# LTA4H extensively associates with mRNAs and lncRNAs indicative of its novel regulatory targets

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## Abstract

The RNA-binding metabolic enzyme LTA4H is a novel target for cancer chemoprevention and chemotherapy. Recent research shows that the increased expression of LTA4H in laryngeal squamous cell carcinoma (LSCC) promotes tumor proliferation, migration, and metastasis. However, its mechanism remains unclear. To investigate the potential role of LTA4H in LSCC, we employed the improved RNA immunoprecipitation and sequencing (iRIP-Seq) experiment to get the expression profile of LTA4H binding RNA in HeLa model cells, a cancer model cell that is frequently used in molecular mechanism research. We found that LTA4H extensively binds with mRNAs/pre-mRNAs and lncRNAs. In the LTA4H binding peak, the frequency of the AAGG motif reported to interact with TRA2β4 was high in both replicates. More notably, LTA4H-binding genes were significantly enriched in the mitotic cell cycle, DNA repair, RNA splicing-related pathways, and RNA metabolism pathways, which means that LTA4H has tumor-related alternative splicing regulatory functions. QRT-PCR validation confirmed that LTA4H specifically binds to mRNAs of carcinogenesis-associated genes, including LTBP3, ROR2, EGFR, HSP90B1, and lncRNAs represented by NEAT1. These results suggest that LTA4H may combine with genes associated with LSCC as an RNA-binding protein to perform a cancer regulatory function. Our study further sheds light on the molecular mechanism of LTA4H as a

33  clinical therapy target for LSCC.

34

## Introduction

36  Laryngeal cancer is not only the most common malignancy of the head and neck, but al
37  so the second most common respiratory system tumor after lung cancer. 95 to 98% of la
38  ryngeal cancers are squamous cell carcinomas (LSCC) (Zuo et al. 2016). Research shows t
39  hat nearly 180,000 new cases of throat cancer and nearly 100,000 throat cancer deaths w
40  ere reported worldwide in one year (Bray et al. 2018). Due to the proneness to recurrence and
41  metastasis, the five-year overall survival rate for LSCC has been approximately 50% in recent ye
42  ars (Cavaliere et al. 2021). Due to the LSCC's uncertain molecular mechanism, over 60% of pati
43  ents were already at late stages when the disease was discovered (Steuer et al. 2017). An increasi
44  ng amount of research demonstrates that the formation and development of LSCC is related
45  to molecular mechanisms. Studies have shown that the prognosis of patients with LSCC
46  with down-regulated HLA class I antigen was worse (Ogino et al. 2006). It has also been re
47  ported that the ANXA1 interaction with FPR2/ALX promote proliferation and metastasis of LSC
48  C (Gastardelo et al. 2014). Studies have revealed lincRNA HOTAIR is highly expressed in LSC
49  C and promotes methylation of PTEN (Li et al. 2013). Hence, to better identify biomarkers and e
50  xplore effective new therapeutic strategies, it is essential to reveal the LSCC's carcinogenic mech
51  anism.

52      Genes, miRNAs and lncRNAs all play key roles in tumor genesis and development. In recent
53  years, researchers have found that many genes, miRNAs and lncRNAs are key factors of LSCC (
54  Zhang et al. 2016). Gao et al detected LTA4H expression in LSCC and normal tissue by q
55  PCR. The results showed that LTA4H was significantly up-regulated in LSCC tissues tha
56  n in normal tissue (Gao et al. 2019). A recent study screened 275 differential proteins associ
57  ated with laryngeal cancer through PPI (Protein-interaction) network analysis. GO function was s
58  ignificantly enriched in RNA processing and respiratory electron transport chains, and LTA4H w
59  as one of the up-regulated proteins (Peyvandi et al. 2018). More importantly, studies have demon
60  strated that increased LTA4H expression in LSCC is associated with a poor prognosis, and knoc
61  kdown of LTA4H successfully suppresses the growth, invasion and migration of laryngeal carcin
62  oma cells (Gao et al. 2019; Peyvandi et al. 2018). However, more study is necessary to fully und
63  erstand the specific molecular mechanism of LTA4H in laryngeal carcinoma.

64    There is increasing evidence that LTA4H is overexpressed in many malignant cancers, which

65    promotes cancer cell proliferation. For example, it has been identified that LTA4H is

66    overexpressed in esophageal adenocarcinoma and through inflammation-augmenting effect and

67    growth-stimulatory effect to promote Carcinogenesis (Chen et al. 2004). Studies have also shown

68    that LTA4H can enhance aminopeptidase and epoxide hydrolase activity to promote colon

69    cancer growth (Jeong et al. 2009). And activation of 5-LOx/LTA4H can stimulate oral epithelial

70    cell proliferation and inflammation, which is the main way to promote oral cancer (Guo et al.

71    2011; Sun et al. 2006).

72    As a zinc-dependent epoxide hydrolase and aminopeptidase, the active site of Leukotriene

73    A4 hydrolase (LTA4H) can be the target action site of related inhibitors (Chen et al. 2004;

74    Haeggström 2004; Vo et al. 2018). LTA4H is being investigated as a new target for cancer

75    treatment due to its role in inflammatory response and tumor progression. As a hydrolase,

76    LTA4H performs its classic biological functions, including chemotaxis, endothelial cell

77    adhesion, and leukocyte activation, by acting on the last step of the arachidonic acid metabolic

78    process (Haeggström 2018; Oh & Olefsky 2016; Snelgrove et al. 2010). As an aminopeptidase, it

79    involves in inflammation and host defensed though grading proline-glycine-proline (PGP), a

80    neutrophil chemokine that is also a biomarker for chronic obstructive pulmonary

81    disease (Haeggström 2004; Snelgrove et al. 2010). Furthermore, LTA4H is believed to function

82    as an RBP in the post-transcriptional control of specific mRNAs (Castello et al. 2012b; Castello

83    et al. 2015). RBPs not only interact with mRNAs directly, but also bound to proteins and other

84    diverse RNAs to play crucial roles (Gerstberger et al. 2014; Hamosh et al. 2005). A growing

85    body of evidence show RBPs can promote cancer cell growth, angiogenesis, and metastasis by

86    regulating numerous target genes related to tumor development (Kang et al. 2020) . We

87    hypothesized that LTA4H might interact with the RNAs of cancer-related critical genes at the

88    transcriptional or post-transcriptional levels to control the expression of those genes, thus

89    affecting the proliferation, invasion and metastasis of tumors (including LCSS) cells. However,

90    whether LTA4H binds to mRNAs in cancer cells remains unclear. We hypothesized that LTA4H

91    might interact with the RNAs of cancer-related critical genes at the transcriptional or post-

92    transcriptional levels to control the expression of those genes.

93    To validate our hypotheses, we used improved RNA immunoprecipitation and sequencing

94    (iRIP-Seq) method (Ke et al. 2021) on LTA4H in modal HeLa cells (Capouillez et al. 2009) to

95   explore its RNA-binding characteristics in cancer cells. The finding demonstrates that LTA4H

96   extensively binds to mRNAs/ pre-mRNAs and lncRNAs. And we have identified some crucial

97   LTA4H-bound genes that regulate cancer development, like NEAT1, LINC00657, LTBP3 and

98   ROR2. These results reveal the underlying molecular mechanisms of LTA4H as a clinical

99   therapeutic target for LCSS, which has significant effects on diagnostic and therapeutic

100  applications.

101

## Materials & Methods

### Cloning and Plasmid Construction

104  Hot Fusion primer pairs were created using CE Design V1.04. Each primer contained a 17-30 bp

105  sequence from the pIRES-hrGFP-1a vector and a gene-specific sequence fragment.

