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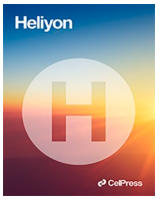

Long non-coding RNA HANR modulates the glucose metabolism of triple negative breast cancer via stabilizing hexokinase 2

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ABSTRACT

Increasing evidence has demonstrated the oncogenic roles of long non-coding RNA (lncRNA) hepatocellular carcinoma (HCC)-associated long non-coding RNA (HANR) in the development of HCC and lung cancer; however, the involvement of HANR in triple-negative breast cancer (TNBC) remains largely unknown. Our results demonstrated the significant overexpression of HANR in TNBC tissues and cells. Higher HANR levels significantly correlated with the poorer phenotypes in patients with TNBC. HANR down-regulation inhibited the proliferation and cell cycle progression and increased the apoptosis of TNBC cells. Mechanistically, immunoprecipitation-mass spectrometry revealed hexokinase II (HK2) as a direct binding target of HANR. HANR binds to and stabilizes HK2 through the proteasomal pathway. Consistent with the important role of HK2 in cancer cells, HANR depletion represses the glucose absorbance and lactate secretion, thus reprogramming the metabolism of TNBC cells. An *in vivo* xenograft model also demonstrated that HANR promoted tumor growth and aerobic glycolysis. This study reveals the role of HANR in modulating the glycolysis in TNBC cells by regulating HK2 stability, suggesting that HANR is a potential drug target for TNBC.

1. Introduction

Triple-negative breast cancer (TNBC) is one of the most common malignant cancers among women, accounting for approximately

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15–20 % of all breast cancer cases [1–3]. Current therapeutic strategies for TNBC include chemotherapy, surgical resection, and radioactive therapy. However, the outcomes of patients with TNBC, especially those with advanced progression, remains poor [4–7]. Therefore, understanding the mechanisms underlying the progression of TNBC and identifying therapeutic targets are critical.

Long non-coding RNAs (lncRNAs) are widely expressed RNA molecules that typically carry >200 nucleotides but do not have protein-coding potential [8–10]. The critical roles of lncRNAs in the regulation of key physiological processes, including cell growth, individual development, and disease progression, are well documented [11–15]. LncRNAs modulated these conditions through epigenetic mechanisms, protein post-translational modifications or micro RNA (miRNA) sponges [13]. Dysregulated lncRNA expression has been found in TNBC, promoting cancer initiation and progression by acting as oncogene or tumor suppressor [10, 16–20]. LncRNAs regulated the cell proliferation, differentiation, and metastasis and reprogram energy metabolism in TNBC cells. HANR was firstly identified in hepatocellular carcinoma (HCC), where it promotes HCC growth and prognosis [21–23]. Increased HANR was correlated with the shorter survival in patients with HCC [21]. Additionally, down-regulation of HANR inhibited HCC growth by suppressing cell proliferation and increasing cell apoptosis. Recent studies also revealed the oncogenic function of HANR in glioma and non-small cell lung cancer (NSCLC) [24,25]. These findings demonstrated the critical function of HANR in tumorigenesis, however, the involvement of HANR in more types of cancer, especially TNBC, has not been explored.

Unlike normal cells, cancer cells exhibit a distinct method for metabolizing glucose, known as the Warburg effect or aerobic glycolysis [26]. Glycolysis enhances the glucose consumption and tumor growth as hallmarks of cancer. Considering the critical role of glycolysis in cancer cell metabolism, disruption of glycolysis may be a promising method to inhibit tumorigenesis. The activation of oncogenes or loss of function of tumor suppressors induces aberrant metabolism in cancer cells by regulating the function of enzymes involved in glycolysis [26]. Hexokinase II (HK2) is a key enzyme that catalyzes the first irreversible step of glycolysis and the conversion of pyruvate to lactate [27,28]. Inhibiting HK2 activation represses glycolysis and causes defects in cancer cell growth [27,29], which has led to investigations into the molecular basis of HK2 regulation. Recently studies have shown that lncRNAs facilitated cancer progression and glucose metabolism by modulating the post-translational modifications of metabolism-related enzymes, including HK2. These findings suggest a potential regulatory function of lncRNAs in cancer development through the reprogramming of glucose metabolism.

