1 Knockdown of linc-OIP5 inhibits proliferation and migration of
2 glioma cells through down-regulation of YAP-NOTCH signaling
3 pathway

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ABSTRACT

Long intergenic noncoding RNAs (lincRNAs) play important roles in regulating the biological functions and underlying molecular mechanisms of glioma. Here, we investigated the expression level and biological function of linc-OIP5 in glioma. In the current study, we used quantitative real-time polymerase chain reaction (qRT-PCR) to determine the expression of linc-OIP5 in glioma tissues and in adjacent normal tissues. Level of linc-OIP5 was up-regulated in glioma tissues and significantly correlated with the advanced tumor stage (III/IV). Subsequently, the efficacy of knockdown of linc-OIP5 by linc-OIP5-small interfering RNA (siRNA) was evaluated in vitro, and we found that knockdown of linc-OIP5 can inhibit glioma cells proliferation, migration in vitro and tumor formation in vivo . Further mechanistic studies revealed the effect of linc-OIP5 knockdown on glioma cell phenotype at least partially through down-regulation of YAP and inhibition of Notch signaling pathway activity. Thus, our study provides evidence that linc-OIP5 is a potential therapeutic target and novel molecular biomarker for glioma.

Keywords: Linc-OIP5; Glioma; Proliferation; Migration; Signaling Pathway

47 **Introduction**

48 Gliomas are regarded as the most prevalent malignant
49 carcinoma in central nervous system (CNS), with an annual
50 incidence of approximately 6 per 100,000 in the USA [1]. Under
51 the current WHO classification, gliomas are divided into four
52 histological grades, from WHO grade I to WHO grade IV [2]. Grade
53 I gliomas are very slow-growing tumours that are potentially
54 curable if completely resected. Grade II gliomas and the more
55 aggressive grade III gliomas have an intermediate clinical course,
56 whereas grade IV gliomas, known as glioblastoma (GBM), is not
57 only the most malignant, but also the most frequent brain tumour.
58 The median survival time of GBM is only 12 to 15 months after
59 diagnosis, despite the use of aggressive treatment with surgery,
60 chemotherapy and postoperative radiotherapy, and adjuvant
61 temozolomide (TMZ)-based chemotherapy [3, 4]. Therefore,
62 identifying the pathogenic mechanisms, finding out more accurate
63 prognostic markers, would not only help gliomas prognosis
64 estimations, but also would provide novel potential targets for
65 therapy.

66 Numerous studies over the past decade have suggested that
67 epigenetic alterations participate in carcinogenesis and progression
68 of malignancies [5, 6]. The epigenetic patterns of gene expression
69 includes methylation of cytosine, posttranslational modification of

70 histone proteins and chromatin remodeling proteins and RNA-
71 based mechanisms, were regulated by the non-codifying material of
72 the genome. These transcription products are referred to non-coding
73 RNAs (ncRNA) and are divided into several large groups [7]. One
74 of them is a class of transcripts at least 200 nucleotides transcribed
75 from the whole genome, called long noncoding RNAs(lncRNAs),
76 which regulate gene expression at epigenetic transcriptional and
77 post-transcriptional levels [8]. As a subtype of lncRNAs, the long
78 intergenic noncoding RNAs (lincRNAs) have demonstrated to be
79 transcript units located within genomic intervals between two
80 protein coding genes [9]. Increasing evidences have indicated that
81 abnormal expression of lincRNA play a critical role in tumor
82 biology, including tumor initiation, progression, and metastasis [10,
83 11]. Our previously research had demonstrated that lincRNA-
84 POU3F3 was overexpressed in glioma and play an important role
85 in prompting glioma cells proliferation and colony formation [12].
86 However, there are thousands of functional lincRNAs yet to be
87 identified.

88 Opa interacting protein 5 (OIP5) is a 23.3 kb transcript with 4
89 exons and is located in the chromosome 15q15.1, increasing
90 evidence shows that OIP5 was consistently upregulated in glioma,
91 renal cell carcinoma, and gastric cancer [13-15]. Ulitsky. et al [16]
92 has demonstrated that linc-OIP5 is overexpressed in the nervous

93 system and is important for controlling neurogenesis during
94 development. The biological function of OIP5 as an oncogene in
95 various human cancers and the highly expression of linc-OIP5 in
96 nervous system suggested that linc-OIP5 may have important role
97 in the development of glioma. However, the expression and detailed
98 function of linc-OIP5 in glioma is still unknown and needs to be
99 investigated.