106  F-primer: agcccgggcggatccgaattcATGCCCGAGATAGTGGATACCTG

107  R-primer: gtcatccttgtagtcctcgagATCCACTTTTAAGTCTTTCCCCAC

108  At 37°C for 2 to 3 hours, we digested the pIRES-hrGFP-1a vector with EcoRI and XhoI (NEB).

109  The enzyme-digested vector was purified on a Qiagen column kit using 1.0% agarose gel. HeLa

110  cells' total RNA was obtained using Trizol. Oligo dT primers were used to transcribe the purified

111  RNA for cDNA. Following that, PCR amplification was used to synthesize the inserted

112  fragment. PCR insert and linearized vector digested with EcoRI and XhoI (NEB) were combined

113  in a PCR microtube and ligated with Vazyme's ClonExpress® II One Step Cloning Kit.

114  Chemical transformation was used to introduce plasmids into Escherichia coli strains. We

115  incubated cells overnight at 37°C on LB agar plates containing 1uL/ml ampicillin. Finally, 28

116  cycles of colony PCR were performed on the backbone vectors using universal primers to screen

117  colonies.

118

### Cell Culture and Transfections

120  CCTCC (China Center for Type Culture Collection, Wuhan, Hubei, China) provides Human CC

121  (Cervical Carcinoma) cell lines, HeLa (CCTCC@GDC0009). In Dulbecco's modified Eagle's

122  medium (DMEM), which contains 10% fetal bovine serum (FBS), 100 ug/mL streptomycin and

123  100 U/mL penicillin, we cultivated HeLa cells at 37°C and 5% CO2. Following the

124  manufacturer's instructions, HeLa cells were transfected using Lipofectamine 2000 (Invitrogen,

125 Carlsbad, CA, USA). Transfected cells were collected after 48 hours for RT-qPCR and western

126 blot analyses.

127

## Assessment of Gene Overexpression

129 To evaluated the effect of LTA4H overexpression, we used GAPDH (glyceraldehyde 3-

130 phosphate dehydrogenase) as a control gene. The synthesis of cDNA was carried out according

131 to standard procedures, and we performed RT-qPCR using Bestar SYBR Green RT-PCR Master

132 Mix (DBI Bioscience, Shanghai, China) on a Bio-Rad S1000. Additional file contains primer

133 information. After normalizing to GAPDH mRNA concentration level, each transcript was

134 quantified using 2- $\Delta\Delta CT$ method (Livak & Schmittgen 2001). The GraphPad Prism software

135 (San Diego, CA) was then used for comparison with the paired Student's t-test.

136

## Immunoprecipitation

138 HeLa cells were lysed on ice for 5 minutes with ice-cold lysis buffer (1×PBS, 0.5% sodium

139 deoxycholate, 0.1% SDS, 0.5% NP40) containing RNase inhibitors (Takara, 2313) and protease

140 inhibitors (Solarbio, 329-98-6). In order to remove cell debris, the mixture was forcefully shaken

141 and centrifuged for 20 minutes at 13,000 x g at 4°C. The centrifuged supernatant was incubated

142 overnight at 4℃ with DynaBeads protein A/G bound to normal IgG or anti-Flag LTA4H

143 antibody. The beads were washed twice with low salt washing buffer, high salt washing buffer

144 and 1X PNK buffer solution respectively, and the samples were suspended in the Elution Buffer

145 to extract RNA from the LTA4H-RNA complex.

146

## Western blot

148 Resuspend sample with 40ul Elution Buffer (50mM Tris-Cl (PH=8.0), 10mM EDTA (PH=8.0),

149 1%SDS, incubate it at 70 °C, 1,400 rpm for 20 min. Put the supernatant in a fresh EP tube. The

150 complex was separated on a 10% SDS-PAGE gel after being boiled in boiling water with 1X

151 SDS sample buffer for 10 min. with TBST buffer (20 mM Tris-buffered saline and 0.1% Tween-

152 20) contained 5% non-fat milk power, we diluted the primary antibody: Flag antibody (1:2,000,

153 Sigma, F7425), actin (1:2,000, 66CUSABIO). The membranes were soaked in the primary

154 antibody incubation solution and incubated at room temperature for 1h. Then the membranes

155 were soaked in the HRP-conjugated secondary antibody incubation solution and incubated at

156  room temperature for 1h. The enhanced chemiluminescence (ECL) reagent (Bio-Rad, 170506)

157  was used to detect the binding secondary antibody (anti-mouse or anti-rabbit 1:10,000) (Abcam).

158

## iRIP-seq Library Preparation and Sequencing

160  TRIzol (Invitrogen) was used to isolate the RBP-bound RNAs from the immunoprecipitation of

161  anti-Flag. In accordance with the manufacturer's instructions, complementary DNA (cDNA)

162  libraries were prepared using KAPA RNA Hyper RNA binding protein connects the future Prep

163  Kit (KAPA, KK8541). On the Illumina HiSeq X Ten platform, the cDNA libraries were

164  sequenced for 150 bp paired-ends.

165

## Data Analysis

167  Only uniquely mapped reads were used for the subsequent analysis after reads were matched

168  onto the genome using TopHat 2 (Kim et al. 2013) ."ABLIRC" strategy was utilized to determine

169  the genomic locations where LTA4H binds (Xia et al. 2017). Peaks were formed from reads that

170  had at least one base pair of overlap. Using computational simulation, reads with the same

171  number and lengths as reads in peaks were generated randomly for each gene. For the purpose of

172  generating random max peak height from overlapping reads, the outputting reads were further

173  mapped to the same genes. The whole procedure was done 500 times. All observed peaks with

174  heights greater than those of random maximum peaks (p-value 0.05) were chosen. The

175  simulation independently analyzed the IP and input samples, removing the IP peaks that

176  overlapped the input peaks. The peaks were used for motifs analysis with the HOMER software

177  (Hypergeometric Optimization of Motif Enrichment) (Heinz et al. 2010).

178

## Functional Enrichment Analysis

180  GO term and KEGG path enrichment analysis was performed using KOBAS 2.0 server (Xie et

181  al. 2011). According to the annotation information of peak associated gene, the GO Term of each

182  gene was counted, and significance of each Term was analyzed by Benjamini-Hochberg FDR

183  (BH) and hypergeometric test to determine the degree of enrichment.

184

## Reverse Transcription qPCR Validation

RT-qPCR was performed using total RNA from the iRIP-seq library preparation. Using the M-MLV Reverse Transcriptase (Vazyme), RNA was reverse transcribed into cDNA. Real-time PCR was carried out with the StepOne RealTime PCR System using the HieffTM qPCR SYBR® Green Master Mix (Low Rox Plus; YEASEN, China). Denaturation at 95 °C for 5 min was followed by 40 cycles of denaturation at 95 °C for 15 s, annealing and extension at 60 °C for 30 s under PCR cycling conditions. PCR amplifications were carried out in triplicate for each sample.

## Statistical Analysis

The statistical software SPSS 16.0 (Chicago, IL, USA) was used to manipulate the experimental data, which were all presented as mean standard deviation (SD). All experiments were run at least three times independently, and P < 0.05 was considered significant.

## Results

## Deregulated Expression of LTA4H in Various Cancers

Previous researches have shown that LTA4H is significantly expressed in several malignancies and affects the initiation and growth of tumors (Chen et al. 2004; Guo et al. 2011; Jeong et al. 2009; Sun et al. 2006). In order to explore the relationship between LTA4H and laryngeal squamous cell carcinoma (LSCC), or more broadly head and neck squamous cell carcinoma (HNSCC), we first studied the expression level of LTA4H in LSCC tissues and normal tissues through TCGA database (Fig.1A). Box plot and scatter diagram were used to display the expression levels of LTA4H (Transcripts Per Million (TPM)). According to the finding, LSCC tissues had lower levels of LTA4H than normal control tissues. The results obtained by Gao et al with TCGA database are also LTA4H downregulation in LSCC. However, the author then detected LTA4H expression in LSCC and normal tissue by qPCR, and have proved that TA4H in LSCC tissues was significantly up-regulated (Gao et al. 2019). In addition, we also studied the association between LTA4H expression level and prognosis in HNSCC and normal tissues (Fig.1B). A blue curve represented the low expression group, while a red curve represented the high expression group. A significant finding was that HNSCC patients with high expression of

214  LTA4H had a poor prognosis. Therefore, the potential function of LTA4H in laryngeal

215  squamous cell carcinoma deserves further study.