The primary aim of this study was attempted to explore the function of HANR and possible mechanisms involved in TNBC, which have not yet been reported. Our results found that HANR was overexpressed in TNBC and correlated with the advanced stage of TNBC patients. Depletion of HANR significantly inhibited the growth of TNBC cells. Mechanism study revealed that HANR bound to and stabilized HK2, which consequently, enhanced the glycolysis of TNBC cells. These findings suggested targeting HANR might be a promising strategy for suppressing TNBC.

2. Materials and methods

2.1. TNBC patients' samples

Fifty pairs of TNBC tissues and adjacent non-cancerous tissues were collected at the Shanxi Province Cancer Hospital between October 2016 and July 2017. The specimens were frozen in liquid nitrogen prior to analysis. Informed consents were provided by all participants. The main characteristics of these TNBC patients were summarized as Supplementary Table 1. This study was approved by the Ethics Committee of the Shanxi Province Cancer Hospital.

2.2. Cell culture and transfection

The TNBC cell lines BT-549, MDA-MB-231, MDA-MB-468, and MDA-MB-453, and normal MCF10A cells were obtained from the American Tissue Culture Collection (ATCC; Rockville, MD, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10 % of fetal bovine serum (FBS; Hyclone, Shanghai, China). The cells were maintained in a 5 % CO_2 incubator at 37 °C. Small interfering RNA (siRNA)-HANR and the corresponding scrambled siRNA were purchased from GenePharma (Shanghai, China). The expression vector plasmid cloning DNA (pcDNA) 3.1-HK2 was constructed by inserting full-length of HK2 complementary DNA (cDNA) into the backbone of pcDNA 3.1 vector (GenePharma, Shanghai, China). Cells were cultured for 48 h, and the transfection efficiency was determined using reverse transcription-polymerase chain reaction (RT-qPCR).

2.3. RT-qPCR

1 μg RNA that extracted from TNBC cells or tissues was reversed transcribed into cDNA using the PrimeScript RT Reagent (TIANGEN, Beijing, China). qPCR was performed using the SYBR Premix Ex *Taq*II on the Light Cycler 480 platform (Roche). The GAPDH expression was detected as the internal reference for HANR or HK2. Primers targeting HANR, HK2 or GAPDH were as follows: HANR: F-5′-AAGTACCAGGCAGTGACAGC-3′ and R-5′-TTCTCCACGTTCTTCTCGGC-3'; HK2: F-5′-CAAAGTGACAGTGGGTGTGG-3′ and R-5′-GCCAGGTCCTTCACTGTCTC-3′. GAPDH: F-5′-GGGAAACTGTGGGGTGAT-3′ and 5′-GAGTGGGTGTCGCTGTTG-3'. The fold changes of HANR and HK2 expression were examined by the $2^{-\triangle\triangle Ct}$ formula.

2.4. Cell growth assay

TNBC cells transfected with the indicated expression vectors were pre-cultured in a 96-well plate, and the cell proliferation was

detected using the Cell Counting Kit-8 (CCK-8, Beyotime, Shanghai) every 24 h for 5 d. Briefly, cells were incubated with CCK-8 reagent (10 μL) at 37 °C for 3 h. Subsequently, the optical density was measured at 450 nm using a Universal Microplate Spectrophotometer (Bio-Tek Instruments, Inc., Winooski, VT, USA). The experiment was performed in triplicate.

2.5. Cell cycle analysis

TNBC cells were harvested using 0.05 % trypsin followed by fixation with 70 % ethanol overnight at 4 °C. After washing thrice with phosphate-buffered saline (PBS), cells were then sequentially incubated for 30 min with 10 mg/L RNase A and 10 g/mL propidium iodide (PI). DNA content was determined using flow cytometry and analyzed using the ModFit software (v3.3, BD Bioscience, USA).

2.6. Western blotting

Total protein was extracted from TNBC cells using radioimmunoprecipitation assay (RIPA) buffer on ice for 15 min protein (20 μg) was separated using 15 % sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by transferring to a polyvinylidene difluoride (PVDF) membrane (Millipore, USA). Membranes were blocked at room temperature (RT) for 1 h using 5 % non-fat milk. The primary antibodies against HK2 (1:1000 dilution; ab209847, Abcam) and GAPDH (used as a control, 1:3000 dilutions; ab8245, Abcam) were incubated with the membrane overnight at 4 °C. After exhausted washing, horseradish peroxidase (HRP) anti-rabbit immunoglobulin (IgG) antibody was added at RT for 1 h. An enhanced chemiluminescence substrate (Beyotime, Shanghai, China) was used to visualize the protein signals.

