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REVIEW

LncRNA HOTAIR promotes aerobic glycolysis by recruiting Lin28 to induce inflammation and apoptosis in acute lung injury

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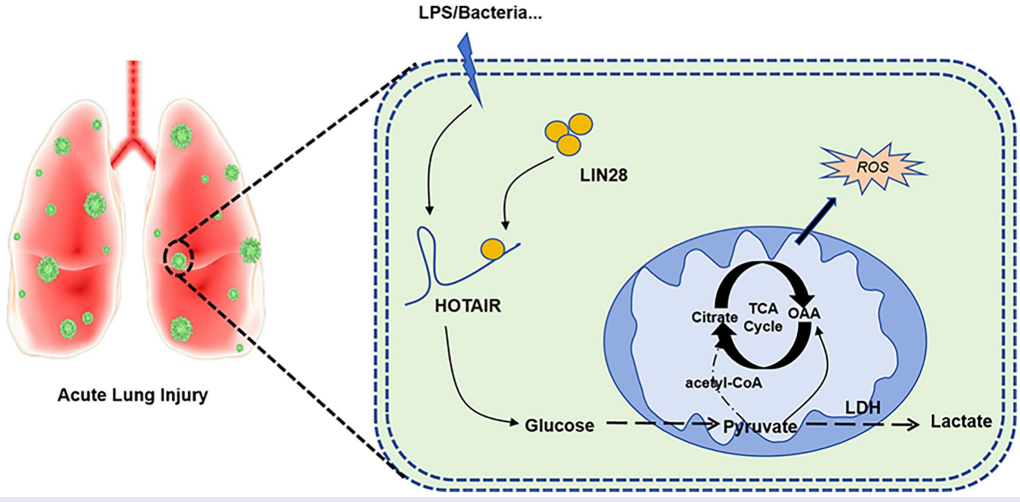
ABSTRACT

Acute lung injury (ALI) is a life-threatening condition with high rates of morbidity and mortality. Recently, there has been growing evidence suggesting a link between lncRNA HOTAIR and ALI. Nonetheless, the precise role and mechanism of lncRNA HOTAIR in ALI remain to be fully elucidated. siHOTAIR transfection, qPCR detection (HOTAIR), ELISA (TNF-α, IL-6, and IL-1β), Lactate detection, Glucose uptake experiment, Cell Apoptosis Analysis, Fluorescence in situ hybridization (FISH) assay. Through siHOTAIR transfection, we discovered that HOTAIR plays a role in the secretion of inflammatory factors in ALI and further regulates glucose uptake and metabolism in lung epithelial cells. Moreover, a comparison between HOTAIR knockdown cells and HOTAIR overexpression cells revealed that HOTAIR promotes cellular aerobic sugar metabolism, leading to increased secretion of inflammatory factors and cell apoptosis. Our in-depth research also identified an interaction between HOTAIR and the LIN28 protein. Knocking down HOTAIR resulted in the downregulation of LIN28 protein expression, which subsequently inhibited the expression of the glucose transporter GLUT1. This indicates that HOTAIR facilitates glucose uptake and boosts cellular aerobic glycolysis by modulating the LIN28 protein, thereby promoting inflammation and apoptosis in acute lung injury. The research findings presented in this article offer significant insights into the function of HOTAIR in ALI and suggest a potential therapeutic target for the treatment of this condition.



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HOTAIR; glycolysis; LIN28; ALI; Inflammation

Introduction

Acute lung injury (ALI) is a rapidly progressing condition characterized by hypoxic respiratory failure due to an uncontrolled inflammatory response in the lungs or the entire body [1,2]. It is caused by an uncontrolled inflammatory response in the lungs or the entire body, resulting in acute diffuse damage to

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alveolar epithelial cells, pulmonary microvascular endothelial cells, and pulmonary interstitium [3,4]. The causes of acute lung injury include infections, inhalation of harmful gases or substances, lung trauma, prolonged hypotension, transfusion-related lung injury, drug reactions, and various other factors. The main symptoms are difficulty breathing, coughing with sputum production, cyanosis and hypoxia, as well as accompanying symptoms such as chest pain, tightness in the chest, and shortness of breath [5]. Acute lung injury has a high mortality rate, with different studies reporting a wide range of mortality rates, from 15% to 72% [6,7]. Despite significant advancements in understanding the aetiology and pathophysiological mechanisms of ALI [8–10], there is still a lack of effective drug treatments. Therefore, it is crucial to conduct in-depth research on the molecular pathogenic mechanisms of ALI and develop effective therapeutic drugs to address this urgent issue in the field of respiratory diseases.

In patients with ALI, there is a high expression of inflammatory factors such as tumour necrosis factor-α (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6) [11,12], which further contribute to lung cell apoptosis [13], resulting in organ failure. Hence, it is of utmost importance to explore and identify effective methods for reducing the release of inflammatory factors and lung cell apoptosis in the treatment of acute lung injury. The reprogramming of glucose metabolism is considered a key regulator of inflammasome activation [14–16]. This metabolic reprogramming, also known as the Warburg effect, was first described in cancer cells and is characterized by increased aerobic glycolysis and decreased oxidative phosphorylation [17]. Glycolysis can induce inflammatory activation to amplify the inflammatory response [18,19]. Increased levels of glycolysis-related reactive oxygen species (ROS) contribute to NLR Family, Pyrin Domain Containing Protein 3 (NLRP3) inflammasome activation and IL-1β secretion [20–22]. Furthermore, inflammasome activation is associated with glucose transporter 1 (GLUT1)-dependent glycolysis [23]. Therefore, it is crucial to explore the regulatory role of glucose metabolism in ALI for a better understanding of the disease's occurrence and development.

Emerging evidence suggests that lncRNAs, particularly HOTAIR, play a crucial role in the resolution of inflammation in Acute respiratory distress syndrome (ARDS) [24,25]. HOTAIR is a 2158-bp long non-coding RNA (lncRNA) identified through a custom tiling array targeting the HOXC gene cluster. Research has demonstrated that HOTAIR suppresses tumour suppressor genes, including HOXD10, PGR, and members of the protocadherin gene family, in breast cancer cells [26,27]. HOTAIR serves as a negative prognostic indicator for various cancers, including breast, liver, colon, pancreatic, and cervical cancers. Additionally, elevated levels of HOTAIR expression have been associated with increased metastasis in breast, liver, colon, and pancreatic cancers remains under investigation [28–31]. Research has shown that HOTAIR can regulate cellular inflammation and apoptosis [32–34]. It promotes the expression of inflammatory factors (TNF-α, IL-1β, IL-6) [35,36] and pro-apoptosis-related proteins (cleaved caspase 3, Bax), while inhibiting the expression of the anti-apoptotic protein Bcl-2 [36,37]. HOTAIR is not regulated in cardiomyocytes of mice with LPS-induced sepsis. Increased HOTAIR levels enhance TNF-α production by activating the NF-κB pathway [35]. Liu et al. have demonstrated that lncRNA HOTAIR contributes to inflammation gene expression in rheumatoid arthritis [38]. Previous studies have shown that HOTAIR, a molecule, plays a critical role in tumour cell glucose metabolism [39–41]. However, the role of HOTAIR in glucose metabolism in ALI has been sparsely reported.

In this study, our objective was to investigate the regulatory mechanism of HOTAIR in acute lung injury. The results revealed that acute lung injury induced by LPS increased the expression of HOTAIR, leading to enhanced glucose uptake in alveolar epithelial cells and promoting cell glycolysis, ultimately resulting in apoptosis. Additionally, we discovered that HOTAIR regulates the induction of aerobic glycolysis by recruiting LIN28, thereby promoting inflammation and apoptosis. These results imply that targeting the lncRNA HOTAIR could represent a potential therapeutic approach for ALI.

Materials and methods

Cell culture

BEAS-2B cells, used as in vitro research subjects for studying ALI, were purchased from American Type Culture Collection (ATCC, Rockville, MD USA) and cultured in DMEM (Hyclone, Logan, UT, USA) containing 10% foetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO_2, 37°C.

qPCR detection of HOTAIR expression

To assess the transcription level of HOTAIR, the total RNA of cell was extracted with TRIzol reagent (Invitrogen). Subsequently, reverse-transcribe the extracted RNA into cDNA. Employ the qRT-PCR detection method to measure the transcription level of HOTAIR. The reaction system (20 μL) consists of Premix Ex Taq (Probe qPCR) 10 μL, upstream and downstream primers (10 μmol/L) 0.4 μL each, Probe 0.8 μL, ROX Reference Dye II 0.4 μL, DNA template 2 μL, and 6 μL of sterilized water. The reaction conditions involve pre-denaturation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 45 s.

