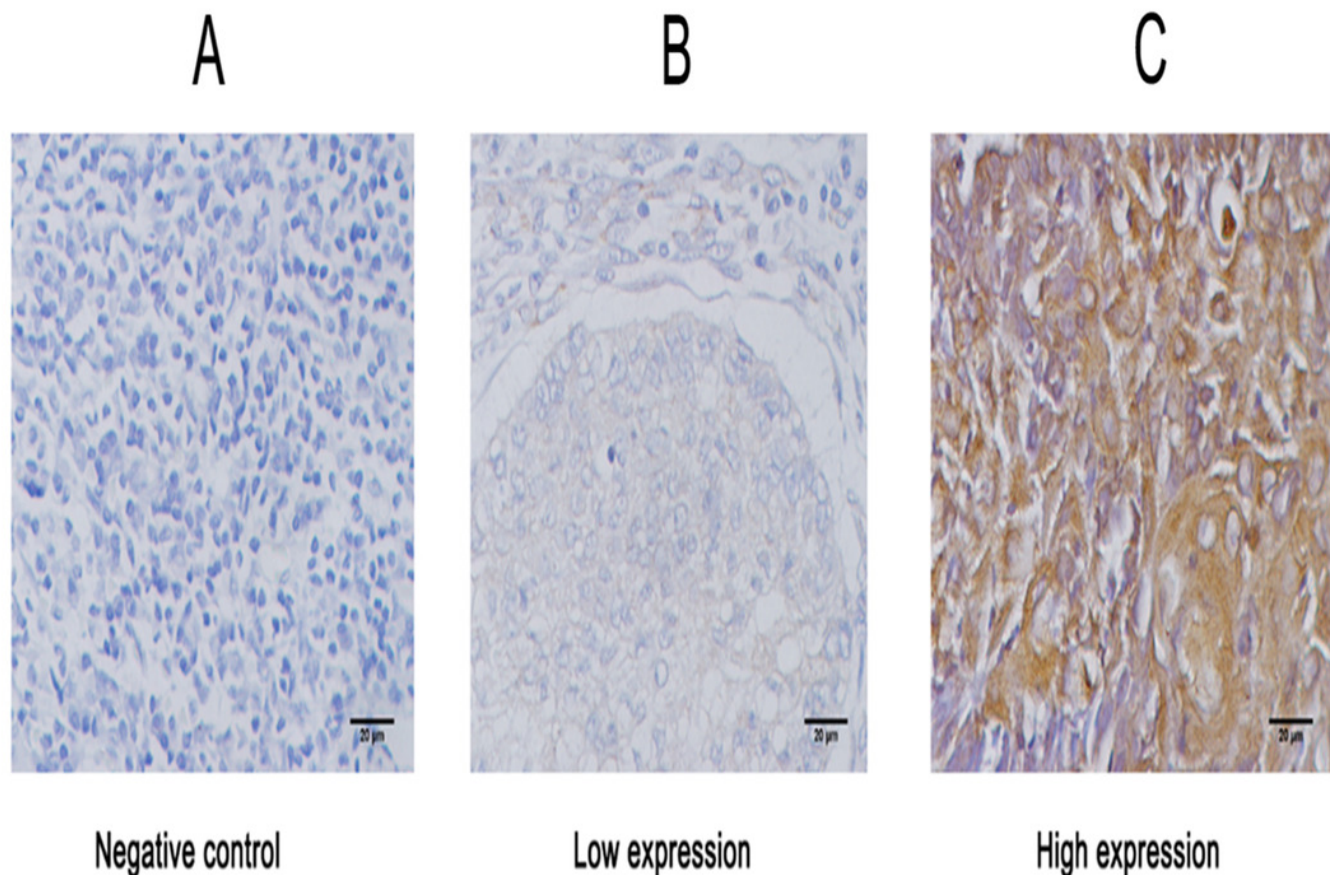