216

217  **Characterization of the LTA4H-RNA Interaction Map by iRIP-seq**

218  **Analysis**

219  To explore the potential function of LTA4H in LSCC, we obtained a LTA4H-bound RNA

220  profile in modal HeLa cells by applying theiRIP-seq approach. The iRIP-seq is an advanced

221  technique for studying RBPs, which achieves the precision of CLIP-seq to obtain both direct and

222  indirect binding sites of protein and RNA accurately, whereas maintains the simplicity of RIP-

223  seq. Labelled antibody and control antibody were used for immunoprecipitation, and two

224  separately replicate experiments were performed. For immunoprecipitation, two separate iRIP

225  repetitions were carried out using flag-tagged LTA4H. The western blots of both IP samples

226  showed the presence of the protein Flag- LTA4H, but the IgG control did not (Fig.2A). Then, we

227  performed paired-end sequencing for the cDNA libraries using the Illumina HiSeq X Ten

228  platform, and obtained the high-quality clean reads. After removing the adapter sequences and

229  low-quality reads, we were left with 30,619,638 and 49,122,624 reads for IP group and input

230  control of replicate 1, and 28,885,522 and 40,002,548 reads from those of the replicate 2 (Table

231  S1). Next, using TopHat 2, we mapped the sequencing reads to reference genomes GRCh38

232  (Kim et al. 2013). About 77.23–78.94 % of them were aligned and about 39.85–83.65 % were

233  matched uniquely. The uniquely mapped reads are overwhelmingly from mature mRNAs. The

234  percentage of splice reads among the uniquely aligned reads was substantially greater in the IP

235  sample compared with the control sample, indicating that LTA4H might have an involvement in

236  the splicing role (Table S2).

237      Correlation analysis of IP and input samples by comparing RPKM (Mortazavi et al. 2008)

238  (Reads per kilo base of a gene per million reads) of the same gene revealed transcripts were

239  obviously enriched in IP samples than input control, which indicated that the specificity of the

240  LTA4H-bound RNA is good (Fig.2B). We also made correlation analysis between the two IP

241  replicates, and the results showed that R=0.917, which indicated that the two groups of IP

242  samples had good repeatability (Fig.2B). The results of these two groups of samples are almost

243  the same, which indicates that the iRIP-seq experiment is reliable. And the reads distribution

244  across reference genomic regions showed LTA4H binding reads tend to concentrate in the CDS,

245 the intron regions than input control reads, as well as in 3'UTR, 5'UTR and noncoding exons

246 regions (Fig.2C).

247     In order to eliminate the interference caused by gene expression quantity for predicting

248 LTA4H specific binding sites, we adopted ABLIRC method (Chi et al. 2009) to identify

249 LTA4H-bound peaks precisely. There were 29242 overlapping peaks in the two replication

250 groups in Hela cells, indicating the overlap of peaks from the two sets of experiments is

251 relatively high (Fig.2D). Interesting, after sorting according to the number of reads on

252 overlapping peaks, the top peaks were mainly located in mRNAs and lncRNAs (Fig.2E). The

253 results demonstrated that LTA4H has an extensive capability for RNA binding and may function

254 as a regulator by interacting to mRNAs and lncRNAs. Peak distribution across reference

255 genomic regions revealed that the LTA4H binding peaks located in the intron region accounted

256 for a large proportion (66.93% and 63.20%), followed by CDS region (Fig.2F).

257     Then, the HOMER was employed to obtain sequence motif enriched within LTA4H peaks.

258 The results showed UG-rich motif and GA-rich motif were presented as the first two motifs of

259 LTA4H peaks of two replicates, which may be the key sites of LTA4H binding to its target

260 (Fig.2G). We found a high frequency of motif AAGG in both repeats in LTA4H binding

261 peaks. It has recently been reported that TRA2B interacts with motif AAGG to promote cancer

262 cell growth by disrupting gene expression processes associated with aging (Kajita et al.

263 2016). Our results suggest that LTA4H may interact with TRA2B for binding of the motif

264 AAGG to regulate gene expression in cancerous cells.

265     In conclusion, the obtained LTA4H-binding RNA map will help our understanding of the

266 overall regulatory mechanism of LTA4H-RNA association during gene expression in Hela cells.

267

268 **Analysis of Pre-mRNA and mRNAs Associated by LTA4H**

269 Further, the LTA4H overlapped peak associated genes were compared to the Gene Ontology

270 database for enrichment biological process analysis. We found that LTA4H-bound genes were

271 involved in gene expression, mitotic cell cycle, viral replication and DNA repair (Fig.3A). Next,

272 the DEseq package was used to identify the LTA4H-bound differentially enriched genes (DEGs)

273 (Anders & Huber 2010). Among the 14,170 DEGs, there were 2,776 enriched genes and 11,394

274 non-enriched genes related to LTA4H. We constructed a volcanic map to show the significantly

275 enriched genes associated with LTA4H, all of which are associated with oncogenesis, including

276  lncRNAs NEAT1 and LINC00657, and mRNAs ROR2, LTBP3, HSP90B1 and EGFR (Fig.3B).

277  To explore the potential biological role of these enriched genes, we continued to analyze the

278  enriched genes using GO database, and it showed they were mainly involved in negative

279  regulation of transcription, mitotic cell cycle, gene expression, and viral replication (Fig.3C).

280      Next, we performed an overlap analysis of LTA4H bound genes from ABLIRC algorithm and

281  DEG from DEseq. Running DEseq identified fewer enriched genes, which were well overlapped

282  by the LTA4H-bound genes by ABLIRC and resulted in 2425 overlapped genes (Fig.3D). The

283  results demonstrated a significant association between LTA4H-bound and enriched gene

284  expression. GO analysis showed the 2425 overlapped genes were mainly clustered at gene

285  expression, mitotic cell cycle, viral replication and DNA repair (Fig.3E).

286      To further verify the presence of Flag-LTA4H protein binding on target genes, we next

287  showed the distribution of reads binding location and coverage depth compared to Peak

288  associated genes. The results across LTBP3 show the two replicates were consistent, and the IP

289  groups were obviously biased towards the intron and exon regions than input control, which was

290  the potential binding region of LTA4H on LTBP3 (Fig.3F left panel). And further, we used this

291  gene to verify that it directly interacts with mRNA by RT-qPCR (Fig.3F right panel). In

292  comparison to the control group, LTBP3 was considerably higher in the IP group. Similarly,

293  mRNAs EGFR, ROR2 and HSP90B1 were distinctly enriched in IP samples compared to the

294  input samples and the results of subsequent validation were as expected (Fig.S1). Taken together,

295  our results suggest that LTA4H and mRNAs are closely interacted in Hela cells.

296

## Analysis of LTA4H-bound lncRNAs

298  We also conducted the reads density landscape for lncRNAs that LTA4H highly enriched. The

299  results across the cancer-promoting gene LINC00657 show there are many LTA4H-bound peaks

300  in IP compared with input (Fig.4A left panel). In the validation experiment, LINC00657 was

301  found to be significantly enriched in IP1, and there was no obvious bias in IP2 compared with

302  the control group, which was considered to be related to experimental error (Fig.4A right panel).

303  It may also be that the peak site is not enriched in IP2, that is, LTA4H may not bind LINC00657

304  specifically, they just bind randomly. The exact mechanism of LTA4H binding to LINC00657

305  has not been fully clarified and needs further study. NEAT1 had an obvious bias in IP groups,

306  and we know that dysregulation of NEAT1 plays a key carcinogenic role (Chen et al. 2015;

307   Wang et al. 2016) (Fig.4B left panel). Besides that, NEAT1 has been verified to be significantly

308   enriched in the IP group (Fig.4B right panel). In summary, we hypothesized that LTA4H is

309   preferentially bound to genes related to tumor formation and progression.