2.7. RNA pull-down

TNBC cells were transfected with His tagged HK2, and total protein was extracted in the presence of protease inhibitor cocktail and RNase inhibitor. The protein concentration was determined using the BCA assay. Afterwards, 50 μL of streptavidin beads were pre-washed with PBS and incubated with 5 μg of biotinylated sense or antisense RNA sequence of HANR in the binding buffer for 2 h at 4 °C. The beads were then incubated with the cell lysates for 4 h at 4 °C followed by washing with PBS for 4 times. After centrifugation, beads were collected and SDS-PAGE loading buffer was added. His-HK2 was detected by Western blot using anti-His antibody.

2.8. RNA immunoprecipitation (RIP)

The total protein of TNBC cells was extracted with the addition of protease inhibitor cocktail and RNase inhibitor. Protein concertation was determined through BCA assay. Primary antibody against HK2 or control IgG was incubated with the same amount of cellular proteins overnight at 4 °C and then isolated using the protein A magnetic beads for 2 h. Beads were washed with PBS for three times and co-precipitated RNAs were extracted with Trizol reagent. The amount of HANR in the elute was quantified by RT-qPCR analysis.

2.9. Mass spectrometry analysis

Proteins eluted from the RNA pull-down assay were separated by 15 % SDS-PAGE gel. When the proteins went into the separating gel, the SDS-PAGE was stopped and the gel was then stained with the staining buffer (0.1 % Coomassie Blue R250 in 40 % ethanol supplemented with 10 % acetic acid). After destaining, the gels were washed and protein bands were excised followed by completely destaining. In-gel digestion was performed using DTT buffer and alkylated with the iodoacetamide. Afterwards, the protein digestion was done by incubating with trypsin at 37 °C overnight followed by extraction using 100 % Acetonitrile (CAN)/3 % Trifluoroacetic acid (TFA) and 40 % ACN/3 % TFA. Peptides were desalted using the C18 StageTips and analyzed using the Dionex Ultimate 3000 nanoRSLC system.

2.10. Glucose metabolism

Glucose consumption was determined using a Glucose Uptake Assay (ab136955; Abcam, Shanghai, China). Forty-eight hours after transfection, TNBC cells were subjected to glucose starvation and then incubated with 2-deoxyglucose (2-DG) for 25 min at 37 °C. TNBC cells were washed to remove exogenous 2-DG, lysed, and pipetted. The lysates were heated at 85 °C for 40 min and cooled on ice 5 min. Thereafter, the lysates were neutralized and the supernatant was incubated with reaction mix A for 1 h at 37 °C. Then, the samples were extracted and heated for 40 min at 90 °C followed by cooling on ice for 5 min. Finally, the lysates were incubated with reaction mix B and analyzed using a microplate reader (Bio-Tek). A lactic acid assay kit (Sigma, MO, USA) was used to quantify the lactate levels in TNBC cells.

2.11. Glycolysis stress test

Glycolysis of TNBC cells was evaluated by measuring the extracellular acidification rate (ECAR) using the Seahorse XF96 Glycolysis Analyzer (Seahorse Bioscience, MA, USA), as previously described.

2.12. Metabolite analysis

TNBC cells were seeded into a six-well plate at a density of 4000 cells/well. After culturing overnight, the medium was replaced with fresh DMEM containing 10 % dialyzed serum for 24 h ^{13}C-labelded or unlabeled glucose (10 mM) was added into the medium and the cells were cultured for an additional 8 h. The labeled metabolites were analyzed with 200 μL of the cell medium.

2.13. In vivo xenograft model

TNBC cells (2×10^6) expressing the scrambled vehicle or HANR were injected subcutaneously into the dorsal flanks of 6-week-old female BALB/c nude mice. The tumor volume was measured using a digital caliper every 5 d and calculated using formula V= (length $\times$ wideth^2)/2. At the conclusion of this assay, tumors were isolated, and lactate levels were measured by normalization to the protein concentrations. The experimental protocol was approved by the Ethics Committee of the Shanxi Province Cancer Hospital (IACUC#089535).

2.14. Statistical analysis

Data analysis was performed using the SPSS software (v21, IBM Corporation, Armonk). Differences between two or multiple groups were analyzed with the Student's *t*-test or One-way ANOVA, respectively. *P* value less than 0.05 was statistically significant.