ELISA

The secretion of TNF-α, IL-6, and IL-1β was detected using the ELISA method. For cell secretion detection, the cell culture media were collected after different treatments. For animal experiments, blood samples were collected and centrifuged to obtain the supernatant. ELISA kits (Merck) were used to detect TNF-α, IL-6, and IL-1β in the cell culture medium and serum, following the manufacturer's protocol.

Lactate detection

The lactate dehydrogenase colorimetric method was used to detect lactate content in cell culture media and serum. After

the cells were treated with different treatments for 24 h, the culture medium was taken.

Glucose uptake experiment

The Glucose Uptake Assay Kit-Green (Dojindo) was employed to measure cellular glucose uptake. Briefly, cells were initially seeded into a cell culture plate and cultured for 24 hours. Subsequently, glucose-free medium was added for a 2-hour period, followed by the addition of different drugs for cell incubation. The glucose-free medium was then replaced with fresh glucose-containing medium. After incubating for 6 hours, the intracellular glucose content was measured using a kit.

Transfection

The HOTAIR overexpression plasmid was created by inserting amplified HOTAIR cDNA into the pcDNA3.1 vector (Invitrogen, Shanghai, China). The empty pcDNA3.1 vector was used as a negative control. The si-HOTAIR and sh-Lin28 plasmids, along with their corresponding negative controls (si-NC), were synthesized by GenePharma (Shanghai, China). Cell transfection was performed using Lipofectamine 3000 following the manufacturer's protocol.

Cell Apoptosis Analysis

Apoptosis detection was performed using Hoechst 33,342 (Beyotime). After various treatments, cells were stained with Hoechst 33,342 and imaged using a confocal microscope. Living cells were represented by blue colour. Additionally, the Annexin V/PI double staining method was employed to detect cell apoptosis in animal lung tissue. Lung tissues from different treatment groups were extracted, ground, and dispersed into single cells. These cells were then stained with an Annexin V/PI kit, and the fluorescence of Annexin V and PI was measured using flow cytometry for detection.

Fluorescence in situ hybridization (FISH) and immunofluorescence assay

The lncRNA HOTAIR-labelled FISH probe (5'-ATCTGTT GGGCGTGAGTGCACTGTCTCTCAAATAAT-3'-biotin) was incubated for 12 h at 37°C perform the hybridization reaction. After hybridization, wash to remove unbound probes. Then add cy3-labelled streptavidin (SA) to incubate with the cells, binding with FISH-biotin to amplify the signal.Cells were then fixed with 4% paraformaldehyde, followed by an overnight incubation with LIN28 antibody at 4°C, and then with 488 fluorescent secondary antibody after washing. And BEAS-2B cells were finally stained with Hoechst 33,258. Images were observed using an EVOS M5000 fluorescence microscope (Thermo Fisher Scientific, MA, USA).

RNA-Binding protein immunoprecipitation (RIP) assay

The RIP assay was conducted using the EZ-Magna RIP kit (Millipore). In summary, whole cell lysates were incubated with RIP buffer that contained magnetic beads conjugated to a HOTAIR/HOTAIR-mut probe, as well as a negative control (normal mouse IgG). The lysed samples were then treated with proteinase K, use the WB assay to immunoprecipitate RNA to detect the presence of proteins bound to the corresponding primers.

LPS-induced ALI rat model

Male Sprague-Dawley (SD) rats weighing 200-250 g were selected and grouped after one week of adaptive feeding. They were then randomly divided into different groups for the experiment: Sham group (Normal group), model group (a group treated with LPS to induce acute lung injury), model + shNC group (a group treated with LPS and non-targeting control shRNA), and model + shHOTAIR group (a group treated with LPS and shHOTAIR). The treatment methods for the model + shNC group and the model + shHOTAIR group were as follows: first, rats were injected with an adenovirus packaging shHOTAIR vector or shNC. Four weeks after the injection, 15 mg/kg of LPS was injected intraperitoneally to simulate ALI, while control mice were treated with an equal volume of normal saline. At the end of the experiment, the mice were euthanized with an overdose of sodium pentobarbital (200 mg/kg), and lung tissue was collected for further testing.

Statistical analysis

Each experiment was repeated in triplicate. Statistical data are presented as mean ± SD. GraphPad Prism7 was utilized for statistical analyses. Student's t test and one-way ANOVA were used to compare two or more groups for statistical analysis, respectively. p value < 0.05 was considered statistically significant.

Results

LPS-induced ALI upregulates HOTAIR expression to promote aerobic glycolysis

Lung epithelial cells BEAS-2B were cultured (Figure 1A) and treated with LPS to create an ALI cell model. To explore the function of HOTAIR in cells, we designed different fragments of interference sequences and selected the most effective sequence based on qPCR results for subsequent experiments (Supplementary Figure S1A). Additionally, the experimental results showed that the expression of HOTAIR decreased significantly after transfection, which verified the successful knockdown efficacy of siRNA after transfection. In addition, the relative expression of HOTAIR increased significantly after LPS intervention, while the increased expression of HOTAIR was inhibited to a certain extent in the LPS intervention and simultaneous transfection of siHOTAIR (Figure 1B). As shown in Figure 1C, siHOTAIR, when not combined with LPS incubation in BEAS-2B cells, downregulated the secretion of IL-1β, IL-6, and TNF-α compared to the control and negative control groups (siNC). Conversely, in the combined group of siHOTAIR and LPS, the secretion of IL-1β, IL-6, and TNF-α by BEAS-2B cells significantly increased. The role of HOTAIR in glucose metabolism in

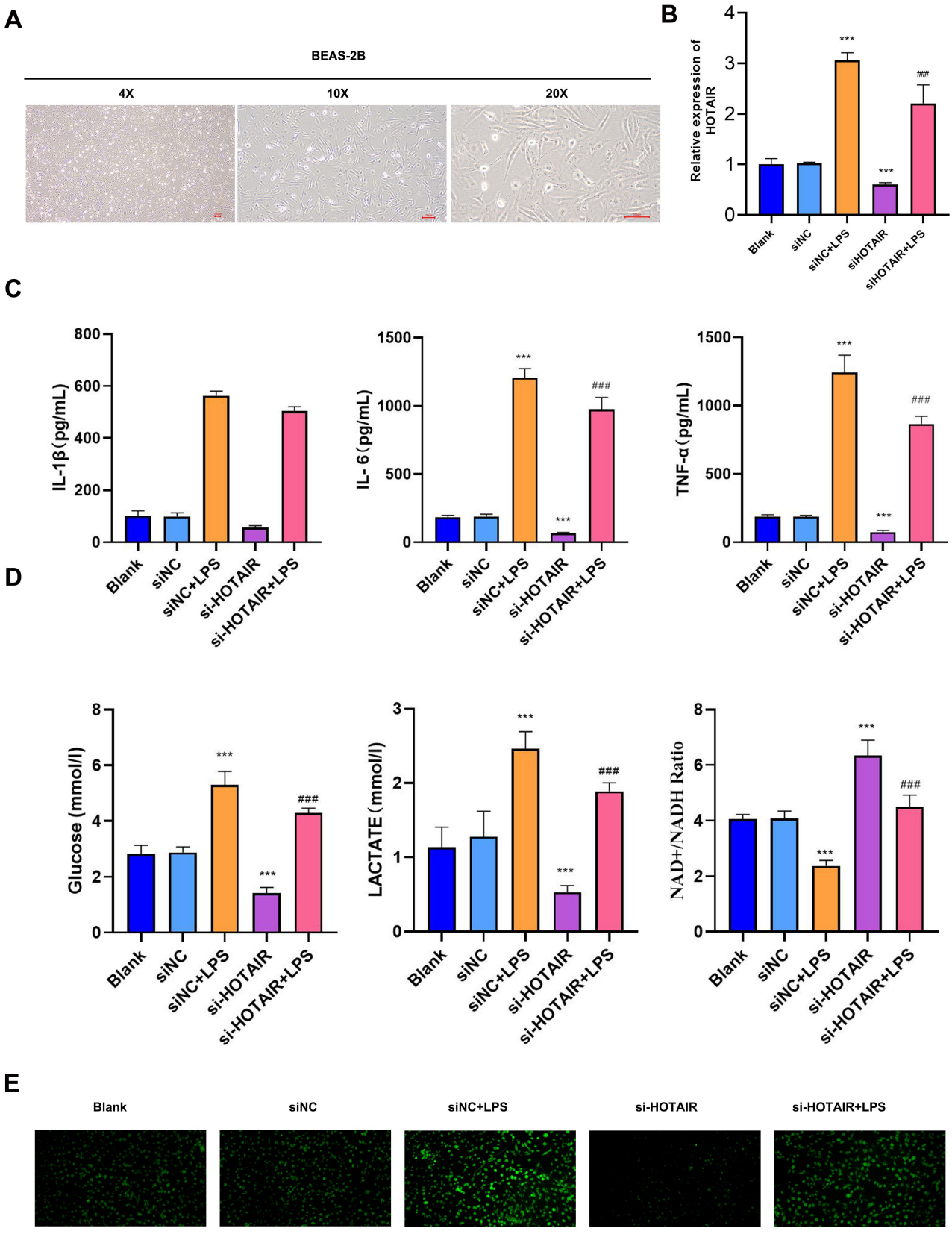

Figure 1. Lps-induced ALI promotes inflammation and cellular glucose uptake by highly expressing HOTAIR. (A) Optical microscope image of BEAS-2B cells. (B) HOTAIR gene expression of BEAS-2B cells after different treatment. (C) The cCytokine secretion of IL-1β, IL-6, and tnf-α for BEAS-2B cells after different treatments. (D) Quantitative detection of the glucose, lactate and NAD^+/NADH content, in BEAS-2B cells after different treated. (E) Fluorescence detection of the glucose content in BEAS-2B cells after different treated. ***,$p < 0.001$,siNC vs si-hotair; ###, $p < 0.001$, si-hotair vs si-hotair+lps.