310

## Discussion

311

312   As an essential hydrolase for LTB4 production (Vo et al. 2018), Upregulated LTA4H has been

313   found to be linked with various malignancies, such as colon, esophageal, and lung cancer (Chen

314   et al. 2003a; Chen et al. 2004; Jeong et al. 2009). In the Cancer Genome Atlas (TCGA) database,

315   the decrease in LTA4H levels in the LSCC was unexpected. We think it could be related to the

316   small number of laryngeal squamous cell carcinoma TCGA database, leading to inconsistency

317   with the previous experimental results (Gao et al. 2019; Peyvandi et al. 2018). The low

318   expression of LTA4H in laryngeal cancer tissues was consistent with previous studies (Gao et al.

319   2019; Rodrigues-Lisoni et al. 2010). Importantly, further data showed that patients' survival

320   times were considerably shorter when their LTA4H expression was higher, suggesting that

321   LTA4H may have a neoplastic role in HNSCC. On the one hand, numerous investigations have

322   revealed that Leukotriene can control tumor growth by influencing interactions between the

323   stromal cells and tumor epithelial cells, creating the favorable conditions for tumor genesis. So

324   inflammatory mediators can be detected in the tumor microenvironment (Colotta et al. 2009;

325   Wang & Dubois 2010). On the other hand, two recent mRNA-interacting protein identification

326   studies reported the activity of LTA4H binding to mRNA (Castello et al. 2012a; Castello et al.

327   2015). Thus, we speculate that LTA4H not only participates in the regulation of cancer through

328   the inflammatory mediator pathway, but also controls the expression of cancer key genes by

329   interacting with mRNA at the transcriptional or post-transcriptional level. However, to

330   understand the specific mechanism of LTA4H in tumor cells, more study is necessary.

331       Herein, we used iRIP-seq to identify interactions between LTA4H and RNAs in Hela

332   cells. We analyzed the binding characteristics of LTA4H as RNA binding protein binding to

333   RNAs and found that IP groups were highly enriched comparing with input groups. It indicates

334   that many pre-mRNAs /mRNAs are specifically bound by LTA4H, confirming the function of

335   LTA4H binding RNA. Surprisingly, we found that LTA4H targets were not only enriched in

336   mRNAs, but also in lncRNAs, suggesting that LTA4H was also involved in non-coding

337   processes.

338   We also analyzed the peaks of LTA4H proteins by using the ABLIRC algorithm from iRIP-

339   seq results. The binding peak of LTA4H was mainly enriched in the Intron region and CDS

340   region, indicating that LTA4H has functional RNA targets. Importantly, GO results revealed that

341   LTA4H- bound proteins were considerably overrepresented in pathways associated with cancer,

342   including mitotic cell cycle, DNA repair, RNA splicing related pathways and RNA metabolism

343   pathways. We know that genomic instability is a common feature of most cancer cells, and DNA

344   damage affects genomic stability (Bröckelmann et al. 2020; Negrini et al. 2010). In addition,

345   defective DNA repair can lead to a predisposition to cancer (Chen et al. 2003b). In eukaryotic

346   cells, RNA splicing is a highly complex fine-tuning step in gene expression, while tumor genes

347   are prone to deactivation mutations at splicing sites (Rhine et al. 2018). It has been studied that

348   RBPs influence the development of various cancers by controlling the metabolism of many

349   transcripts, which confirms the relevance of our findings (Pereira et al. 2017). These results gave

350   us a new hint that LTA4H may bind to cancer-related lncRNAs and mRNAs and regulate their

351   expression and splicing levels, which may be a previously unknown molecular regulatory

352   mechanism of LTA4H in cancer.

353   In our study, we obtained six enriched genes associated with carcinogenesis from 14,170

354   DEGs. Among them, studies have been shown that mRNAs such as ROR2, LTBP3, HSP90B1,

355   and EGFR have some close links with the occurrence and treatment of LSCC. Upregulated

356   ROR2 and Wnt5a have shown to represent poor tumor stage and lymphatic metastasis in LSCC,

357   suggesting that ROR2 was an independent prognostic factor (Zhang et al. 2017). And Wnt5a,

358   which interacts with RoR2 physically and functionally, has been demonstrated to be related to

359   the growth of many different cancers (Asem et al. 2016; Oishi et al. 2003). Likewise, early-stage

360   head and neck neoplasm patients with high levels of LTBP3 have a poor prognosis for survival

361   (Deryugina et al. 2018). It has been shown that HSP90B1 is regulated by Mir-99a-3p to

362   participate in the pathogenesis of head-neck cancer (Okada et al. 2019), and the highly expressed

363   HSP90B1 represents the poor prognosis of many tumors, including breast cancer and lung cancer

364   (Lin et al. 2020; Liu et al. 2019). EGFR knockdown suppressed LSCC cell growth, infiltration

365   and migration, and EGFR inhibitors were proved to have anti-laryngeal cancer effects in vitro

366   and in vivo (Ren et al. 2021; Yang et al. 2020). These results show that LTA4H interacting

367   mRNAs are involved in regulating cancer proliferation, invasion, and metastasis in LSCC.

368    We also found LTA4H widely binds to lncRNAs such as NEAT1 and LINC00657, which

369    have recently been investigated for a variety of cellular roles (Mercer et al. 2009; Wilusz et al.

370    2009). Previous studies have reported that many lncRNAs interact with RBPs to play regulatory

371    functions. For example, MALAT1 binds serine/arginine (SR) proteins to regulate alternative

372    splicing (Tripathi et al. 2010). And NEAT1 can regulate transcription, miRNA processing and

373    alternative splicing by binding RBP (Cooper et al. 2014; Imamura et al. 2014; Jiang et al. 2017).

374    We also found some links between these targeted genes and laryngeal cancer and other similar

375    cancers. NEAT1 levels were significantly upregulated in LSCC, which predicted a poor

376    prognosis (Wang et al. 2016). Others have shown activation of NEAT1 expression also promoted

377    proliferation, migration, and invasion of Esophageal squamous cell carcinoma (Chen et al. 2015).

378    LINC00657 has been shown to play a regulatory function as an oncogene in ESCC (Sun et al.

379    2018). These results suggest that LTA4H may bind lncRNAs to participate in transcriptional and

380    post-transcriptional regulation to promote cancer development and result in a poor prognosis. It

381    could reveal a novel mechanism by which LTA4H regulates LSCC and may become a possible

382    target for clinical treatment of laryngeal carcinoma. If further studies in LSCC cells and clinical

383    samples clarify the regulatory function of LTA4H and lncRNA interaction, new ideas will be

384    provided for clinical treatment. In addition, we plan to conduct functional studies of LTA4H in

385    the future, such as comparing laryngeal cancer cells with cells that overexpress LTA4H/LTA4H

386    knockdown, to validate the findings from the RIP-seq analyses.

387

## 388    Conclusion

389    In summary, this is the first time we found that LTA4H preferentially binds to the mRNAs and

390    lncRNAs of cancer-related functional pathway genes in tumor cells by iRIP-Seq experiments and

391    shows enriched binding in specific intron and CDS regions of these genes. Therefore, we

392    speculated that LTA4H not only participates in the regulation of cancer through the

393    inflammatory mediator pathway, but also influences the production of different subtypes of

394    proteins by binding the RNA of target genes to regulate the alternative splicing process, thus

395    regulating the proliferation, migration, and invasion of LSCC. To better understand how LTA4H

396    regulates the alternative splicing of target genes, more research should be done. Therefore, our

397    discoveries could shed light on the underlying mechanism of LTA4H in promoting tumor

398    development and provide a new potential anti-tumor target.