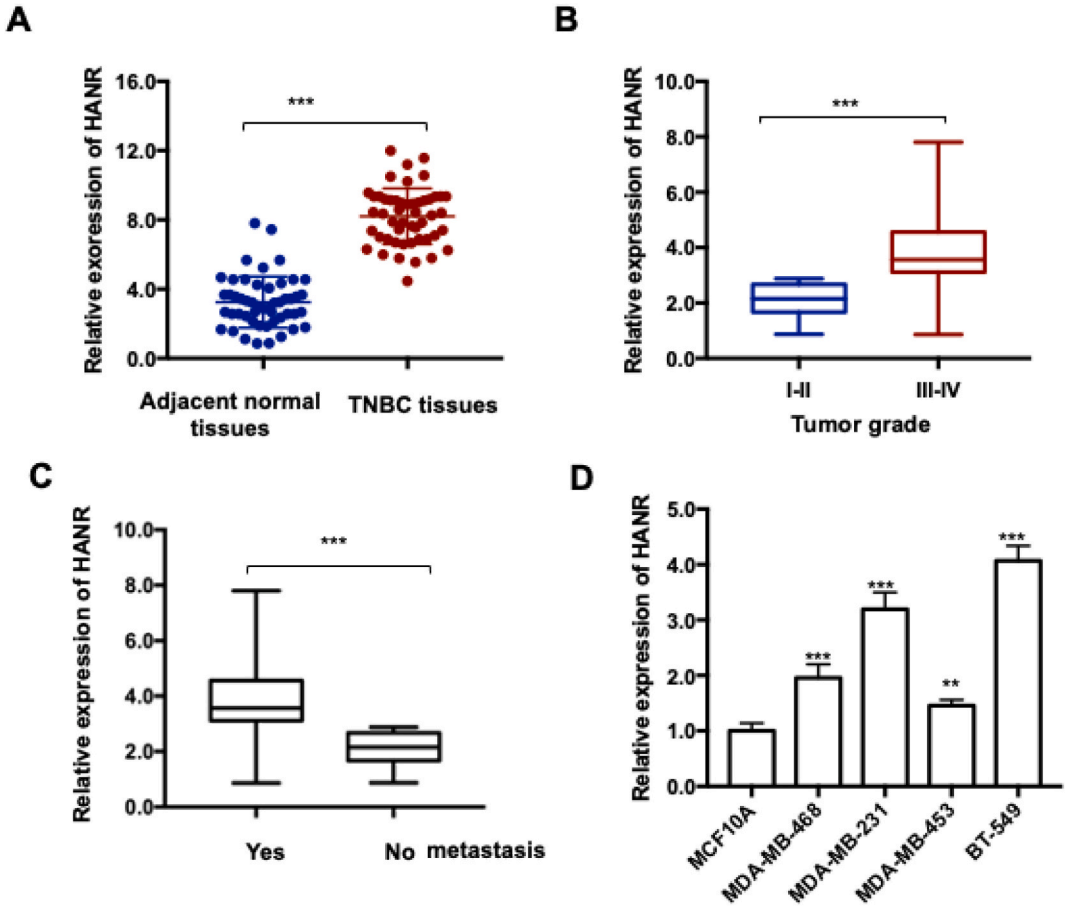

Fig. 1. Increased HANR expression in TNBC was correlated with the patients' advanced stage. (A) Significantly higher HANR expression in TNBC than matched non-cancer samples. (B, C) Higher HANR levels were found in TNBC patients with higher tumor grade and lymph node metastasis. (D) HANR levels in TNBC cells were increased compared with normal MCF10A cells. $^{**}P < 0.01$; $^{***}P < 0.001$.

3. Results

3.1. HANR was up-regulated and correlated with the aggressive progression of TNBC

Differential expression of HANR was detected using RT-qPCR in 50 paired TNBC tissues and healthy controls. HANR levels were significantly higher in TNBC tissues than in non-cancerous tissues (Fig. 1A), particularly in patients within advanced tumor grade (Fig. 1B) and bone metastasis (Fig. 1C). HANR expression was observed in both TNBC and normal cells. As indicated in Fig. 1D, all four TNBC cell lines exhibited a relatively higher abundance of HANR than the normal cells. Collectively, these data suggest that HANR is dysregulated in TNBC.