ALI has been sparsely reported. Therefore, we aimed to investigate the relationship between HOTAIR and glucose metabolism in an ALI model. Glucose detection kit was used to measure intracellular glucose content and examine the impact of HOTAIR on glucose metabolism in BEAS-2B cells. As shown in Figure 1D, the glucose uptake in the siHOTAIR group was significantly reduced, nearly half of that in the Blank group and siNC group. Conversely, in the combined group of siHOTAIR and LPS, the glucose uptake in BEAS-2B cells increased. We further employed fluorescence to assess the glucose content of BEAS-2B cells following different treatments, obtaining consistent results (Figure 1E). These findings demonstrate that HOTAIR can enhance glucose uptake in alveolar epithelial cells.

It is well-known that lactate production increases during glycolysis. As shown in Figure 1D the lactate production in the Blank group and siNC group was roughly 2.5 times higher than that in the siHOTAIR group, indicating that siHOTAIR affects the glycolysis process of cells. Moreover, in the combined group of siHOTAIR and LPS, lactic acid production was relatively high, suggesting that in the acute lung injury model, cellular glycolytic metabolism is highly active.The NAD+/NADH ratio serves as a crucial marker for cellular redox status. Infection with LPS results in the accumulation of NADH, thereby decreasing the NAD+/NADH ratio. On the other hand, the excessive accumulation of NADH due to LPS infection hampers its timely oxidation, potentially inhibiting enzyme activity and consequently impacting the glycolytic process. This substantiates the potential role of HOTAIR in cellular glycolysis.

Next, to investigate the role of HOTAIR in cellular glucose uptake and aerobic glycolysis, we generated BEAS-2B cells that overexpressed HOTAIR (Supplement Figure S1B). Control groups included cells transfected with Vector, siNC, and siHOTAIR. The findings, presented in Figure 2A, indicate that cells overexpressing HOTAIR (Over-HOTAIR group) exhibited the highest glucose uptake, while cells with inhibited HOTAIR expression (siHOTAIR group) showed the lowest glucose uptake. This result was further supported by fluorescence detection of intracellular glucose content (Figure 2B). These findings confirm the involvement of HOTAIR in cellular glucose uptake. Moreover, analysis of lactic acid content revealed that Over-HOTAIR significantly increased intracellular lactic acid levels compared to the siHOTAIR group Additionally, the NAD^+/NADH ratio in BEAS-2B cells treated with siHOTAIR was relatively high, whereas cells overexpressing HOTAIR exhibited a relatively low ratio (Figure 2A). Collectively, these results provide further evidence that HOTAIR regulates the aerobic glycolysis process in cells.

HOTAIR regulates cellular inflammation and apoptosis

To further investigate the role of HOTAIR in ALI, we examined its effect on inflammation in BEAS-2B cells. Figure 2C shows that the relative expression levels of IL-1β, IL-6, and TNF-α were lower in BEAS-2B cells transfected with si-HOTAIR compared to cells transfected with si-NC. Conversely, the relative expression levels of IL-1β, IL-6, and TNF-α were significantly higher in BEAS-2B cells overexpressing HOTAIR compared to cells transfected with si-NC and si-HOTAIR. Additionally, we investigated whether HOTAIR induces apoptosis by labelling living cells with Hoechst. The results are presented in Figure 2D, Where the apoptosis rate of BEAS-2B cells treated with si-HOTAIR was lower than that of cells treated with sh-NC. Conversely, the apoptosis rate of BEAS-2B cells treated with overexpressed HOTAIR was significantly higher than that of cells treated with si-NC (Supplement Figure S1C).

HOTAIR regulates LIN28 protein expression

Recently, several studies have demonstrated that lncRNAs regulate signalling pathways by interacting with specific proteins [42]. According to the Starbase database, HOTAIR is predicted to interact with Lin28 (Figure 3A). Existing reports have elucidated a regulatory relationship between HOTAIR and LIN28, with the Lin28 protein plays a role in regulating cellular metabolic processes [25,43]. To investigate whether HOTAIR influences inflammation and apoptosis through Lin28, we initially developed a FISH probe to detect the distribution of HOTAIR and determine if they interact spatially. As shown in Figure 3B, HOTAIR is predominantly located in the cytoplasm, where Lin28 accumulates. Hence, HOTAIR and Lin28 can interact in spatial locations. RIP assay was conducted to further confirm this interaction, which demonstrated that HOTAIR was enriched in Lin28 (Figure 3C). Subsequently, we aimed to determine the potential effects of HOTAIR on Lin28 expression. The findings revealed that the expression of Lin28 was down-regulated in cells with HOTAIR knockdown (Figure 3D), while a significant up-regulation of Lin28 was observed in HOTAIR-overexpressing BEAS-2B cells (Figure 3E). Collectively, these data suggest that HOTAIR recruits and regulates LIN28 protein expression. Interestingly, HOTAIR also appears to influence the expression of the glucose transporter Glut1. In BEAS-2B cells overexpressing HOTAIR, Glut1 expression was significantly upregulated. Conversely, knocking down HOTAIR led to a down-regulation of Glut1 expression. This implies that HOTAIR may additionally impact cellular glucose uptake by recruiting and regulating LIN28 protein expression.

HOTAIR mediates aerobic glycolysis through Lin28 to promote cell apoptosis

Western blotting experiments results showed that interfering with LIN28 expression in cells (siLIN28 group) decreased the protein expression of LIN28, while interfering with LIN28 expression in HOTAIR overexpression cells (siLIN28 + over-HOTAIR group) increased LIN28 protein expression (Figure 4A). This suggests that HOTAIR has a regulatory effect on the expression of Lin28. Subsequently, the study examined the changes in LIN28 expression, cellular glucose uptake, and lactate production under different conditions. It was observed that interfering with LIN28 expression led to a decrease in glucose uptake by cells, indicating the importance of LIN28 in the cellular glycolysis process (Figure 4B). Interestingly, when LIN28 expression was disrupted in