399

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403

## References

Anders S, and Huber W. 2010. Differential expression analysis for sequence count data. *Genome Biol* 11:R106. 10.1186/gb-2010-11-10-r106

Asem MS, Buechler S, Wates RB, Miller DL, and Stack MS. 2016. Wnt5a Signaling in Cancer. *Cancers (Basel)* 8. 10.3390/cancers8090079

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, and Jemal A. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 68:394-424. 10.3322/caac.21492

Bröckelmann PJ, de Jong MRW, and Jachimowicz RD. 2020. Targeting DNA Repair, Cell Cycle, and Tumor Microenvironment in B Cell Lymphoma. *Cells* 9. 10.3390/cells9102287

Capouillez A, Debauve G, Decaestecker C, Filleul O, Chevalier D, Mortuaire G, Coppée F, Leroy X, Belayew A, and Saussez S. 2009. The helicase-like transcription factor is a strong predictor of recurrence in hypopharyngeal but not in laryngeal squamous cell carcinomas. *Histopathology* 55:77-90. 10.1111/j.1365-2559.2009.03330.x

Castello A, Fischer B, Eichelbaum K, Horos R, Beckmann Benedikt M, Strein C, Davey Norman E, Humphreys David T, Preiss T, Steinmetz Lars M, Krijgsveld J, and Hentze Matthias W. 2012a. Insights into RNA Biology from an Atlas of Mammalian mRNA-Binding Proteins. *Cell* 149:1393-1406. 10.1016/j.cell.2012.04.031

Castello A, Fischer B, Eichelbaum K, Horos R, Beckmann BM, Strein C, Davey NE, Humphreys DT, Preiss T, Steinmetz LM, Krijgsveld J, and Hentze MW. 2012b. Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. *Cell* 149:1393-1406. 10.1016/j.cell.2012.04.031

Castello A, Hentze MW, and Preiss T. 2015. Metabolic Enzymes Enjoying New Partnerships as RNA-Binding Proteins. *Trends Endocrinol Metab* 26:746-757. 10.1016/j.tem.2015.09.012

Cavaliere M, Bisogno A, Scarpa A, D'Urso A, Marra P, Colacurcio V, De Luca P, Ralli M, Cassandro E, and Cassandro C. 2021. Biomarkers of laryngeal squamous cell carcinoma: a review. *Ann Diagn Pathol* 54:151787. 10.1016/j.anndiagpath.2021.151787

Chen X, Kong J, Ma Z, Gao S, and Feng X. 2015. Up regulation of the long non-coding RNA NEAT1 promotes esophageal squamous cell carcinoma cell progression and correlates with poor prognosis. *Am J Cancer Res* 5:2808-2815.

Chen X, Li N, Wang S, Wu N, Hong J, Jiao X, Krasna MJ, Beer DG, and Yang CS. 2003a. Leukotriene A4 hydrolase in rat and human esophageal adenocarcinomas and inhibitory effects of bestatin. *J Natl Cancer Inst* 95:1053-1061. 10.1093/jnci/95.14.1053

Chen X, Wang S, Wu N, and Yang CS. 2004. Leukotriene A4 hydrolase as a target for cancer prevention and therapy. *Curr Cancer Drug Targets* 4:267-283. 10.2174/1568009043333041

Chen Z, Xu XS, Yang J, and Wang G. 2003b. Defining the function of XPC protein in psoralen and cisplatin-mediated DNA repair and mutagenesis. *Carcinogenesis* 24:1111-1121. 10.1093/carcin/bgg051

Chi SW, Zang JB, Mele A, and Darnell RB. 2009. Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. *Nature* 460:479-486. 10.1038/nature08170

Colotta F, Allavena P, Sica A, Garlanda C, and Mantovani A. 2009. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* 30:1073-1081. 10.1093/carcin/bgp127

Cooper DR, Carter G, Li P, Patel R, Watson JE, and Patel NA. 2014. Long Non-Coding RNA NEAT1 Associates with SRp40 to Temporally Regulate PPARγ2 Splicing during Adipogenesis in 3T3-L1 Cells. *Genes (Basel)* 5:1050-1063. 10.3390/genes5041050

Deryugina EI, Zajac E, Zilberberg L, Muramatsu T, Joshi G, Dabovic B, Rifkin D, and Quigley JP. 2018. LTBP3 promotes early metastatic events during cancer cell dissemination. *Oncogene* 37:1815-1829. 10.1038/s41388-017-0075-1

Gao W, An C, Xue X, Zheng X, Niu M, Zhang Y, Liu H, Zhang C, Lu Y, Cui J, Zhao Q, Wen S, Thorne RF, Zhang