100 In the present study, we identified the expression pattern of
101 linc-OIP5 in glioma patients and its correlation with
102 clinicopathological factors of glioma. Furthermore, we investigated
103 the biological function of linc-OIP5 regulate glioma cell
104 proliferation, migration in vitro and tumorigenicity in vivo. At last,
105 we researched the potential signal pathways involved in linc-OIP5'
106 biological function.

107 **Materials and methods**

108 **Ethics statement**

110 The protocols employed in this study and the use of human
111 tissues was approved by the Ethics Committee of the Second
112 Affiliated Hospital of Nanchang University and conducted in full
113 accordance with ethical principles, including the World Medical
114 Association Declaration of Helsinki, and the local legislation. All
115 patients were informed and consent to use excess pathological

specimens for research purposes. In addition, all experiments protocols were carried out in accordance with the relevant guidelines and regulations.

119

Patients and tissue samples

Patients with glioma (n = 167) who underwent initial surgery in the Second Affiliated Hospital of Nanchang University were retrospectively selected for this study. No patients had received therapy before resection. All tumors were classified on the basis of the WHO criteria for tumors of the central nervous system. The clinical characteristics of all patients were summarized in Table 1. Glioma tissues and adjacent normal tissues were immediately frozen in liquid nitrogen and were stored at –80 °C until further use.

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Cell lines and culture conditions

Two human glioma cell lines (T98G and A172) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco) in humidified air at 37 °C with 5% CO₂.

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RNA extraction and Quantitative real-time polymerase chain reaction (qRT-PCR) assay

Total RNA from glioma tissues, adjacent normal tissues and T98G, A172 cell lines and was extracted using the Trizol Total RNA Reagent (Invitrogen, Carlsbad, CA, USA). The primers were obtained from GenePharma (Shanghai, China), the following human primers were used: linc-OIP5, yes-associated-protein (YAP), Jagged-1 (Jag-1), Notch1, hairy and enhancer of split-1 (Hes-1), and β -actin. The primer sequences used in this study were summarized in Table 2. First-strand cDNA synthesis was performed with 2 μ g total RNA using the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Takara, Otsu, Japan). qRT-PCR was performed using the SYBR PrimeScript RT-PCR kit (Takara) in an Applied Biosystems 7500 Fluorescent Quantitative PCR System (Applied Biosystems, Foster City, CA, USA) according to the instructions. β -actin was used as an internal reference gene to normalize RNA levels between different samples for an exact comparison of transcription levels. Each sample was analyzed in triplicate for yield validation. The $2^{-\Delta\Delta C_t}$ method was used to determine the relative quantitation of gene expression levels.

Knockdown of linc-OIP5 expression

Small interfering RNAs (siRNAs) duplexes targeting linc-OIP5 and the negative control siRNA duplexes were purchased

163 from GenePharma (Shanghai, China). The effective sequences of
164 siRNAs were as follows: siRNA 1: GGC UGA GUU UCA UUU
165 GAA ACA GGT G, CAC CUG UUU CAA AUG AAA CUC AGC
166 CUU; siRNA 2: CAU GCA GUG CCA UCU GAC UUU AUG G,
167 CCA UAA AGU CAG AUG GCA CUG CAU GAG; siRNA 3:
168 CAC CAA ACA GGC UUU GUG UUC CUT A, UAA GGA ACA
169 CAA AGC CUG UUU GGU GGU. The siRNAs used in this study
170 were mixtures of the three siRNAs. The negative control (NC)
171 siRNA duplexes was AAT TCT CCG AAC GTG TCA CGT. T98G
172 and A172 cells were seeded at a density of 1.5×10^5 cells per well
173 in 6-well plates. Twenty-four hours later, cells were transfected with
174 mixed siRNAs using Lipofectamine 3000 Transfection Reagent
175 (Life Technologies, Grand Island, NY, USA)[17]. The effectiveness
176 of siRNA knockdown was assessed by qRT-PCR.