3.2. Silencing of HANR inhibited TNBC cell growth

Considering the increased levels of HANR in TNBC, to assess the function of HANR in regulating the malignancy of TNBC, both BT-549 and MDA-MB-231 cells were transfected with siRNA-HANR (Fig. 2A). The CCK-8 assay was performed to determine the proliferation of both BT-549 and MDA-MB-231 cells. HANR depletion obviously inhibited TNBC cell viability compared to that in the control (Fig. 2B and C). Knockdown of HANR also significantly inhibited the proliferation of MDA-MB-468 (Supplementary Figs. 1A and 1B). In contrast, the proliferation of MCF10A cells was not significantly affected with the down-regulation of HANR (Supplementary Figs. 2A and 2B). Cell cycle progression of both BT-549 and MDA-MB-231 cells was detected using flow cytometry. siRNA-HANR significantly increased the number of cells in the G_1 phase, suggesting G_1 cell cycle arrest compared to the control group (Fig. 2D and E). The percentage of apoptotic TNBC cells was determined. Cells with down-regulated HANR exhibited increased apoptosis compared with cells expressing the control vector (Fig. 2F). These results suggested the essential function of HANR in modulating the malignant behaviors of TNBC.

3.3. HANR interacted with and stabilized HK2 in TNBC cells

To understand the function of HANR in TNBC, the binding proteins of HANR were identified using an RNA pull-down assay,

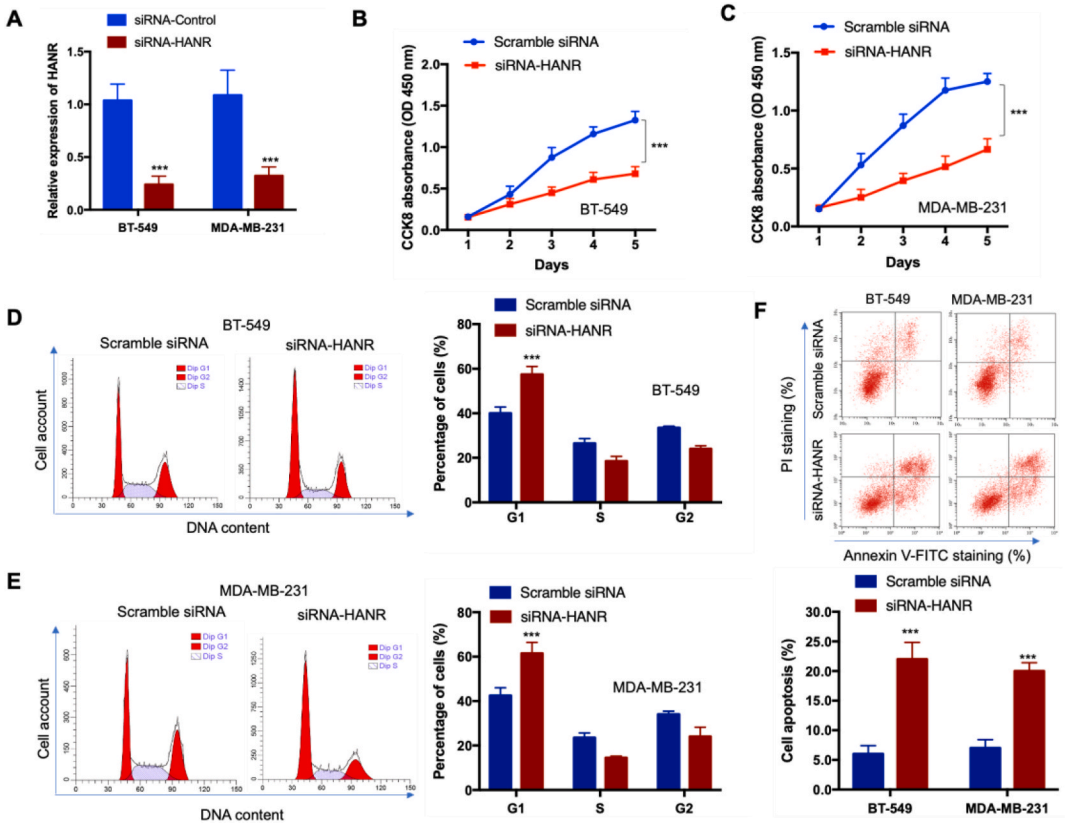

Fig. 2. siRNA-HANR suppressed TNBC growth. (A) The knockdown efficacy of HANR in TNBC cells. (B, C) Proliferation of both BT-549 and MDA-MB-231 cells transfected with siRNA-HANR was inhibited. (D, E) The population of TNBC cells in G1 stage was remarkably increased with siRNA-HANR. (F) HANR depletion promoted the early and late apoptosis of BT-549 and MDA-MB-231 cells. ***$P < 0.001$ vs. Scramble siRNA.