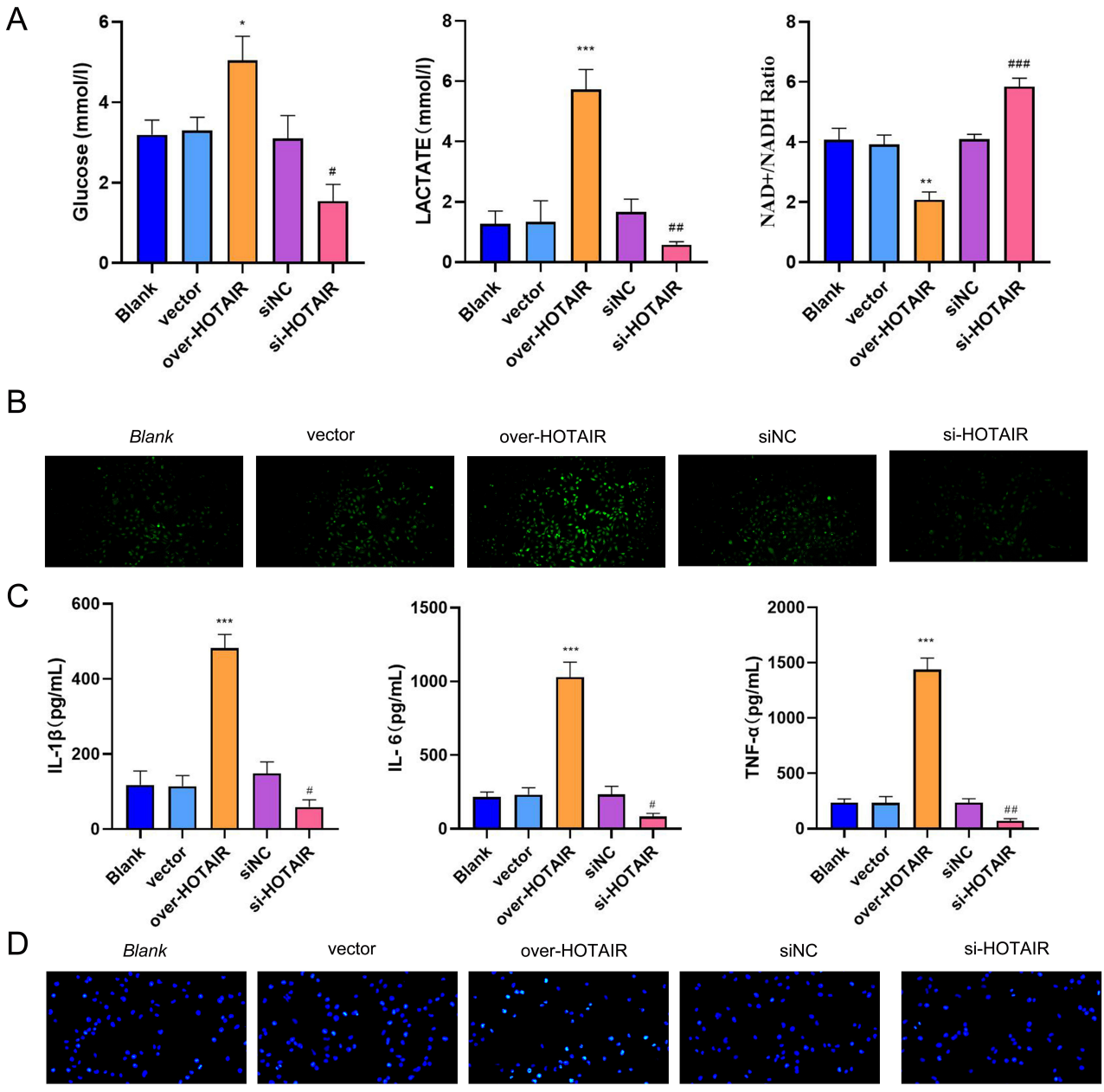

Figure 2. HOTAIR expression promotes aerobic glycolysis and regulates cellular inflammation and apoptosis of cells. (A) Quantitative detection of the glucose, the lactate and NAD+/NADH content in BEAS-2B cells after different treated. (B) Fluorescence detection of the glucose content in BEAS-2B cells after different treatment. C) the Cytokine secretion of IL-1β, IL-6, and tnf-α for BEAS-2B cells after different treatments. (D) Detecting cell apoptosis through Hoechst assay. ***, $p < 0.001$, vector vs over-hotair; ###, $p < 0.001$, siNC vs si-hotair.

HOTAIR-overexpressing cells, the amount of glucose uptake by the cells increased significantly, suggesting that HOTAIR mediates glucose uptake by regulating Lin28. In the lactate production experiment, the siLIN28 group exhibited a decrease in cellular lactate content compared to the siNC and Blank groups, while the Over-HOTAIR + siLIN28 group showed a significant increase in intracellular lactate content. Moreover, the ratio of NAD^+/NADH in BEAS-2B cells of the siLIN28 group was relatively high, but the ratio of BEAS-2B cells in the Over-HOTAIR + siLIN28 group was relatively low. These findings collectively demonstrate that HOTAIR mediates aerobic glycolysis through its regulation of Lin28.

Furthermore, we conducted additional investigations on cell apoptosis, which are presented in Figure 4C,D. Upon reducing the expression of LIN28 in cells (siLIN28 group), we observed a decrease in the rate of cell apoptosis (1.54%). However, when Over-HOTAIR + siLIN28 group BEAS-2B cells were analysed, a significant increase in the apoptosis rate was observed. These findings suggest that HOTAIR plays a role in promoting apoptosis in ALI through its interaction with Lin28.

HOTAIR induces inflammation and apoptosis in ALI by promoting aerobic glycolysis via Lin28 *in vivo*

We investigated whether *in vivo* inflammatory HOTAIR induces inflammation and apoptosis in acute lung injury by promoting aerobic glycolysis through Lin28. To construct a rat ALI model, rats were treated with LPS and divided into four groups with different treatments: the normal control

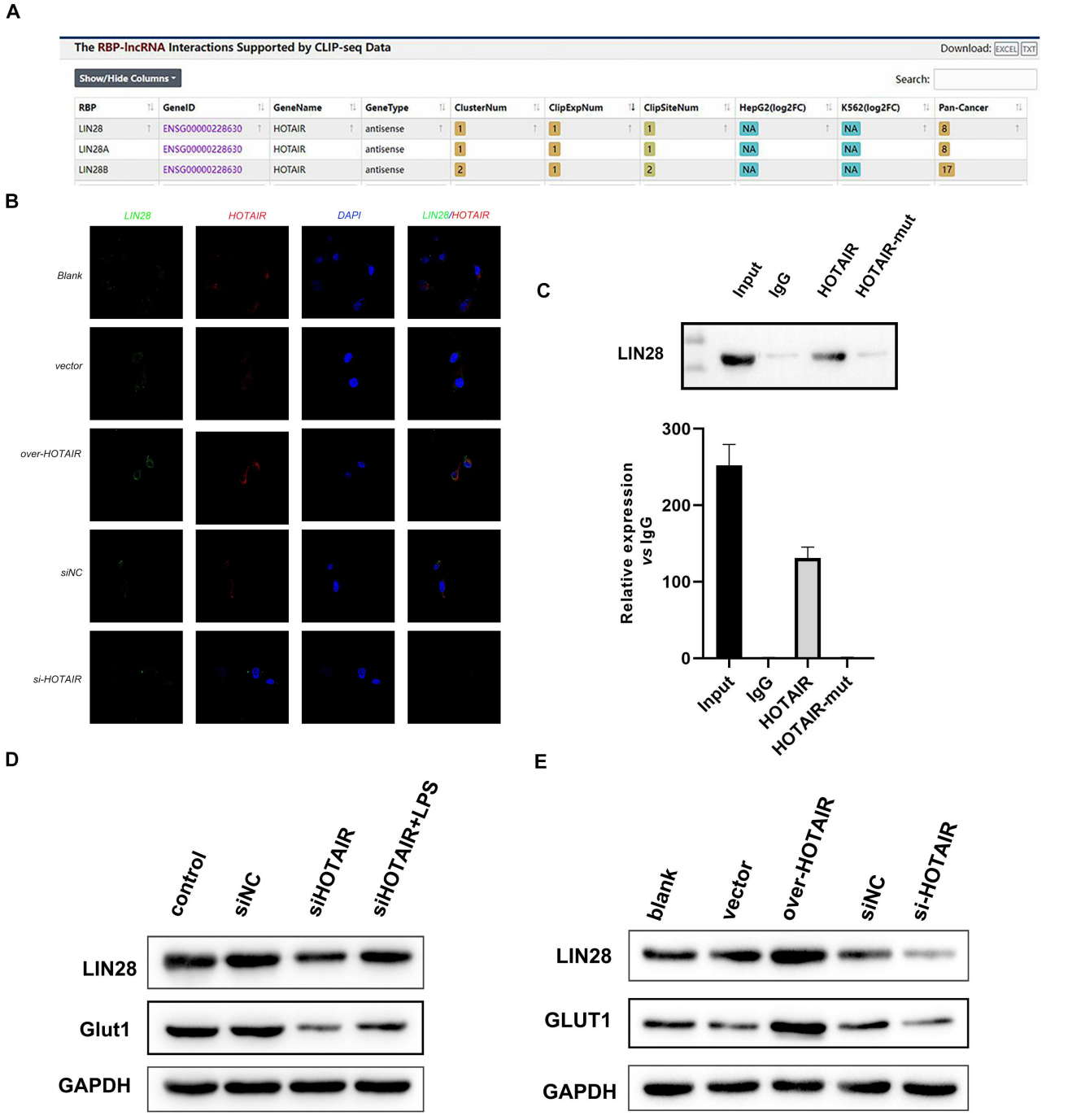

Figure 3. HOTAIR recruits and regulates LIN28 protein expression. (A) Database predicts the interaction of HOTAIR and LIN28. (B) FISH detects the distribution of HOTAIR in cells. (C) The interaction between HOTAIR and Lin28 was detected by RIP assay. (D) The expression level of LIN28 protein after interfering with HORTAIR expression. (E)Comparison of LIN28 protein expression in HORTAIR overexpressing cells and HORTAIR low expressing cells.