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178 **Cell proliferation assay**

179 A Cell Counting Kit-8 (CCK-8) assay (Dojindo, Kyushu
180 Island, Japan) was performed to assess cell proliferation. Cells were
181 collected at 24 h after treatment of siRNAs. Briefly, 2000 cells per
182 well were seeded in 96-well plates, After quiescence for 12 hours,
183 CCK-8 solution (10 μ L) was added into medium and incubated for
184 3 hours at 37°C. The amount of formazan dye generated by cellular
185 dehydrogenase activity was measured for absorbance at 450 nm by

186 using a microplate reader. The measurement of cell proliferation
187 was conducted every 24 hours for 4 days. The optical density values
188 of each well represented the survival/proliferation of cells. All of
189 these experiments were performed in triplicate and repeated at least
190 three times.

191

192 **Colony formation assay**

193 For the colony formation assay, cells treated by siRNAs for 24
194 hours and were routinely trypsinized and seeded in 6-well plates
195 (1000 cells/well). Cells were cultured in DMEM medium
196 containing 10% FBS. The medium was changed every two days.
197 After 14 days, the cells were washed with PBS, fixed with 4%
198 paraformaldehyde for 30 minutes, and then stained with crystal
199 violet for 30 minutes for visualization and counting. All of these
200 experiments were performed in triplicate and repeated at least three
201 times. The number of colonies containing ≥ 50 cells was counted
202 under the microscope using the formula: plate clone formation
203 efficiency = (number of colonies/number of cells
204 inoculated) $\times 100\%$.

205

206 **Cell migration assay**

207 The real-time cell analyzer (RTCA) migration assay and
208 scratched wound assay were used to analyze the migration effect of

209 T98G and A172 cells treated by siRNAs. The xCELLigence system
210 (Roche Applied Sciences, Basel, Switzerland) used impedance as a
211 readout and can continuously monitor the cellular migration ability,
212 producing time-dependent cellular response profiles. The electronic
213 readout of cellsensor impedance is displayed in real-time as CI, a
214 value directly influenced by cell attachment, spreading, or cell
215 proliferation or a combination of these. The CI value at each time
216 point is defined as $R_n - R_b / R_b$, where R_n is the cell-electrode
217 impedance of the well with the cells and R_b is the background
218 impedance of the well with only medium[18]. Cells (4×10^4 cells
219 per well) were seeded into the upper chamber, and DMEM medium
220 containing 2% FBS was added into the lower chamber. The cells
221 were incubated at 37°C in 5% CO₂ and monitored for 24 hours. For
222 the scratched wound assay, 2×10^5 cells were seeded into 12-well
223 plates after transfection by siRNA and maintained at 37°C to permit
224 cell adhesion and the formation of a confluent monolayer. Next,
225 these confluent monolayers were ‘scratch’-wounded by using the
226 tip of a p200 pipet tip. The medium was removed and rinsed once
227 with PBS to remove the debris and smooth the edge of the scratch
228 and then replaced with fresh DMEM medium containing 2% FBS.
229 Wound closure was monitored by collecting digital images at 0-,
230 12-, and 24-hour intervals after the scratch, and digital images were
231 captured by using an inverted microscope (Leica, Solms, Germany).

232 The images were obtained at the same position before and after
233 incubation. The experiment was repeated three times. The level of
234 wound closure was assessed by the ratio of closure area to initial
235 wound (0 hours) as follows:

$$Rn = \frac{(A0) - An}{A0} \times 100\%$$

237 where Rn represents the percentage of wound closure, An
238 represents the residual area of wound at the metering point (nh), and
239 A0 represents the area of initial wound (0 hours)[19].

240

241 **In vivo assay**

242 Animal study protocols were approved by the Institutional
243 Animal Care and Use Committee at Nanchang University. Female,
244 6 weeks old nude mice were purchased from Chinese Academy of
245 Science Shanghai Experimental Animal Center. As described
246 previously[20], a burr hole was drilled into the skull 3.5 mm lateral
247 to the bregma, T98G cells and A172 cells (NC siRNA and linc-
248 OIP5 siRNA, 5×10^5 in 5ul medium) were slowly injected over 5
249 minutes into the basal ganglia using a 30-gauge needle attached to
250 a 25-AL Hamilton syringe. Forty-eight mice were randomized
251 divided into two groups to receive cell transplantation. The animals
252 were killed at every 1 week to calculate tumor volume. Tumor
253 volumes were estimated using the formula: (tumor volume=length
254 $\times$ width $2 / 2$).