followed by mass spectrometry. Several of the top candidates, including HK2, exhibited the potential for binding to HANR (Fig. 3A). Notably, HK2 is one of the three rate-limiting enzymes involved in glycolysis. To confirm binding between HANR and HK2, RNA-binding protein immunoprecipitation (RIP) was performed by lysing TNBC cells, followed by incubation with an anti-HK2 antibody. The binding of HK2 to HANR was further determined via qPCR using HANR primers. As shown in Fig. 3B, the enrichment of HANR in anti-HK2 antibody immunoprecipitates was significantly higher than that in the IgG control, indicating a specific interaction between HK2 and HANR. Furthermore, the *in vitro* his-pull down assay also indicated the binding between HANR and HK2 (Fig. 3C).

To determine whether the HANR-HK2 interaction affects HK2 expression, we first detected the mRNA levels of HK2. No significant differences int the HK2 mRNA levels were observed following HANR depletion (Fig. 3D). Additionally, the protein expression of HK2 was determined in both BT-549 and MDA-MB-231 cells after HANR depletion. HK2 expression were reduced following HANR depletion in TNBC cells (Fig. 3E). To further characterize the mechanism underlying the effect of HANR on HK2 expression, MG132 was applied to block the protein degradation. As indicated in Fig. 3F, exposure to MG132 recued the decreased levels of HK2 upon HANR downregulation. These data suggest that HANR regulates HK2 expression through the proteasomal pathway. The abundance of HK2 was consistently higher in TNBC than in the normal tissues (Fig. 3G). High HK2 expression was also found in TNBC cell lines (Fig. 3H). These results demonstrated that HANR is a novel regulatory of HK2 and modulates HK2 expression in TNBC.

3.4. Knockdown of HANR suppressed the glycolytic flux in TNBC cells

Because of the important role of HK2 in glucose metabolism, we evaluated the effects of HANR on the glycolysis in TNBC cells. ECAR analysis was performed to determine the glycolytic flux of TNBC cells. The ECAR was significantly decreased with HANR downregulation (Fig. 4A and B). A notable reduction in glucose consumption and lactate levels was observed in TNBC cells with the HANR depletion (Fig. 4C and D and Supplementary Fig. 1C), suggesting an essential role of HANR in TNBC cell glycolysis. Unlike cancer cells, normal cells rely on mitochondrial oxidative phosphorylation for glucose metabolism. Compared with the control cell, depletion of HANR in normal MCF10A cells did not affect the glucose consumption (Supplementary Fig. 2C). The ^{13}C-metabolite flux