group (sham group), the LPS-induced ALI model group (model group), the LPS-induced ALI model group with ALI model + shNC treatment (model + NC group), and the LPS-induced ALI model with shHOTAIR treatment (Supplementary figure S2). The results demonstrated a significant increase in the expression of IL-6 (1515.929 pg/mL) in the serum of rats in the model group, which was approximately three times higher than that of the control group (519.626 pg/mL). However, when the expression of HOTAIR was knocked down in the model + shHOTAIR group, the IL-6 level in rat serum decreased to 786.9443 pg/mL, showing a significant difference compared to the model + NC group (Figure 5A). The inflammatory factors IL-1β and TNF-α exhibited a similar change trend. These findings suggest that HOTAIR plays a role in promoting inflammation in ALI. The H&E staining results revealed that in the model group (including the model group and model + NC group) of rats induced with LPS, a significant number of inflammatory cells were observed in the lung tissue, indicating damage. However, when HOTAIR knockdown rats were treated with LPS (model + shHOTAIR group), the inflammatory infiltration in the lungs was reduced compared to the model group and

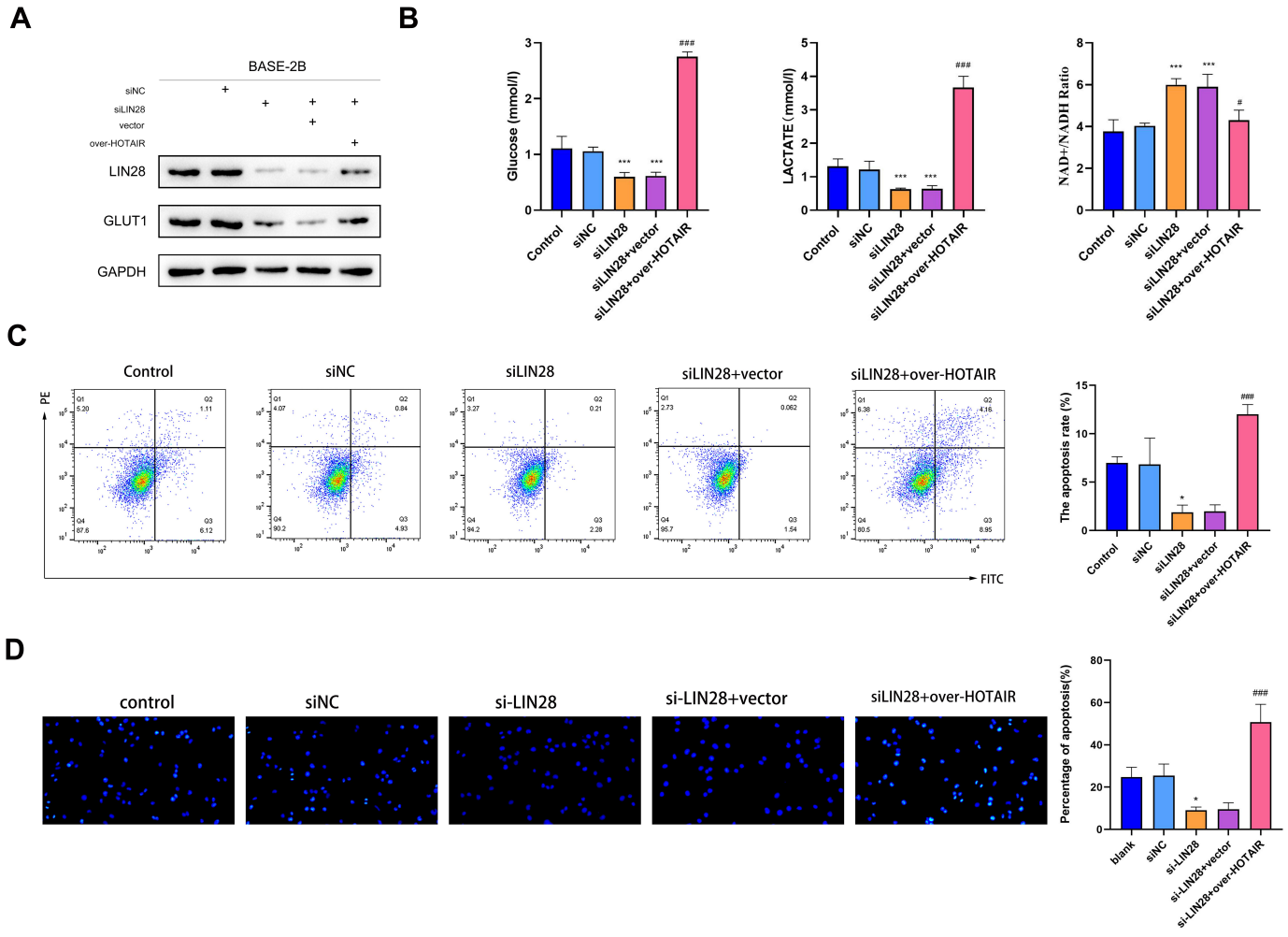

Figure 4. HOTAIR mediates aerobic glycolysis through Lin28 to promote cell apoptosis. (A) Western blotting analysed the expression of LIN28 and GLUT1 in BEAS-2B cells after different treatments. (B)The glucose content, the lactate and the ratio of NAD^+/NADH in BEAS-2B cells after different treatment. (C) Cell flow cytometry to detect cell apoptosis after different treatments. (D) Hoechst assay to detect cell apoptosis after different treatments. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, siNC vs siLIN28; ##, $p < 0.01$, ###, $p < 0.001$, siLin28+vector vs siLin28+over-hotair.

model + NC group (Figure 5B). This finding provides additional evidence that HOTAIR plays a role in promoting inflammation in ALI.

Subsequently, we proceeded to analyse the glucose and lactic acid levels in the blood of rats. The findings revealed that the glucose content in the blood of rats belonging to the model group and model + NC group was higher. Conversely, the glucose content in the blood of rats in the model + shHOTAIR group, where the expression of HOTAIR was suppressed, exhibited a reduction (Figure 5C). Additionally, we observed that the ratio of NAD^+/NADH in the serum of rats from the model group and model + NC group was comparatively low. However, the serum of rats with increased HOTAIR expression exhibited a relatively elevated content of NAD^+/NADH. This shows that HOTAIR is involved in the metabolic process of glucose in rats, which is consistent with the results of cell-level detection.

Next, the co-localization of HOTAIR gene expression and LIN28 protein expression in the lung tissue of rats in different treatment groups was examined (Figure 6A). In the Sham group, HOTAIR gene expression and LIN28 protein expression were relatively low, but after LPS treatment in the model group, their expressions significantly increased. Interestingly, interfering with HOTAIR gene expression in the lung tissue of rats treated with LPS (model + shHOTAIR group) not only reduced the amount of HOTAIR but also decreased the expression of LIN28 protein. This suggests that HOTAIR has a regulatory effect on the expression of LIN28 protein, which was confirmed by western blotting experiments (Figure 6B). Furthermore, Western blotting experiments revealed that the expression of GLUT1 in the lungs of rats in the model group significantly increased, but after inhibiting the expression of HOTAIR gene, the expression of GLUT1 decreased. This indicates that HOTAIR has a regulatory effect on the expression of GLUT1 protein. These findings demonstrate that HOTAIR mediates the expression of GLUT1 protein through LIN28, thereby affecting cellular glucose metabolism. Additionally, analysis of cell apoptosis in lung tissue of different treatment groups showed a higher proportion of cell apoptosis in the lung tissue of rats in the model group compared to the Sham group, indicating that ALI can induce apoptosis of lung cells (Figure 6C,D). Knocking down HOTAIR (model + shHOTAIR group) further increased the proportion of cell apoptosis in rat lung tissue compared to the