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Protein extraction and western blotting

Cells were washed with PBS and lysed for 10 min on ice in RIPA buffer (Thermo Scientific, Waltham, MA, USA). Equal amount of protein was separated by SDS-PAGE using a 10% polyacrylamide gel. The proteins were transferred electrophoretically onto a nitrocellulose membranes (Whatman, Maidstone, Kent, UK). The membranes were blocked in 5 % skimmed milk in Tris-buffered saline with tween (TBST) buffer for 2 h at room temperature, then exposed to antibodies polyclonal antibodies against YAP (1:300, Cell Signaling Technology, Beverly, MA, USA), Jag-1(1:500, Santa Cruz Biotechnology, Santa Cruz, CA), Notch-1 (1:500, Santa Cruz Biotechnology) and Hes-1 (1:500, Abcam, Cambridge, UK) overnight at 4°C. The membranes were washed three times in 1×TBST for 5 minutes and incubated for 1 hour in TBST containing HRP-linked secondary antibody (Abcam, Cambridge, UK) at room temperature. β -actin (1:10000, Cell Signaling Technology) was used as an internal control. Proteins were detected by using enhanced chemiluminescence (Thermo Scientific) and imaged by using an Image Quant LAS 4000 mini bio-molecular imager (GE Healthcare, Uppsala, Sweden).

278 **Statistical analysis**

279 Statistical analysis was performed using the SPSS Graduate
280 Pack, version 11.0, statistical software (SPSS). Differences between
281 two groups were analyzed by Student's t-test. Data were expressed
282 as means $\pm$ standard deviation (SD) of three independent
283 experiments. P values less than 0.05 was considered to be
284 significant.

285

286 **Results**

287 **Increased expression of linc-OIP5 in human glioma samples**

288 The expression level of linc-OIP5 was assessed in 167 paired
289 glioma samples and corresponding adjacent normal tissues using
290 qRT-PCR, with normalization to β -actin. Firstly, we studied the
291 correlation between linc-OIP5 expression and clinical pathological
292 features, as shown in Table 1, linc-OIP5 expression increased
293 correlated with the WHO grade, while did not correlate with
294 patients' sex, age, and tumor size. Compared with their
295 corresponding adjacent normal tissues, linc-OIP5 expression in
296 glioma was increased (fold change ≥ 2) in 111 cases (66.5%),
297 whereas 56 cases (33.5%) showed decrease or no significant
298 difference (Fig. 1A and Table 1, $P < 0.01$). Furthermore, as shown in
299 Fig. 1B and Table 2, linc-OIP5 expression level significantly
300 increased with the increasing of glioma WHO grade. In WHO III/IV

glioma, there was 82 cases (49.1%) showing linc-OIP5 highly expression (fold change ≥ 2), whereas only 29 cases (17.4%) in WHO I/II glioma showing highly expression ($P < 0.01$). This result implied that linc-OIP5 overexpression might participate in the development of glioma and might serve as a novel marker for poor prognosis or progression of glioma.

Knockdown of linc-OIP5 suppressed the proliferation and migration of glioma cell lines

qRT-PCR analysis was performed to examine the expression levels of linc-OIP5 in T98G cells and A172 cells treated by linc-OIP5 siRNAs and NC siRNA. As shown in Fig 2A, linc-OIP5 expression was significantly downregulated treated by linc-OIP5 siRNA compared with NC siRNA ($P < 0.01$), which means linc-OIP5 siRNA can efficiently silence linc-OIP5 expression in T98G cells and A172 cells. We further investigated whether knockdown linc-OIP5 can affect T98G cells and A172 cells proliferation using CCK8 assay and colony formation assay. As shown in Fig. 2B and 2C, siRNA-mediated knockdown of linc-OIP5 impaired the proliferation function of T98G cells and A172 cells, the number of T98G cells and A172 cells was significantly decreased after transfection with linc-OIP5 siRNA compared with the negative controls ($P < 0.05$). Accordingly, consistent with the proliferation assay, the ability to form colonies by T98G cells and A172 cells was

also suppressed significantly after knockdown of linc-OIP5 when compared with that by the negative controls (Fig. 2D and 2E, $P < 0.05$). These results showed that linc-OIP5 depletion had an obvious inhibitory effect on the growth of glioma cells.