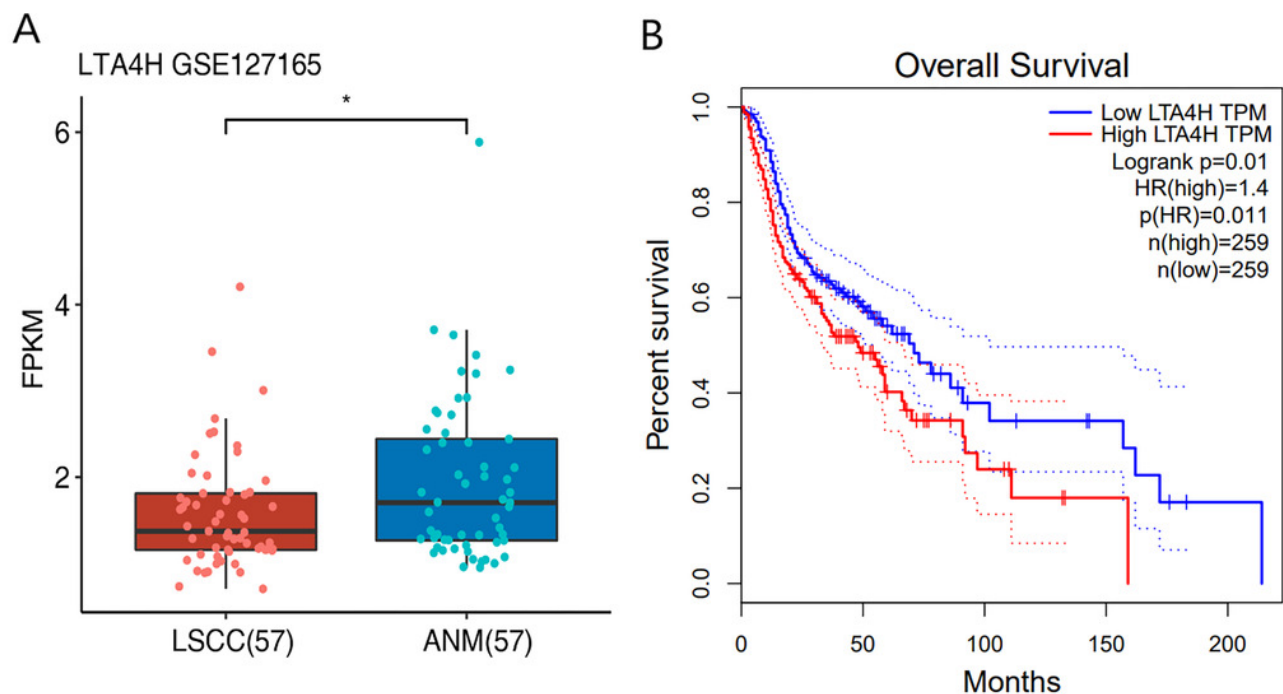
450  X, Wu Y, and Wang B. 2019. Mass Spectrometric Analysis Identifies AIMP1 and LTA4H as FSCN1-Binding
451       Proteins in Laryngeal Squamous Cell Carcinoma. *Proteomics* 19:e1900059. 10.1002/pmic.201900059
452  Gastardelo TS, Cunha BR, Raposo LS, Maniglia JV, Cury PM, Lisoni FC, Tajara EH, and Oliani SM. 2014.
453       Inflammation and cancer: role of annexin A1 and FPR2/ALX in proliferation and metastasis in human
454       laryngeal squamous cell carcinoma. *PLoS One* 9:e111317. 10.1371/journal.pone.0111317
455  Gerstberger S, Hafner M, and Tuschl T. 2014. A census of human RNA-binding proteins. *Nat Rev Genet* 15:829-845.
456       10.1038/nrg3813
457  Guo Y, Wang X, Zhang X, Sun Z, and Chen X. 2011. Ethanol promotes chemically induced oral cancer in mice
458       through activation of the 5-lipoxygenase pathway of arachidonic acid metabolism. *Cancer Prev Res (Phila)*
459       4:1863-1872. 10.1158/1940-6207.CAPR-11-0206
460  Haeggström JZ. 2004. Leukotriene A4 hydrolase/aminopeptidase, the gatekeeper of chemotactic leukotriene B4
461       biosynthesis. *J Biol Chem* 279:50639-50642. 10.1074/jbc.R400027200
462  Haeggström JZ. 2018. Leukotriene biosynthetic enzymes as therapeutic targets. *J Clin Invest* 128:2680-2690.
463       10.1172/jci97945
464  Hamosh A, Scott AF, Amberger JS, Bocchini CA, and McKusick VA. 2005. Online Mendelian Inheritance in Man
465       (OMIM), a knowledgebase of human genes and genetic disorders. *Nucleic Acids Res* 33:D514-517.
466       10.1093/nar/gki033
467  Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, Cheng JX, Murre C, Singh H, and Glass CK. 2010. Simple
468       combinations of lineage-determining transcription factors prime cis-regulatory elements required for
469       macrophage and B cell identities. *Mol Cell* 38:576-589. 10.1016/j.molcel.2010.05.004
470  Imamura K, Imamachi N, Akizuki G, Kumakura M, Kawaguchi A, Nagata K, Kato A, Kawaguchi Y, Sato H, Yoneda
471       M, Kai C, Yada T, Suzuki Y, Yamada T, Ozawa T, Kaneki K, Inoue T, Kobayashi M, Kodama T, Wada Y,
472       Sekimizu K, and Akimitsu N. 2014. Long noncoding RNA NEAT1-dependent SFPQ relocation from
473       promoter region to paraspeckle mediates IL8 expression upon immune stimuli. *Mol Cell* 53:393-406.
474       10.1016/j.molcel.2014.01.009
475  Jeong CH, Bode AM, Pugliese A, Cho YY, Kim HG, Shim JH, Jeon YJ, Li H, Jiang H, and Dong Z. 2009. [6]-
476       Gingerol suppresses colon cancer growth by targeting leukotriene A4 hydrolase. *Cancer Res* 69:5584-5591.
477       10.1158/0008-5472.Can-09-0491
478  Jiang L, Shao C, Wu QJ, Chen G, Zhou J, Yang B, Li H, Gou LT, Zhang Y, Wang Y, Yeo GW, Zhou Y, and Fu XD.
479       2017. NEAT1 scaffolds RNA-binding proteins and the Microprocessor to globally enhance pri-miRNA
480       processing. *Nat Struct Mol Biol* 24:816-824. 10.1038/nsmb.3455
481  Kajita K, Kuwano Y, Satake Y, Kano S, Kurokawa K, Akaike Y, Masuda K, Nishida K, and Rokutan K. 2016.
482       Ultraconserved region-containing Transformer 2β4 controls senescence of colon cancer cells. *Oncogenesis*
483       5:e213. 10.1038/oncsis.2016.18
484  Kang D, Lee Y, and Lee JS. 2020. RNA-Binding Proteins in Cancer: Functional and Therapeutic Perspectives.
485       *Cancers (Basel)* 12. 10.3390/cancers12092699
486  Ke J, Liu F, Tu Y, Cai Z, Luo Y, and Wu X. 2021. PARP1-RNA interaction analysis: PARP1 regulates the expression
487       of extracellular matrix-related genes in HK-2 renal proximal tubular epithelial cells. *FEBS Lett* 595:1375-
488       1387. 10.1002/1873-3468.14065
489  Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, and Salzberg SL. 2013. TopHat2: accurate alignment of
490       transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* 14:R36. 10.1186/gb-
491       2013-14-4-r36
492  Li D, Feng J, Wu T, Wang Y, Sun Y, Ren J, and Liu M. 2013. Long intergenic noncoding RNA HOTAIR is
493       overexpressed and regulates PTEN methylation in laryngeal squamous cell carcinoma. *Am J Pathol* 182:64-
494       70. 10.1016/j.ajpath.2012.08.042
495  Lin T, Qiu Y, Peng W, Peng L, and Harrison P. 2020. Heat Shock Protein 90 Family Isoforms as Prognostic
496       Biomarkers and Their Correlations with Immune Infiltration in Breast Cancer. *BioMed Research*
497       *International* 2020:1-15. 10.1155/2020/2148253
498  Liu K, Kang M, Li J, Qin W, and Wang R. 2019. Prognostic value of the mRNA expression of members of the HSP90
499       family in non‑small cell lung cancer. *Experimental and Therapeutic Medicine*. 10.3892/etm.2019.7228
500  Livak KJ, and Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and
501       the 2(-Delta Delta C(T)) Method. *Methods* 25:402-408. 10.1006/meth.2001.1262
502  Mercer TR, Dinger ME, and Mattick JS. 2009. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 10:155-
503       159. 10.1038/nrg2521
504  Mortazavi A, Williams BA, McCue K, Schaeffer L, and Wold B. 2008. Mapping and quantifying mammalian
505       transcriptomes by RNA-Seq. *Nat Methods* 5:621-628. 10.1038/nmeth.1226