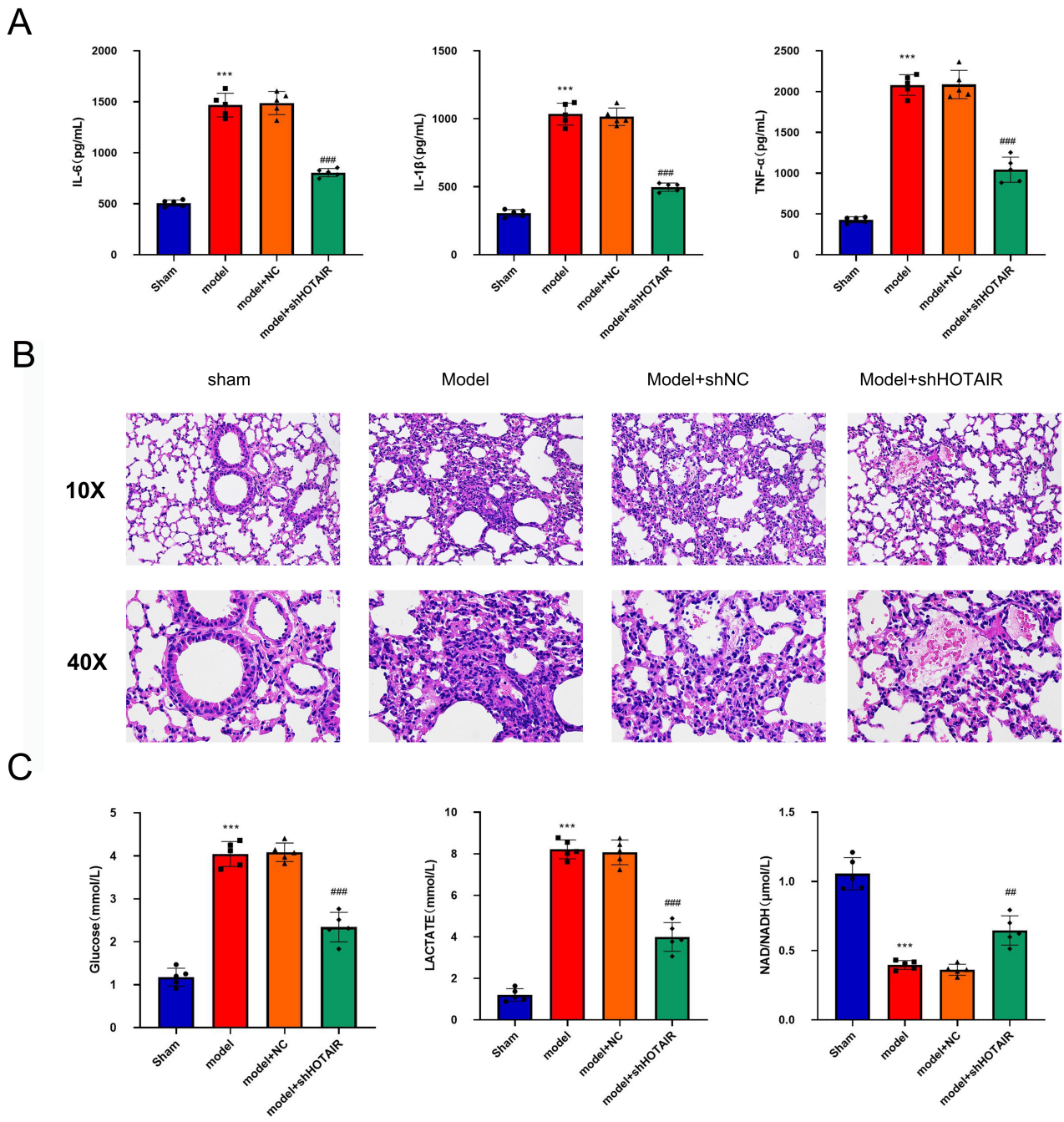

Figure 5. HOTAIR induces inflammation in ALI by promoting aerobic glycolysis via Lin28 *in vivo*. (A) Inflammatory factors IL-6, IL-1β and tnf-α in the serum of rats in different treatment groups. (B)H&E staining images of rat lung tissue in different treatment groups. (C) Glucose, lactic acid contents and NAD^+/NADH ratio in serum of rats in different treatment groups. ***, $p < 0.001$, sham vs model; ##, $p < 0.01$, ###, $p < 0.001$, model+nc vs model+ shHOTAIR.

model group, suggesting that HOTAIR regulates cell apoptosis in ALI. Based on the results obtained at the cellular level, it can be concluded that HOTAIR promotes aerobic glycolysis, which leads to ALI inflammation and apoptosis through Lin28 in vivo.

Discussion

It is widely accepted that the pathogenesis of ALI is associated with pulmonary or systemic inflammatory response [10,44]. Numerous studies have demonstrated that patients with ALI exhibit elevated secretion of inflammatory factors, such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) [45,46]. The increase of IL-1β, IL-6, and TNF-α after siHOTAIR and LPS treatment, supporting the role of HOTAIR in regulating inflammatory factors. Meanwhile, Metabolic abnormalities in ALI can lead to blood sugar disorders, causing cells to adjust their own metabolic pathways and accelerate glucose consumption [47]. Previous studies have shown that HOTAIR plays a critical role in tumour cell glucose metabolism [39–41]. By interfering with HOTAIR and overexpressing HOTAIR, we found HOTAIR promoted the glucose uptake of BEAS-2B cells, increased the production of lactic acid, and further increased

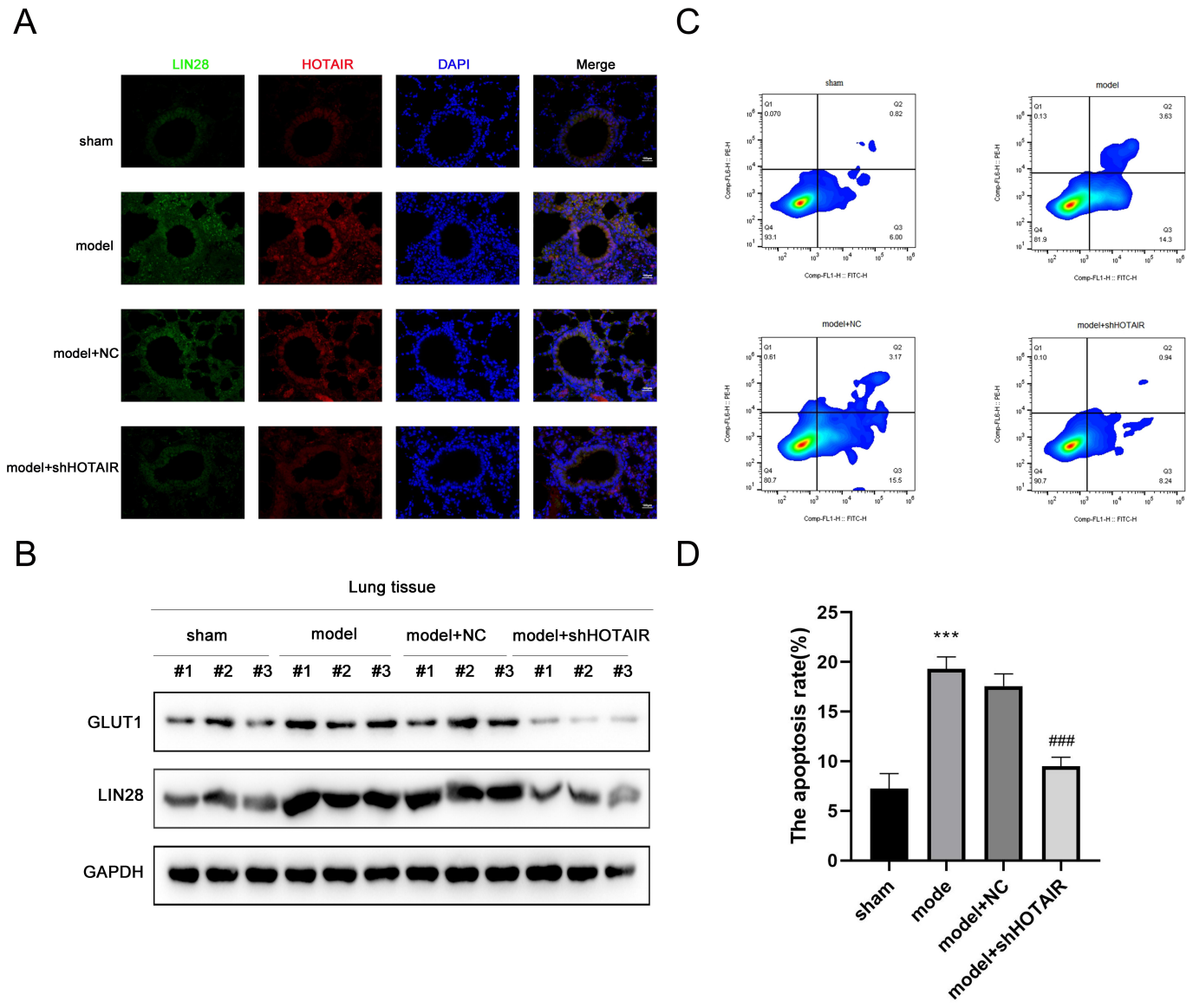

Figure 6. HOTAIR induces apoptosis in ALI via Lin28 *in vivo*. (A) Immunofluorescence detection of HOTAIR gene expression and Lin28 protein expression in lung tissue of rats in different treatment groups. (B) Western blotting analysis of GLUT1 and Lin28 protein expression in rat lung tissues after different treatments. (C) Flow cytometry analysis of cell apoptosis in rat lung tissues after different treatments. (D) Quantitative analysis of cell apoptosis in rat lung tissues after different treatments. ***, $p < 0.001$, sham vs model; ###, $p < 0.001$, model+NC vs model+ shHOTAIR.Scale bar, 100 μm.

the ratio of NAD^+/NADH. Nicotinamide adenine dinucleotide (NAD^+) is a crucial coenzyme involved in intracellular energy metabolism and closely associated with the glycolysis process [48,49]. Our results suggest indicating the role of HOTAIR in regulating the aerobic glycolysis process of cells.