Having found the effect of linc-OIP5 downregulation on the proliferation of glioma cell lines, we then examined the impact of decreased expression of linc-OIP5 on cell migration in glioma cells. We first explored the role of linc-OIP5 knockdown in glioma cells migration via the wound healing assay. The si-linc-OIP5-treated T98G cells and A172 cells showed comparatively slower migration towards the wound space; however, cells treated with NC siRNA migrated more aggressively and nearly closed the wound at 24 h after scratching (Fig 3A-D, $P < 0.05$). The involvement of linc-OIP5 in migration ability of glioma cells was also investigated by RTCA assay. As shown in Fig 3E of the real-time growth curves, T98G cells and A172 cells treated with linc-OIP5 reached the log phase later and had lower mean cell indices than those treated with NC siRNA (Fig 3E, $P < 0.05$), which means linc-OIP5 can significantly suppress the motility of glioma cell lines.

344

345 **Knockdown of linc-OIP5 suppressed tumor growth in vivo**

Cell proliferation and migration are hallmarks of cancer cell tumorigenesis and the development of metastasis [21]. Given our in

348 vitro data that linc-OIP5 inhibited proliferation and migration of
349 glioma cell lines, we therefore investigated whether knockdown
350 linc-OIP5 in T98G cells and A172 cells can reduce their tumor
351 progression in vivo in nude mice. We found that knockdown linc-
352 OIP5 in both T98G cells and A172 cells strikingly reduced tumor
353 growth compared to control group (Fig 3F and 3G, $P<0.01$).

354

355 **Knockdown of linc-OIP5 decreased the genes and protein** 356 **expression of YAP and Notch signaling pathway**

357 It has been well demonstrated that YAP was upregulated
358 expressed in many types of gliomas such as ependymoma [22],
359 astrocytoma, and oligodendroglioma [23]. YAP can promote
360 glioblastoma cell lines proliferation, migration in vitro, and high
361 levels of YAP gene expression were associated with aggressive
362 molecular subsets of glioblastoma and worst median survival in
363 human astrocytoma patients[23]. So in our research, we firstly
364 explored the expression level in human glioma tissues, we found
365 that YAP gene was upregulated in glioma tissues compared to the
366 adjacent normal tissues, and the increased YAP gene expression
367 was correlated with glioma histology grade (Fig. 4A and B, $P<0.01$).
368 These results in accordance with other previously reports and co-
369 suggested that YAP participate in the development of glioma.
370 Furthermore, we explored the causal link between linc-OIP5

371 expression and YAP mRNAs expression level, and we observed a
372 significant positive correlation between YAP expression and the
373 linc-OIP5 level (Fig.4C, $R^2=0.7458$; $P<0.01$). These results
374 suggested that YAP expression level was positively affected with
375 linc-OIP5 level.

376 As Felix. et al [24] had demonstrated that YAP can up-
377 regulated Jag-1 to activate Notch signaling pathway in Human
378 Hepatocellular Carcinoma, and Notch signaling pathway had been
379 proven to involve in glioma growth[25]. So we further explored
380 whether knockdown of linc-OIP5 can down-regulate YAP
381 expression and suppress Notch signaling pathway. The effects of
382 linc-OIP5 reduction on YAP and Notch axis components were
383 researched on gene expression level and protein expression level.
384 As shown in Fig.5A and B, linc-OIP5 knockdown in both T98G cell
385 and A172 cell could significantly suppressed YAP gene expression,
386 reduced Jag-1, Notch-1 gene expression level and Notch target gene
387 Hes-1 gene expression ($P < 0.05$). Besides, western blot analysis
388 also showed that knockdown of linc-OIP5 significantly decreased
389 the levels of YAP and downstream Notch signaling pathway
390 components Jag-1, Notch-1 and Hes-1 (Fig 5C-F, $P<0.05$). Taken
391 together, these results demonstrated that inhibitory effect of down-
392 regulated expression of linc-OIP5 on malignant phenotype of
393 glioma cells may be through a repression of functionally relevant