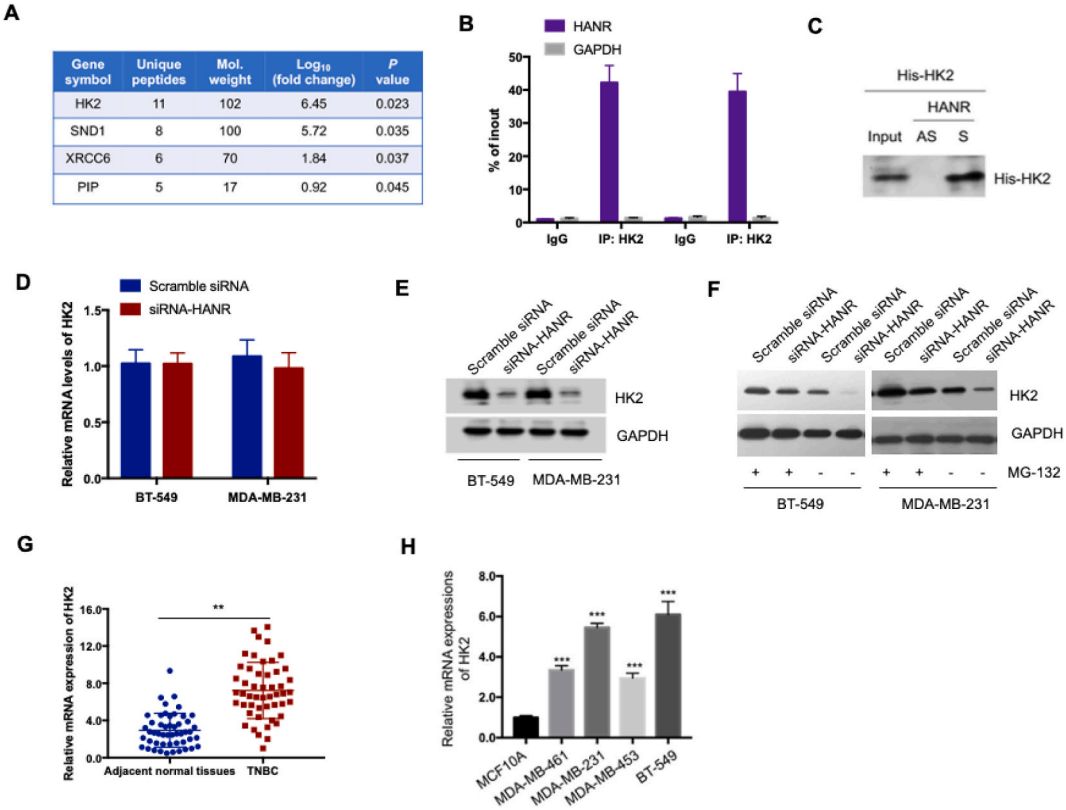

Fig. 3. HANR interacted and stabilized HK2 in TNBC cells. (A) The top four potential binding candidates of HANR detected by mass spectrum. (B) The binding of HANR with HK2 was detected via RIP assay. The signaling of HANR enrichment in anti-HK2 group was significantly higher than that of the control IgG. (C) *In vitro* pull-down assay suggested the direct binding of HK2 and HANR. (D) The mRNA levels of HK2 was not significantly changed with HANR depletion. (E) HANR depletion suppressed HK2 protein levels in TNBC cells. (F) TNBC cells expressing siRNA-control or siRNA-HANR were treated with 20 μM MG-132 for 8 h. The protein expression of HK2 was examined by Western blot. (G) HK2 expression was increased in TNBC tissues with that of non-cancer tissues as control. (H) HK2 was overexpressed in TNBC cell lines. $^{**}P < 0.01$; $^{***}P < 0.001$. The original gel blots for panel C, E, and F were provided as supplementary materials.