Interestingly, HOTAIR has been found to impact the secretion of inflammatory factors and the occurrence of apoptosis in BEAS-2B cells. It has been reported that HOTAIR can regulate the expression of cytokines and the inflammatory response in macrophages when exposed to LPS [50]. The up-regulation of HOTAIR has been shown to promote the development of osteoarthritis [51]. Our study shown that, knocking down HOTAIR reduces the ability of BEAS-2B cells to secrete IL-1β, IL-6, and TNF-α. On the other hand, overexpression of HOTAIR significantly increases the secretion of these inflammatory factors by BEAS-2B cells. These findings suggest that HOTAIR plays a role in modulating the secretion of inflammatory factors and promoting inflammation. Additionally, HOTAIR has been found to be involved in apoptosis [52]. Down-regulation of HOTAIR leads to a reduction in the expression of tumour necrosis factor-α (TNF-α), thereby inhibiting apoptosis [35]. Knockdown of HOTAIR has also been shown to partially inhibit LPS-induced activation, inflammation, and apoptosis [25], which aligns with our own results. We observed that down-regulation of HOTAIR inhibits apoptosis in BEAS-2B cells, while up-regulation of HOTAIR promotes apoptosis. This indicates that the expression of HOTAIR can influence cell apoptosis. In summary, our results provide evidence that HOTAIR plays a crucial role in regulating cell inflammation and apoptosis.

How does HOTAIR regulate inflammation and apoptosis? Recently, several studies have demonstrated that lncRNAs regulate signalling pathways by interacting with specific

proteins [42]. HOTAIR is predicted to interact with Lin28 according to the Starbase database. Lin28 induces the release of inflammatory cytokines and plays an important role in peripheral T-cell lymphoma [53]. Zhou et al. found that Lin28b promotes foetal B cell lymphopoiesis by increasing the expression of the transcription factor Arid3a [54]. Whether HOTAIR influences inflammation and apoptosis through Lin28? HOTAIR is predominantly located in the cytoplasm, where Lin28 accumulates. Hence, HOTAIR and Lin28 can interact in spatial locations. Our findings demonstrate that HOTAIR regulates the expression of Lin28 protein, which is known to play a crucial role in cellular metabolic processes [55]. Furthermore, our results indicate a strong association between Lin28 protein and the expression of GLUT1, suggesting that HOTAIR can modulate glucose metabolism via Lin28. Previous studies have demonstrated that the Lin28/Let-7 pathway is involved in the regulation of mammalian glucose metabolism, and this pathway itself is regulated by various complex factors [55]. Can HOTAIR regulate glycolysis through Lin28? It was observed that LIN28 expression influence the glucose uptake in cells, further regulate the production of lactic acid and the intracellular ratio of NAD^+/NADH *in vitro* and *in vivo*, indicating the importance of LIN28 in the cellular glycolysis process. Therefore, it can be proven that HOTAIR promotes aerobic glycolysis by regulating LIN28, further promoting inflammation and apoptosis.

In conclusion, we conducted an in-depth investigation into the role of lncRNA HOTAIR in the development of ALI in this study. Our findings revealed the following: I) Cells treated with LPS exhibited high expression of HOTAIR. II) HOTAIR plays a regulatory role in the expression of LIN28, which in turn promotes glucose uptake and lactic acid production in alveolar epithelial cells. Moreover, III) HOTAIR mediates oxygen glycolysis through LIN28, leading to increased inflammation and apoptosis in ALI. These results provide valuable insights and offer a potential new target for the treatment of ALI.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Ethics approval and consent to participate

All the experimental protocols involving animals were approved by the Animal Ethics Committee of Maternal and child health hospital of Sanshui District Experimental Animal Center.

References

[1] Fischer I, Dulin JN, Lane MA. Transplanting neural progenitor cells to restore connectivity after spinal cord injury. Nat Rev Neurosci. 2020;21(7):366–383. doi: 10.1038/s41583-020-0314-2

[2] Wang YH, Yan Z-Z, Luo S-D, et al. Gut microbiota-derived succinate aggravates acute lung injury after intestinal ischaemia/reperfusion in mice. Eur Respir J. 2023;61(2):2200840. doi: 10.1183/13993003.00840-2022

[3] Zhang H, Liu J, Zhou Y, et al. Neutrophil extracellular traps mediate m6A modification and regulates sepsis-associated acute lung injury by activating ferroptosis in alveolar epithelial cells. Int J Biol Sci. 2022;18(8):3337–3357. doi: 10.7150/ijbs.69141

[4] Bhattacharya J, Matthay MA. Regulation and repair of the alveolar-capillary barrier in acute lung injury. Annu Rev Physiol. 2013;75(1):593–615. doi: 10.1146/annurev-physiol-030212-183756

[5] Long ME, Mallampalli RK, Horowitz JC. Pathogenesis of pneumonia and acute lung injury. Clin Sci (Lond). 2022;136(10):747–769. doi: 10.1042/CS20210879

[6] Clifford L, Jia Q, Subramanian A, et al. Characterizing the epidemiology of postoperative transfusion-related acute lung injury. Anesthesiology. 2015;122(1):12–20. doi: 10.1097/ALN.0000000000000514

[7] Mowery NT, Terzian WTH, Nelson AC. Acute lung injury. Curr Probl Surg. 2020;57(5):100777. doi: 10.1016/j.cpsurg.2020.100777

[8] Long M, Mallampalli R, Horowitz J. Pathogenesis of pneumonia and acute lung injury. Clin Sci (Lond). 2022;136(10):747–769. doi: 10.1042/CS20210879

[9] He YQ, Zhou C-C, Yu L-Y, et al. Natural product derived phytochemicals in managing acute lung injury by multiple mechanisms. Pharmacol Res. 2021;163:105224. doi: 10.1016/j.phrs.2020.105224

[10] Butt Y, Kurdowska A, Allen TC. Acute lung injury: a clinical and molecular review. Arch Pathol Lab Med. 2016;140(4):345–350. doi: 10.5858/arpa.2015-0519-RA

[11] Chen Y, Peng M, Li W, et al. Inhibition of inflammasome activation via sphingolipid pathway in acute lung injury by Huanglian Jiedu decoction: an integrative pharmacology approach. Phytomedicine. 2022;107:154469. doi: 10.1016/j.phymed.2022.154469

[12] Wang JF, Wang Y-P, Xie J, et al. Upregulated PD-L1 delays human neutrophil apoptosis and promotes lung injury in an experimental mouse model of sepsis. Blood. 2021;138(9):806–810. doi: 10.1182/blood.2020009417

[13] Gu Z, Sun M, Liu J, et al. Endothelium-derived engineered extracellular vesicles protect the pulmonary endothelial barrier in acute lung injury. Adv Sci (Weinh). 2024;11(6):e2306156. doi: 10.1002/advs.202306156

[14] Paik S, Kim JK, Silwal P, et al. An update on the regulatory mechanisms of NLRP3 inflammasome activation. Cell Mol Immunol. 2021;18(5):1141–1160. doi: 10.1038/s41423-021-00670-3

[15] Mo Y, Mo L, Zhang Y, et al. High glucose enhances the activation of NLRP3 inflammasome by ambient fine particulate matter in alveolar macrophages. Part Fibre Toxicol. 2023;20(1):41. doi: 10.1186/s12989-023-00552-8

[16] Chen J, Xie S, Qiu D, et al. The NLRP3 molecule influences the therapeutic effects of mesenchymal stem cells through Glut1-mediated energy metabolic reprogramming. J Adv Res. 2024;65:125–136. doi: 10.1016/j.jare.2023.12.006

[17] Fresquet V, Garcia-Barchino MJ, Larrayoz M, et al. Endogenous retroelement activation by epigenetic therapy reverses the Warburg effect and elicits mitochondrial-mediated cancer cell death. Cancer Discov. 2021;11(5):1268–1285. doi: 10.1158/2159-8290.CD-20-1065

[18] Kim B, Kim HY, Yoon BR, et al. Cytoplasmic zinc promotes IL-1β production by monocytes and macrophages through mTORC1-induced glycolysis in rheumatoid arthritis. Sci Signal. 2022;15(716):eabi7400. doi: 10.1126/scisignal.abi7400

[19] Xie M, Yu Y, Kang R, et al. PKM2-dependent glycolysis promotes NLRP3 and AIM2 inflammasome activation. Nat Commun. 2016;7(1):13280. doi: 10.1038/ncomms13280

[20] Lin HC, Chen Y-J, Wei Y-H, et al. Cbl negatively regulates NLRP3 inflammasome activation through GLUT1-dependent glycolysis inhibition. Int J Mol Sci. 2020;21(14):5104. doi: 10.3390/ijms21145104