394 YAP expression and Notch signaling pathway activity.

395

396 **Discussion**

397 In the present study, we examined the expression level of linc-
398 OIP5 in a large cohort of human glioma tissue samples, we found
399 that linc-OIP5 was overexpressed in glioma tissues compared with
400 the adjacent normal tissues and positively correlated with the tumor
401 WHO grade. In addition, linc-OIP5 function was investigated in
402 siRNA mediated knockdown studies and we demonstrated that
403 knockdown of linc-OIP5 significantly inhibited glioma cell
404 proliferation, migration in vitro, and tumorigenesis in vivo.
405 Moreover, knockdown expression of linc-OIP5 could down-
406 regulation of YAP and Notch signaling pathway by mediating the
407 YAP and downstream genes Jag-1, Notch-1, and Hes-1 expression
408 in glioma cells. Taken together, these results suggested that linc-
409 OIP5 could be a promising diagnosis, therapeutic target and novel
410 molecular biomarker for glioma.

411 As a newly discovered class of non-coding genes, lncRNAs
412 have been recently found to be pervasively transcribed in the
413 genome. Alterations in the primary structure, secondary structure
414 and expression levels of lncRNAs as well as their cognate RNA-
415 binding proteins are often associated with human diseases, in
416 particular cancer[26]. Accumulating studies have demonstrated the
417 aberrant expression of lncRNAs in various human cancers,

418 including glioma[27], oesophageal squamous cell carcinoma[28],
419 breast cancer[29], ovarian Cancer[30], and lung cancer[31]. For
420 instance, lnc-MALAT1 (Metastasis-associated lung
421 adenocarcinoma transcript 1) was over-expressed in multiple
422 tumors such as glioblastoma [32], breast cancer [33], and lung
423 cancer[34]. As in glioblastoma, MALAT1 interacted with epithelia-
424 mesenchymal transition (EMT) and confers invasive capacity to
425 malignant cells, reduced MALAT1 expression inhibit glioblastoma
426 cell migration and invasion [32]. Another lncRNA-H19 was widely
427 studied in cancer biology [35-37], the oncogenic properties of H19
428 were strongly by virtue of antagonism let-7 to influence HMGA2
429 to mediate EMT [36]. These research indicated that there is a
430 growing number of deregulated lncRNAs contribute to
431 carcinogenesis and metastasis, an absolute requirement is need to
432 awareness of more lncRNAs functions and intrinsic mechanisms.

433 OIP5 is a 23.3 kb transcript with 4 exons and is located in the
434 chromosome 15q15.1, increasing evidence shows that OIP5 was
435 consistently upregulated in glioma, renal cell carcinoma, and gastric
436 cancer [13-15]. Linc-OIP5, known as cyrano in zebrafish, is
437 overexpressed in the nervous system and is important for
438 controlling neurogenesis during development [16]. Until now, little
439 is known about the expression level and biological function of linc-
440 OIP5 in glioma. In the current study, we found that the expression

441 level of linc-OIP5 was significantly upregulated in most glioma
442 tissues when compared with the matched adjacent normal tissues,
443 intriguingly, when tumor tissues were stratified based on clinical
444 progression, we found that linc-OIP5 expression levels were
445 remarkably increased in WHO III/IV stage glioma compared with
446 those in WHO I/II stage glioma tissues, which suggested that linc-
447 OIP5 might play a role in glioma metastasis. To further understand
448 the biological function of linc-OIP5 in glioma, we performed a
449 siRNA mediated knockdown studies and we demonstrated that the
450 decreased linc-OIP5 expression led to the inhibition of cell
451 proliferation, colony formation and cell migration. Similar
452 efficiency of cellular growth behaviors of glioma was also observed
453 in vivo that the linc-OIP5 knockdown decreased tumor formation.