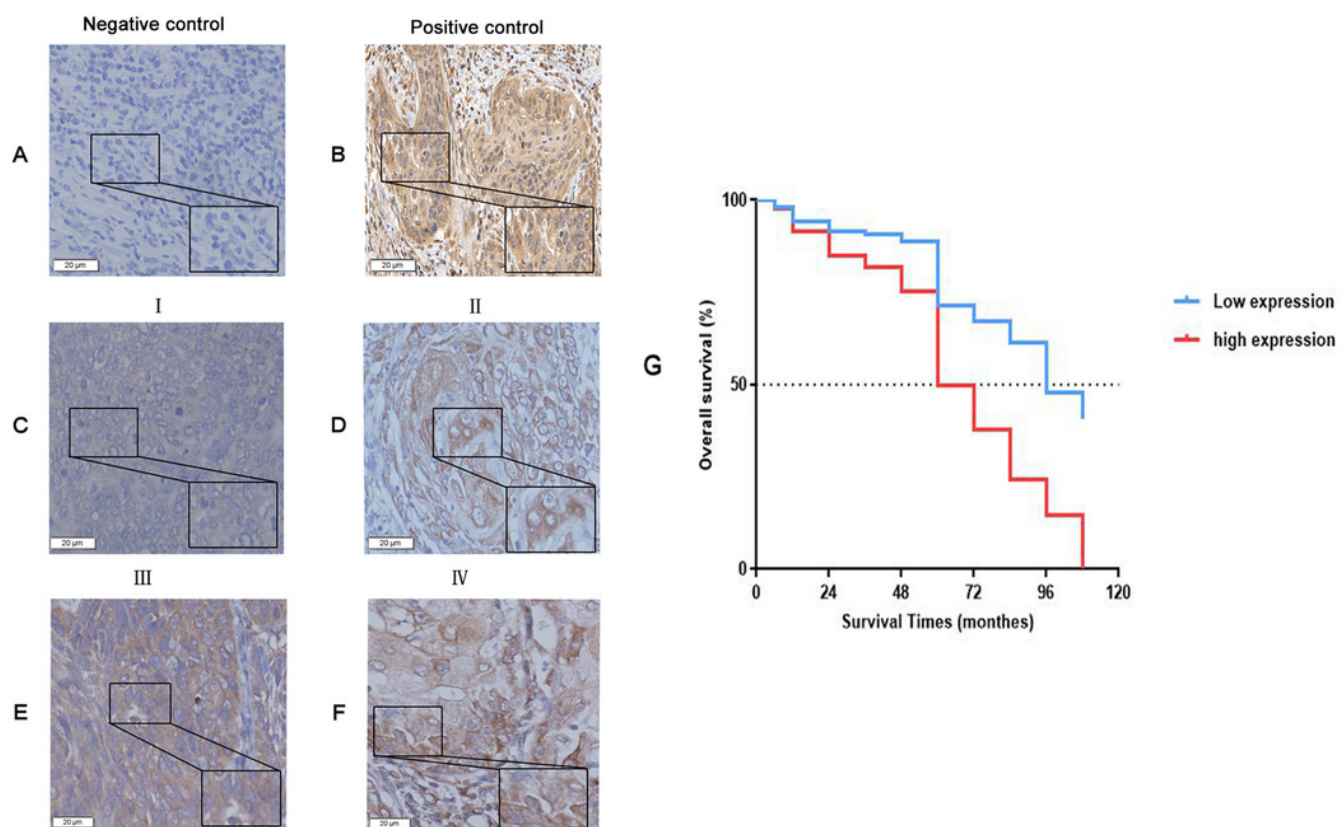