[21] Youm YH, Nguyen KY, Grant RW, et al. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome–mediated

inflammatory disease. Nat Med. 2015;21(3):263–269. doi: 10.1038/nm.3804
[22] Dai Y, Zhang J, Xiang J, et al. Calcitriol inhibits ROS-NLRP3-IL-1β signaling axis via activation of Nrf2-antioxidant signaling in hyperosmotic stress stimulated human corneal epithelial cells. Redox Biol. 2019;21:101093. doi: 10.1016/j.redox.2018.101093
[23] Vinaik R, Barayan D, Auger C, et al. Regulation of glycolysis and the Warburg effect in wound healing. JCI Insight. 2020;5(17). doi: 10.1172/jci.insight.138949
[24] Dolina JS, Van Braeckel-Budimir N, Thomas GD, et al. CD8(+) T cell exhaustion in cancer. Front Immunol. 2021;12:715234. doi: 10.3389/fimmu.2021.715234
[25] Ni SY, Xu W-T, Liao G-Y, et al. LncRNA HOTAIR promotes LPS-Induced inflammation and apoptosis of cardiomyocytes via Lin28-mediated PDCD4 stability. Inflammation. 2021;44(4):1452–1463. doi: 10.1007/s10753-021-01431-0
[26] Yuan C, Ning Y, Pan Y. Emerging roles of HOTAIR in human cancer. J Cell Biochem. 2020;121(5–6):3235–3247. doi: 10.1002/jcb.29591
[27] Mozdarani H, Ezzatizadeh V, Rahbar Parvaneh R. The emerging role of the long non-coding RNA HOTAIR in breast cancer development and treatment. J Transl Med. 2020;18(1):152. doi: 10.1186/s12967-020-02320-0
[28] Cantile M, Di Bonito M, Cerrone M, et al. Long non-coding RNA HOTAIR in breast cancer therapy. Cancers (Basel). 2020;12(5):1197. doi: 10.3390/cancers12051197
[29] Hsu CY, Jamal A, Kamal MA, et al. Pathological roles of lncRNA HOTAIR in liver cancer: an updated review. Gene. 2025;940:149180. doi: 10.1016/j.gene.2024.149180
[30] Tufail M. HOTAIR in colorectal cancer: structure, function, and therapeutic potential. Med Oncol. 2023;40(9):259. doi: 10.1007/s12032-023-02131-5
[31] Jiang D, Xu L, Ni J, et al. Functional polymorphisms in LncRNA HOTAIR contribute to susceptibility of pancreatic cancer. Cancer Cell Int. 2019;19(1):47. doi: 10.1186/s12935-019-0761-x
[32] Wang B, Sun Y, Liu N, et al. LncRNA HOTAIR modulates chondrocyte apoptosis and inflammation in osteoarthritis via regulating miR-1277-5p/SGTB axis. Wound Repair Regen. 2021;29(3):495–504. doi: 10.1111/wrr.12908
[33] Zhang L, Yu Z, Qu Q, et al. Exosomal lncRNA HOTAIR promotes the progression and angiogenesis of endometriosis via the miR-761/HDAC1 axis and activation of STAT3-mediated inflammation. Int J Nanomedicine. 2022;17:1155–1170. doi: 10.2147/IJN.S354314
[34] Wang Y, Yi K, Liu X, et al. HOTAIR Up-Regulation Activates NF-κB to Induce Immunoescape in Gliomas. Front Immunol. 2021;12:785463. doi: 10.3389/fimmu.2021.785463
[35] Wu H, Liu J, Li W, et al. LncRNA-hotair promotes tnf-α production in cardiomyocytes of LPS-induced sepsis mice by activating NF-κB pathway. Biochem Biophys Res Commun. 2016;471(1):240–246. doi: 10.1016/j.bbrc.2016.01.117
[36] Yu Y, Zhang X, Li Z, et al. LncRNA HOTAIR suppresses TNF-α induced apoptosis of nucleus pulposus cells by regulating miR-34a/Bcl-2 axis. Biomed Pharmacother. 2018;107:729–737. doi: 10.1016/j.biopha.2018.08.033
[37] Jiang ZJ, Zhang M-Y, Fan Z-W, et al. Influence of lncRNA HOTAIR on acute kidney injury in sepsis rats through regulating miR-34a/Bcl-2 pathway. Eur Rev Med Pharmacol Sci. 2019;23(8):3512–3519. doi: 10.26355/eurrev_201904_17717
[38] Elhai M, Micheroli R, Houtman M, et al. The long non-coding RNA HOTAIR contributes to joint-specific gene expression in rheumatoid arthritis. Nat Commun. 2023;14(1):8172. doi: 10.1038/s41467-023-44053-w
[39] Obaid M, Udden SMN, Alluri P, et al. LncRNA HOTAIR regulates glucose transporter Glut1 expression and glucose uptake in macrophages during inflammation. Sci Rep. 2021;11(1):232. doi: 10.1038/s41598-020-80291-4
[40] Ramya V, Shyam KP, Angelmary A, et al. Lauric acid epigenetically regulates lncRNA HOTAIR by remodeling chromatin H3K4 tri-methylation and modulates glucose transport in SH-SY5Y human neuroblastoma cells: lipid switch in macrophage activation. Biochim Biophys Acta Mol Cell Biol Lipids. 2024;1869(1):159429. doi: 10.1016/j.bbalip.2023.159429
[41] Wei S, Fan Q, Yang L, et al. Promotion of glycolysis by HOTAIR through GLUT1 upregulation via mTOR signaling. Oncol Rep. 2017;38(3):1902–1908. doi: 10.3892/or.2017.5840
[42] Herman AB, Tsitsipatis D, Gorospe M. Integrated lncRNA function upon genomic and epigenomic regulation. Mol Cell. 2022;82(12):2252–2266. doi: 10.1016/j.molcel.2022.05.027
[43] Li H, Liu D, Liu L, et al. The role of HOTAIR/miR-152-3p/LIN28B in regulating the progression of endometrial squamous carcinoma. Arch Med Sci. 2021;17(2):434–448. doi: 10.5114/aoms.2019.89632
[44] Kumar V. Pulmonary innate immune response determines the outcome of inflammation during pneumonia and sepsis-associated acute lung injury. Front Immunol. 2020;11:1722. doi: 10.3389/fimmu.2020.01722
[45] Qian Y, Wang Z, Lin H, et al. TRIM47 is a novel endothelial activation factor that aggravates lipopolysaccharide-induced acute lung injury in mice via K63-linked ubiquitination of TRAF2. Signal Transduct Target Ther. 2022;7(1):148. doi: 10.1038/s41392-022-00953-9
[46] Yuan R, Li Y, Han S, et al. Fe-curcumin nanozyme-mediated reactive oxygen species scavenging and anti-inflammation for acute lung injury. ACS Cent Sci. 2022;8(1):10–21. doi: 10.1021/acscentsci.1c00866
[47] Singer M. The role of mitochondrial dysfunction in sepsis-induced multi-organ failure. Virulence. 2014;5(1):66–72. doi: 10.4161/viru.26907
[48] Lopaschuk GD, Karwi QG, Tian R, et al. Cardiac energy metabolism in heart failure. Circ Res. 2021;128(10):1487–1513. doi: 10.1161/CIRCRESAHA.121.318241
[49] Hopp AK, Grüter P, Hottiger MO. Regulation of glucose metabolism by NAD(+) and ADP-Ribosylation. Cells. 2019;8(8):890. doi: 10.3390/cells8080890
[50] Obaid M, Udden SMN, Deb P, et al. LncRNA HOTAIR regulates lipopolysaccharide-induced cytokine expression and inflammatory response in macrophages. Sci Rep. 2018;8(1):15670. doi: 10.1038/s41598-018-33722-2
[51] Chen WK, Yu X-H, Yang W, et al. lncRnas: novel players in intervertebral disc degeneration and osteoarthritis. Cell Prolif. 2017;50(1). doi: 10.1111/cpr.12313
[52] Yang SZ, Xu F, Zhou T, et al. The long non-coding RNA HOTAIR enhances pancreatic cancer resistance to TNF-related apoptosis-inducing ligand. J Biol Chem. 2017;292(25):10390–10397. doi: 10.1074/jbc.M117.786830
[53] Beachy SH, Onozawa M, Chung YJ, et al. Enforced expression of Lin28b leads to impaired T-cell development, release of inflammatory cytokines, and peripheral T-cell lymphoma. Blood. 2012;120(5):1048–1059. doi: 10.1182/blood-2012-01-401760
[54] Zhou Y, Li Y-S, Bandi SR, et al. Lin28b promotes fetal B lymphopoiesis through the transcription factor Arid3a. J Exp Med. 2015;212(4):569–580. doi: 10.1084/jem.20141510
[55] Jun-Hao ET, Gupta RR, Shyh-Chang N. Lin28 and let-7 in the metabolic physiology of aging. Trends Endocrinol Metab. 2016;27(3):132–141. doi: 10.1016/j.tem.2015.12.006