454 Furthermore, we investigated the mechanisms of linc-OIP5
455 regulates glioma biological function. Given that YAP exerted pro-
456 oncogenic activities in glioma [23], YAP acted as a molecular
457 sponge to bind with Jag-1 to activate Notch signaling pathway [24],
458 as well as the Notch signaling pathway regulated glioma growth
459 [25]. We investigated the relationship between linc-OIP5, YAP, and
460 Notch signaling pathway. We found that YAP mRNAs was
461 upregulated in glioma tissues when compared with the adjacent
462 normal tissues, and the increased YAP gene expression was
463 correlated with glioma histology grade. These results means that

464 YAP may involve in the development of glioma. Besides, linc-OIP5
465 expression was significantly positive with YAP mRNAs expression
466 indicated linc-OIP5 is associated with YAP. So we postulated that
467 linc-OIP5 can regulate YAP-Notch signaling pathway to contribute
468 glioma metastasis. To confirm our hypothesis, we detected the
469 expression level of YAP, Jag-1, Notch-1, and Hes-1 after treated
470 glioma cell lines T98G and A172 with linc-OIP5-siRNA, and we
471 proved that linc-OIP5 indeed suppressed YAP expression, reduced
472 downstream Jag-1, Notch-1, and Hes-1 expression both at gene and
473 protein level, which indicated that abnormal expression of linc-
474 OIP5 could influence YAP and Notch signaling pathway by
475 mediating YAP gene and the downstream genes expression of
476 Notch signaling pathway in glioma cells, thus play significant roles
477 in glioma origination and development.

478

479 **Conclusion**

480 Taken together, the findings from our study demonstrated that
481 the down-regulation of the linc-OIP5 expression inhibits glioma
482 cell proliferation, migration, and tumorigenesis potential by
483 influencing the YAP-Notch signaling pathway. Thus, linc-OIP5
484 could be a promising therapeutic target and novel molecular
485 biomarker for glioma.

486

487 **Abbreviations**

488 CCK8: Cell Counting Kit-8; CNS: Central Nervous System;
489 DMEM: Dulbecco's modified Eagle's medium; GBM:
490 Glioblastoma; Hes-1: Hairy and Enhancer of Split-1; Jag-1: Jagged-
491 1; LincRNAs: Long intergenic noncoding RNAs; LncRNAs: Long
492 noncoding RNAs; MALAT1: Metastasis-associated lung
493 adenocarcinoma transcript 1; ncRNAs: non-coding RNAs; OIP5:
494 Opa interacting protein 5; qRT-PCR: Quantitative real-time
495 polymerase chain reaction; RTCA: Real-Time Cell Analyzer;
496 siRNA : small interfering RNA; TMZ: Temozolomide; YAP: yes-
497 associated-protein.

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502 **Author's contributions**

503 LW, XGZ, and HG conceived the study, designed the experiments,
504 and provided their funds to the study; GWH and HLL participated
505 in cell culture, siRNAs infection; PZ and WK were responsible for
506 animal experiment and performed statistical analysis of all
507 experimental data. All authors read and approved the final
508 manuscript.

509

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Figure legends

Fig 1 - Abnormal linc-OIP5 expression is associated with glioma.

(A) Relative expression level of linc-OIP5 expression in glioma tissues and adjacent normal tissues was measured by qRT-PCR (N=167). RNA relative expression levels were normalized against the gene β -actin transcript expression levels. 111 cases (66.5%) showed increased linc-OIP5 expression, whereas 56 cases (33.5%) showed decrease or no significant difference. (B) 82 cases (49.1%) of 98 WHO III/IV glioma patients showed linc-OIP5 highly expression (fold change ≥ 2), whereas 29 cases (17.4%) of 69 WHO I/II glioma showing highly expression. A fold change of ≥ 2 was defined as linc-OIP5 high, and the rest was indicated as linc-OIP5

627 low. $P < 0.01$.

628

629 **Fig 2 - Knockdown of linc-OIP5 in glioma cell lines suppressed**
630 **glioma cells proliferation in vitro.**

631 (A) T98G and A172 glioma cells were transfected with mixed
632 linc-OIP5 siRNA, and linc-OIP5 expression were significantly
633 decreased measured by qRT-PCR. (B-C) Proliferation was
634 measured by using the CCK8 , Knockdown of linc-OIP5
635 significantly suppressed T98G cells and A172 cells proliferation (*
636 represents $P < 0.05$ when compared to negative controls). (D-E)
637 Proliferation was measured by testing colony formation ability,
638 Knockdown of linc-OIP5 significantly suppressed T98G cells and
639 A172 cells colony formation (* represents $P < 0.05$ when compared
640 to negative controls).