506  Negrini S, Gorgoulis VG, and Halazonetis TD. 2010. Genomic instability--an evolving hallmark of cancer. *Nat Rev*
507      *Mol Cell Biol* 11:220-228. 10.1038/nrm2858
508  Ogino T, Shigyo H, Ishii H, Katayama A, Miyokawa N, Harabuchi Y, and Ferrone S. 2006. HLA class I antigen
509      down-regulation in primary laryngeal squamous cell carcinoma lesions as a poor prognostic marker. *Cancer*
510      *Res* 66:9281-9289. 10.1158/0008-5472.Can-06-0488
511  Oh DY, and Olefsky JM. 2016. G protein-coupled receptors as targets for anti-diabetic therapeutics. *Nat Rev Drug*
512      *Discov* 15:161-172. 10.1038/nrd.2015.4
513  Oishi I, Suzuki H, Onishi N, Takada R, Kani S, Ohkawara B, Koshida I, Suzuki K, Yamada G, Schwabe GC, Mundlos
514      S, Shibuya H, Takada S, and Minami Y. 2003. The receptor tyrosine kinase Ror2 is involved in non-canonical
515      Wnt5a/JNK signalling pathway. *Genes Cells* 8:645-654. 10.1046/j.1365-2443.2003.00662.x
516  Okada R, Koshizuka K, Yamada Y, Moriya S, Kikkawa N, Kinoshita T, Hanazawa T, and Seki N. 2019. Regulation
517      of Oncogenic Targets by miR-99a-3p (Passenger Strand of miR-99a-Duplex) in Head and Neck Squamous
518      Cell Carcinoma. *Cells* 8. 10.3390/cells8121535
519  Pereira B, Billaud M, and Almeida R. 2017. RNA-Binding Proteins in Cancer: Old Players and New Actors. *Trends*
520      *Cancer* 3:506-528. 10.1016/j.trecan.2017.05.003
521  Peyvandi H, Peyvandi AA, Safaei A, Zamanian Azodi M, and Rezaei-Tavirani M. 2018. Introducing Potential Key
522      Proteins and Pathways in Human Laryngeal Cancer: A System Biology Approach. *Iran J Pharm Res* 17:415-
523      425.
524  Ren K, Wang B, and Qi Q. 2021. Development of a new EGFR antibody antagonist which exhibits potential biological
525      effects against laryngeal cancer. *Ann Transl Med* 9:964. 10.21037/atm-21-1839
526  Rhine CL, Cygan KJ, Soemedi R, Maguire S, Murray MF, Monaghan SF, and Fairbrother WG. 2018. Hereditary
527      cancer genes are highly susceptible to splicing mutations. *PLoS Genet* 14:e1007231.
528      10.1371/journal.pgen.1007231
529  Rodrigues-Lisoni FC, Peitl P, Jr., Vidotto A, Polachini GM, Maniglia JV, Carmona-Raphe J, Cunha BR, Henrique T,
530      Souza CF, Teixeira RA, Fukuyama EE, Michalaurt P, Jr., de Carvalho MB, Oliani SM, Tajara EH, Cury PM,
531      de Carvalho MB, Dias-Neto E, Figueiredo DL, Fukuyama EE, Góis-Filho JF, Leopoldino AM, Mamede RC,
532      Michaluart-Junior P, Moyses RA, Nóbrega FG, Nóbrega MP, Nunes FD, Ojopi EF, Serafini LN, Severino P,
533      Silva AM, Silva WA, Jr., Silveira NJ, Souza SC, Tajara EH, Wünsch-Filho V, Amar A, Bandeira CM,
534      Braconi MA, Brandão LG, Brandão RM, Canto AL, Cerione M, Cicco R, Chagas MJ, Chedid H, Costa A,
535      Cunha BR, Curioni OA, Fortes CS, Franzi SA, Frizzera AP, Gazito D, Guimarães PE, Kaneto CM, López
536      RV, Macarenco R, Magalhães MR, Meneses C, Mercante AM, Pinheiro DG, Polachini GM, Rapoport A,
537      Rodini CO, Rodrigues-Lisoni FC, Rodrigues RV, Rossi L, Santos AR, Santos M, Settani F, Silva FA, Silva
538      IT, Souza TB, Stabenow E, Takamori JT, Valentim PJ, Vidotto A, Xavier FC, Yamagushi F, Cominato ML,
539      Correa PM, Mendes GS, Paiva R, Ramos O, Silva C, Silva MJ, and Tarlá MV. 2010. Genomics and
540      proteomics approaches to the study of cancer-stroma interactions. *BMC Med Genomics* 3:14. 10.1186/1755-
541      8794-3-14
542  Snelgrove RJ, Jackson PL, Hardison MT, Noerager BD, Kinloch A, Gaggar A, Shastry S, Rowe SM, Shim YM,
543      Hussell T, and Blalock JE. 2010. A critical role for LTA4H in limiting chronic pulmonary neutrophilic
544      inflammation. *Science* 330:90-94. 10.1126/science.1190594
545  Steuer CE, El-Deiry M, Parks JR, Higgins KA, and Saba NF. 2017. An update on larynx cancer. *CA Cancer J Clin*
546      67:31-50. 10.3322/caac.21386
547  Sun Y, Wang J, Pan S, Yang T, Sun X, Wang Y, Shi X, Zhao X, Guo J, and Zhang X. 2018. LINC00657 played
548      oncogenic roles in esophageal squamous cell carcinoma by targeting miR-615-3p and JunB. *Biomed*
549      *Pharmacother* 108:316-324. 10.1016/j.biopha.2018.09.003
550  Sun Z, Sood S, Li N, Ramji D, Yang P, Newman RA, Yang CS, and Chen X. 2006. Involvement of the 5-
551      lipoxygenase/leukotriene A4 hydrolase pathway in 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral
552      carcinogenesis in hamster cheek pouch, and inhibition of carcinogenesis by its inhibitors. *Carcinogenesis*
553      27:1902-1908. 10.1093/carcin/bgl039
554  Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, Freier SM, Bennett CF, Sharma A, Bubulya PA, Blencowe
555      BJ, Prasanth SG, and Prasanth KV. 2010. The nuclear-retained noncoding RNA MALAT1 regulates
556      alternative splicing by modulating SR splicing factor phosphorylation. *Mol Cell* 39:925-938.
557      10.1016/j.molcel.2010.08.011
558  Vo TTL, Jang WJ, and Jeong CH. 2018. Leukotriene A4 hydrolase: an emerging target of natural products for cancer
559      chemoprevention and chemotherapy. *Ann N Y Acad Sci* 1431:3-13. 10.1111/nyas.13929
560  Wang D, and Dubois RN. 2010. Eicosanoids and cancer. *Nat Rev Cancer* 10:181-193. 10.1038/nrc2809
561  Wang P, Wu T, Zhou H, Jin Q, He G, Yu H, Xuan L, Wang X, Tian L, Sun Y, Liu M, and Qu L. 2016. Long noncoding

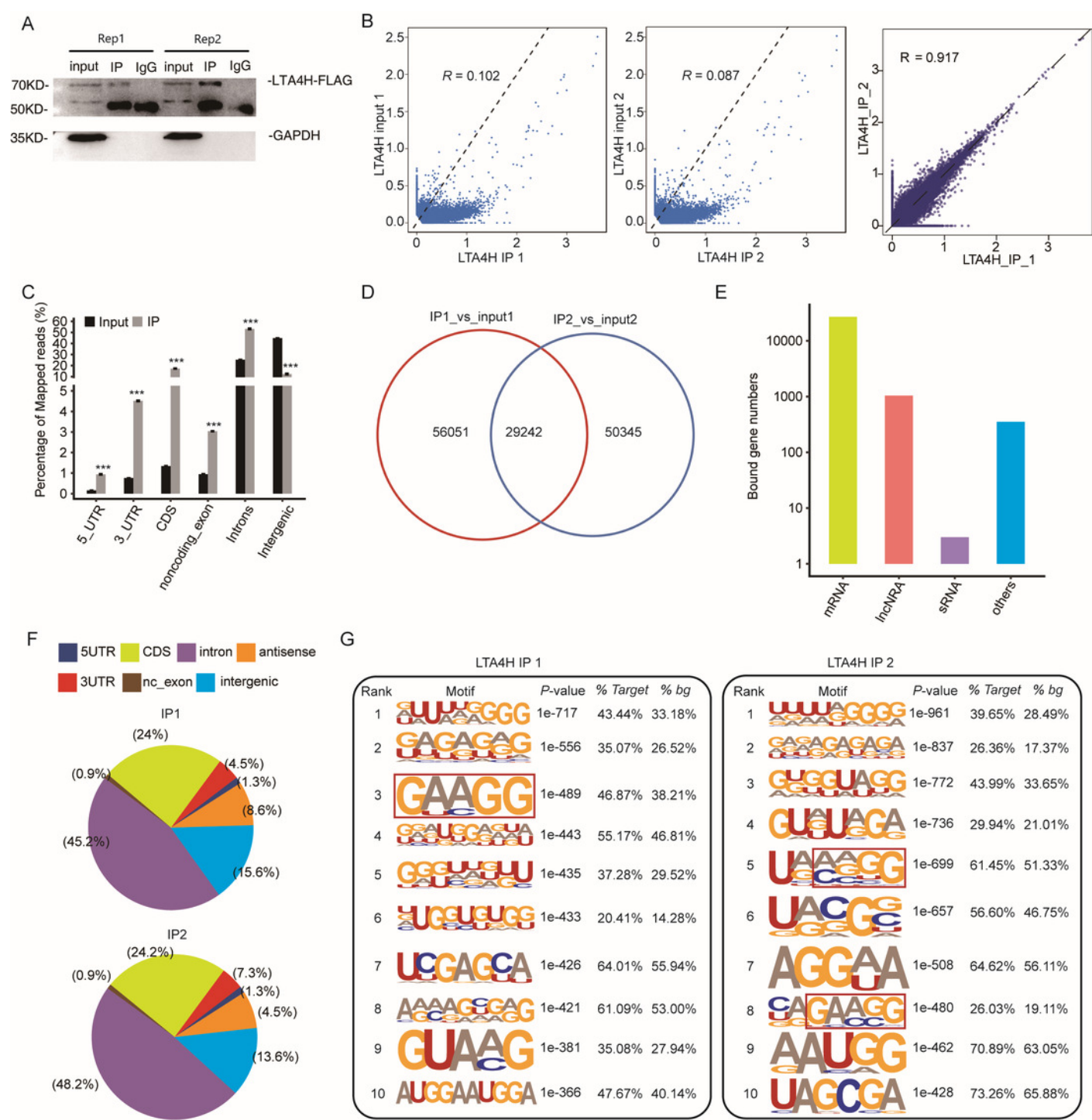
562      RNA NEAT1 promotes laryngeal squamous cell cancer through regulating miR-107/CDK6 pathway. *J Exp*
563          *Clin Cancer Res* 35:22. 10.1186/s13046-016-0297-z
564  Wilusz JE, Sunwoo H, and Spector DL. 2009. Long noncoding RNAs: functional surprises from the RNA world.
565          *Genes Dev* 23:1494-1504. 10.1101/gad.1800909
566  Xia H, Chen D, Wu Q, Wu G, Zhou Y, Zhang Y, and Zhang L. 2017. CELF1 preferentially binds to exon-intron
567          boundary and regulates alternative splicing in HeLa cells. *Biochim Biophys Acta Gene Regul Mech* 1860:911-
568          921. 10.1016/j.bbagrm.2017.07.004
569  Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, Kong L, Gao G, Li CY, and Wei L. 2011. KOBAS 2.0: a web server
570          for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res* 39:W316-322.
571          10.1093/nar/gkr483
572  Yang T, Li S, Liu J, Yin D, Yang X, Tang Q, and Wang S. 2020. Long non-coding RNA KRT16P2/miR-1294/EGFR
573          axis regulates laryngeal squamous cell carcinoma cell aggressiveness. *Am J Transl Res* 12:2939-2955.
574  Zhang C, Gao W, Wen S, Wu Y, Fu R, Zhao D, Chen X, and Wang B. 2016. Potential key molecular correlations in
575          laryngeal squamous cell carcinoma revealed by integrated analysis of mRNA, miRNA and lncRNA
576          microarray profiles. *Neoplasma* 63:888-900. 10.4149/neo_2016_608
577  Zhang W, Yan Y, Gu M, Wang X, Zhu H, Zhang S, and Wang W. 2017. High expression levels of Wnt5a and Ror2
578          in laryngeal squamous cell carcinoma are associated with poor prognosis. *Oncol Lett* 14:2232-2238.
579          10.3892/ol.2017.6386
580  Zuo J, Wen J, Lei M, Wen M, Li S, Lv X, Luo Z, and Wen G. 2016. Hypoxia promotes the invasion and metastasis
581          of laryngeal cancer cells via EMT. *Med Oncol* 33:15. 10.1007/s12032-015-0716-6
582