Figure 3

Figure 3 The expression of KIAA1199 in LSCC tissues and survival surve.

(A-F) KIAA1199 expression by immunohistochemical staining. (A) Adjacent nonc-ancerous tissue as the negative control. (B) Gastric cancer tissue as the positive control. (C) I stage LSCC tissue. (D) Ⅱstage LSCC tissue. (E) Ⅲ stage LSCC tissue. (F) Ⅳ stage LSCC tissue. (G) Kaplan-Meier survival curves analysis of ov-erall survival for all patients with KIAA1199 negative and positive LSCC tissue.

Clinicopathological characteristics of patient samples and expression of KIAA1199 in LSCC

1   **Table1: Clinicopathological characteristics of patient samples and expression of KIAA1199 in LSCC**

Parameters		Case number / n (%)
Gender	Male	103 (98.1)
	Female	2 (1.9)
Age(y)	≤60	44 (41.9)
	＞60	61 (58.1)
Pathologic differentiation	Poorly	20 (19.05)
	Moderately	29 (27.62)
	Highly	56 (53.33)
Clinic Region	Supraglottic type	10 (9.52)
	Trans glottic type	5 (4.76)
	Glottic type	87 (82.86)
	Subglottic type	3 (2.86)
T stage	T1-T2	70 (66.6)
	T3-T4	35 (33.4)
N stage	N0	85 (81)
	N1-N3	20 (19)
Clinical Stages	I	52 (49.5)
	II	15 (14.3)
	III	11 (10.5)

	IV	27 (25.7)
Operation	Total laryngectomy	28 (26.7)
	The partial laryngetomy	77 (73.3)
Neck lymph dissection	No	40 (38.1)
	Radical cervical clearing	26 (24.8)
	Selective/functional neck cleanser	39 (37.1)
Smoke	No	30 (28.6)
	Yes	75 (71.4)
Drink	No	53 (50.5)
	Yes	52 (49.5)
Expression of KIAA1199	Low expression	50 (47.6)
	High expression	55 (52.4)

Correlation between KIAA1199 expression and clinicopathologic characteristics of LSCC patients

1   **Table 2: correlation between KIAA1199 expression and clinicopathologic characteristics of LSCC patients**

Parameters		Expression of KIAA1199 (No.)		P
		Low	High	
Gender	Male	48	55	0.224
	Female	2	0	
Age(year)	≤60	21	23	1.000
	＞60	29	32	
Pathologic differentiation	Poorly	1	19	<0.001
	Moderately	18	11	
	Highly	31	25	
Clinic Region	Supraglottic type	3	7	0.072
	Trans glottic type	0	5	
	Glottic type	46	41	
	Subglottic type	1	2	
T stage	T1-T2	48	22	<0.001
	T3-T4	2	33	
N stage	N0	50	35	<0.001
	N1-N3	2	18	
Clinical Stage	Ⅰ-Ⅱ	48	19	<0.001
	Ⅲ-Ⅳ	2	36	
Smoke	No	18	12	0.132
	Yes	32	43	

Drink	No	26	27	0.846
	Yes	24	28	
Survival status	survive	43	7	<0.001
	death	7	48	
Survival times (month)	≤12	2	7	0.008
	>12, ≤36	0	8	
	>36, ≤60	23	22	
	>60	25	18	

Univariate analyses of various prognostic parameters in patients with LSCC

1 **Table 3: Univariate analyses of various prognostic parameters in patients with LSCC**

Parameters	Univariate Cox		
	Hazard ratio	95% CI	p-Value
Gender	0.048	0-25.791	0.422
Age(y)	1.032	1.001-1.063	0.040
Pathologic differentiation	0.643	0.524-0.789	<0.001
Clinic Region	0.068	0.49-1.026	0.068
T stage	1.402	1.139-1.724	0.001
N stage	1.679	1.148-2.457	0.008
Clinical Stages	1.445	1.180-1.769	<0.001
Operation	0.380	0.222-0.650	<0.001
Neck lymph dissection	0.957	0.7106-1.291	0. 774
Smoke	1.028	0.560-1.885	0.930
Drink	0.782	0.460-1.330	0.365
expression of KIAA1199	12.165	5.434-27.233	<0.001

Multivariate analyses of various prognostic parameters in patients with LSCC

1   **Table 4: Multivariate analyses of various prognostic parameters in patients with LSCC**

Parameters	Multivariate Cox		
	Hazard ratio	95% CI	p-Value
Age(y)	1.039	1.003-1.077	0.035
Clinic Stage	0.704	0.581-0.960	0.023
Operation	0.285	0.093-0.870	0.027
T stage	0.68	0.529-0.874	0.003
Smoke	0.400	0.204-0.785	0.008
expression of KIAA1199	27.937	10.600-73.632	0.001