641

642 **Fig 3 - Knockdown of linc-OIP5 in glioma cell lines suppressed**
643 **glioma cells migration in vitro and tumor growth in vivo.**

644 (A-D) Migration ability was measured by using scratched wound
645 assay , Knockdown of linc-OIP5 significantly suppressed T98G
646 cells and A172 cells migration (* represents $P < 0.05$ when compared
647 to negative controls). (E) Migration ability was measured by using
648 RTCA assay, Knockdown of linc-OIP5 remarkably suppressed the
649 motility of glioma cell lines (* represents $P < 0.05$ when compared

650 to negative controls). (F-G) Tumor volumes were measured in linc-
651 OIP5 siRNA groups and negative control groups. Downregulated
652 of linc-OIP5 significantly inhibited tumor growth in vivo (*
653 represents $P < 0.05$) .

654

655 **Fig 4 - Quantitative determination of YAP by qRT-PCR in**
656 **glioma tissues and adjacent normal tissues.**

657 Relative expression level of linc-OIP5 expression in glioma tissues
658 and adjacent normal tissues was measured by qRT-PCR (N=167).
659 RNA relative expression levels were normalized against the gene β -
660 actin transcript expression levels. (A) The YAP expression levels in
661 the glioma group were higher than those in the adjacent normal
662 tissues. (B) The YAP expression levels in the WHO III/IV glioma
663 group were higher than those in the WHO I/II glioma group. (C)
664 The YAP mRNA levels were plotted against linc-OIP5 expression,
665 and a strong positive correlation was detected between linc-OIP5
666 expression and YAP expression ($R^2 = 0.7458$; $p < 0.01$).

667

668 **Fig 5 - Knockdown of linc-OIP5 inhibits YAP and downstream**
669 **Notch signaling pathway expression.**

670 qRT-PCR and Western blot were used to detect the expression level
671 of YAP, Jag-1, Notch-1, and Hes-1 treated by linc-OIP5 siRNAs
672 and siRNA negative control. (A-B) Knockdown of linc-OIP5

significantly suppressed YAP, Jag-1, Notch-1, and Hes-1 genes expression in T98G cell and A172 cell. (C-F) Knockdown of linc-OIP5 significantly suppressed YAP, Jag-1, Notch-1, and Hes-1 protein levels in T98G cell and A172 cell. β -actin was used as a control. Data were presented as the mean $\pm$ SD (n=3). *P<0.05

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682 **Table 1 Association between lncRNA-OIP5 expression and clinicopathological features in glioma.**

Characteristic	No. of Patients (%)	Relative OIP5 expression		<i>P value</i> *
		High	Low	
Sex				
male	118 (70.7%)	69 (41.3%)	49 (29.3%)	0.207
female	49 (29.3%)	28 (16.8%)	21 (12.6%)	
Age				
<55 (42.1±9.9)	64 (38.3%)	39 (23.4%)	25 (15.0%)	0.428
≥55 (64.2±4.5)	103 (61.7%)	58 (34.7%)	45 (26.9%)	
Tumor size (cm)				
<5	113 (67.7%)	59 (35.3%)	54 (32.3%)	0.174
≥5	54 (32.3%)	32 (19.2%)	22 (13.2%)	
WHO grade				
I/II	69 (41.1%)	29 (17.4%)	40 (24.0%)	0.007
III/IV	98 (58.9%)	82 (49.1%)	16 (9.5%)	

**P values* were determined using a 2-sided chi-square test or a 1-way analysis of variance

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Table 2 Primers used for quantitative reverse-transcriptase polymerase chain reaction (RT-PCR)

Genes	Forward Primer (5'-3')	Reverse Primer (5'-3')
h-linc-OIP5	TGCGAAGATGGCGGAGTAAG	TAGTTCCTCTCCTCTGGCCG
h-YAP	CCTGCGTAGCCAGTTACCAA	CCATCTCATCCACACTGTTC
h-Jag1	GAGACATCGATGAATGTGCC	GAGCAGTTCTTGCCCTCATA
h-Notch1	GCAGTTGTGCTCCTGAAGAA	CGGGCGGCCAGAAAC
h-Hes1	ACCGGACAAACAAAGACAGCCTCTG	CTGCAGGTTCCGGAGGTGCTTCACTG
h-β-actin	CGTGGGCCCGCCCTAGGCACCAAGG	GGGAGGAAGAGGATGCGGCAGTGG

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Fig 1

Fig 2

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775 Fig 5

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