# Figure 1

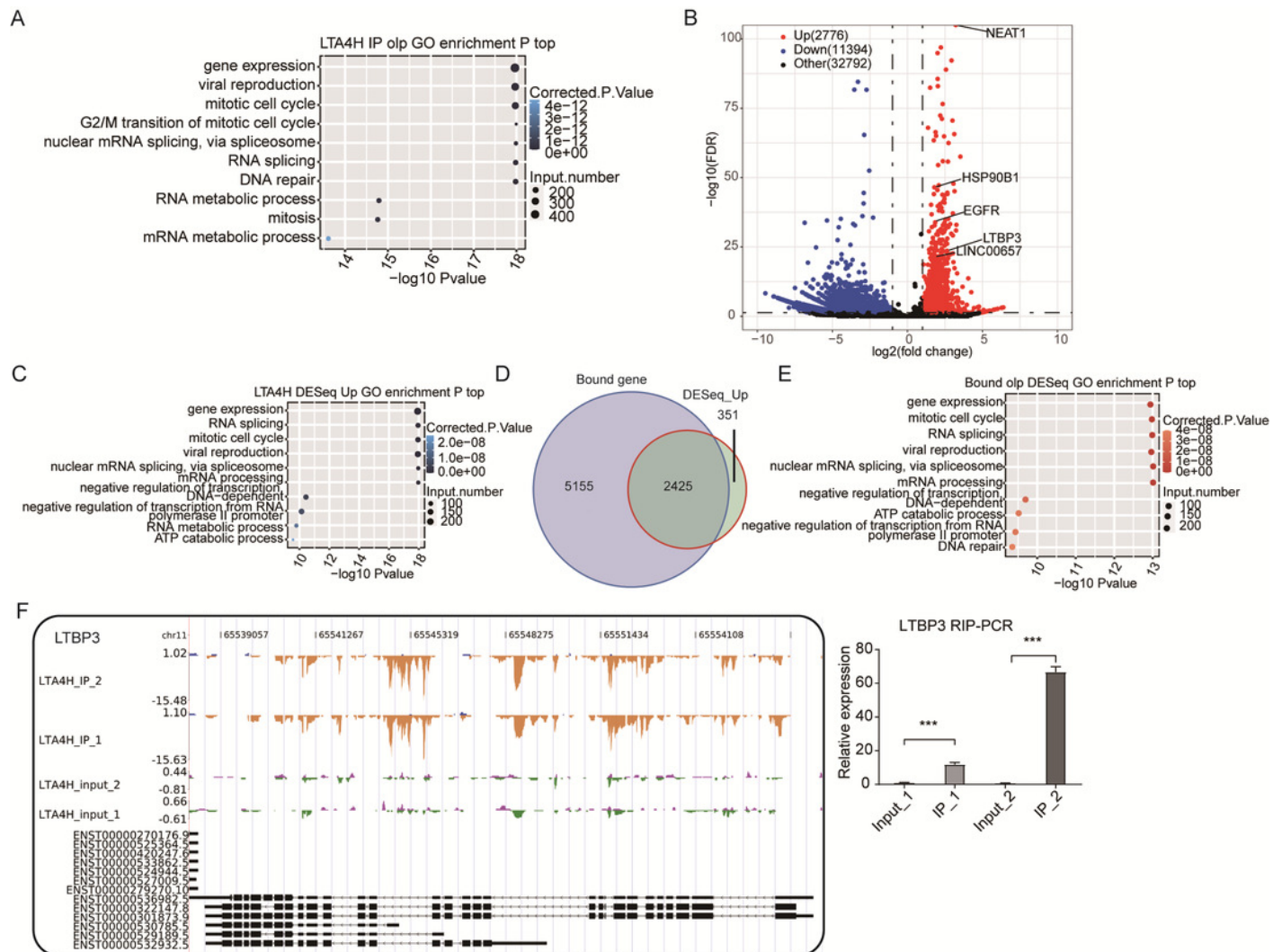
Expression and survival analysis of LTA4H.

(A) Transcription levels of LTA4H in LSCC and normal samples from The Cancer Genome Atlas (TCGA) database. (B) Survival analysis of LTA4H in HNSCC from The Cancer Genome Atlas (TCGA) database.

# Figure 2

Transcriptome-wide identification of LTA4H binding targets using iRIP-seq method.

(A) Western blotting analysis of LTA4H expression. (B) Scatter plot showing Pearson correlation between IP and input samples and between the two IP replicates. (C) Reads distribution across reference genome. Error bars represent mean ± SEM. ***$p < 0.001$, **$p < 0.01$. (D) Venn diagram showing the overlap of LTA4H binding peaks obtained from two replicates of iRIP-seq. The peaks were called by ABLIRC algorithm. (E) Bar plot showed the classification of LTA4H targets in common in two replicates. (F) Distribution of peaks across reference genome. (G) The top ten over-represented motifs in LTA4H binding peaks.

# Figure 3

Analysis of the targets bound by LTA4H.

(A) The top 10 enriched GO biological processes of the LTA4H-bound genes by ABLIRC algorithm in two replicates. (B) Potential targets identified by DEseq. (C) The top 10 enriched GO biological processes of the LTA4H-bound genes by DEseq. (D) Venn diagram showing the overlap of LTA4H bound genes obtained from two replicates by ABLIRC algorithm and DEseq. (E) The top 10 enriched GO biological processes of the LTA4H- bound genes by both ABLIRC algorithm and DEseq. (F) The reads density landscape of LTA4H- binding peaks across LTBP3 (left). Quantification of LTBP3 expression by qRT-PCR using iRIP-seq data (right).

# Figure 4

LTA4H binds to lncRNA involved in laryngeal squamous cell carcinoma.

(A-B) The reads density landscape of LTA4H-binding peaks across lncRNAs (left).

Quantification of LINC00657 and NEAT1 expression by qRT-PCR using iRIP-seq data (right)