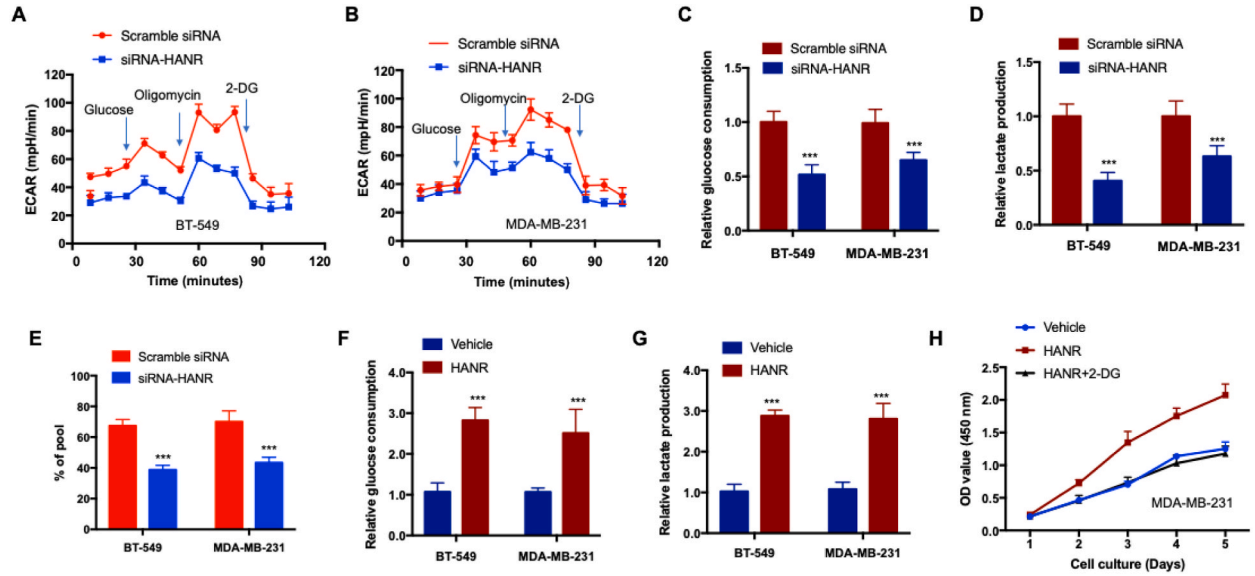

Fig. 4. HANR depletion inhibited TNBC cell glucose metabolism. (A, B) The glycolysis flux was decreased with HANR down-regulation compared with scramble siRNA-control group. (C, D) HANR silencing inhibited the glucose consumption (C) and lactate generation (D) of TNBC cells. (E) ^{13}C-metobolic labeling assay showed the ^{13}C-glucose vs. unlabeled glucose was decreased in siRNA-HANR TNBC cells. (F, G) HANR overexpression significantly enhance the glucose consumption and lactate generation. (H) 2-DG treatment attenuated the promoting-function of HANR in TNBC cell proliferation. $^{***}P < 0.001$.

analysis showed significantly decreased production of ^{13}C-labeled metabolites in the medium of cells carrying siRNA-HANR compared to the controls (Fig. 4E). HANR overexpression consistently promoted both the glucose consumption and lactate production (Fig. 4F and G). To assess whether HANR regulates TNBC cell proliferation through aerobic glycolysis, cells were treated with the glycolytic inhibitor 2-DG. The CCK8 assay showed that the proliferation of HANR-overexpressing cells was suppressed by the addition of 2-DG (Fig. 4H). These results suggest that HANR facilitates TNBC cell growth by enhancing glycolysis.

3.5. *HK2 rescued the glucose metabolism and proliferation of TNBC cells induced by HANR-depletion*

To further explore whether the suppression of cell growth by HANR depletion was mediated by HK2, TNBC cells were co-transfected with siRNA-HANR and pcDNA 3.1-HK2 to rescue reduced HK2 expression (Fig. 5A). The inhibitory effects of siRNA-HANR on TNBC cell proliferation were abolished by HK2 overexpression (Fig. 5B and C). Additionally, cell cycle arrest caused by siRNA-HANR was significantly reversed by transfection with HK2 (Fig. 5D and E). Consistently, both glucose uptake and lactate secretion increased in TNBC cells co-transfected with HK2 (Fig. 5F and G). To further demonstrate the essential role of HK2 in mediating the function of HANR, HK2 expression in TNBC cells was down-regulated by transfecting siRNA-HK2 (Supplementary Fig. 3A). Both BT-549 and MDA-MB-231 cells were transfected with pcDNA-HANR and siRNA-HK2, or siRNA-control vector, respectively. The CCK-8 assay showed that HANR overexpression enhanced the proliferation of TNBC cells, while depletion of HK2 significantly attenuated the promoting effects of HANR on TNBC cell proliferation (Supplementary Figs. 3B and 3C). Consistently, down-regulation of HK2 suppressed the function of HANR in enhancing the glucose metabolism of TNBC cells (Supplementary Fig. 3D). Collectively, these findings indicated the essential role of HK2 in the regulation of TNBC cell growth and glucose metabolism by HANR. To further characterize the function of HANR, an *in vivo* xenograft mouse model was established by subcutaneous implantation of MDA-MB-231 cells. HANR overexpression significantly increased tumor growth (Fig. 5H). Notably, higher lactate levels were found in tumors formed by HANR-overexpressing MDA-MB-231 cells (Fig. 5I). Collectively, these findings suggested that HANR elevates glycolysis and cell proliferation both *in vitro* and *in vivo*.

4. Discussion

TNBC is a common malignancy with frequent recurrence and high mortality rate [1,2]. The involvement of lncRNAs in TNBC has been uncovered by increasing evidence [17,19]. Increased expression and oncogenic functions of HANR have been observed in gliomas, NSCLC, and HCC [21–25]. In the present study, HANR was shown to be upregulated in TNBC tissues and cells. Higher levels of HANR are associated with the poorer TNBC progression, suggesting the involvement of HANR in TNBC development. Previous studies have shown that HANR promotes the proliferative, migratory, and invasive capacities of HCC, NSCLC, and glioma [21–25]. In the current study, HANR down-regulation repressed the TNBC cell proliferation, cell cycle progression, and enhanced apoptosis. These results indicated that HANR is a potential target for disrupting the progression of TNBC, which also explains the clinical association

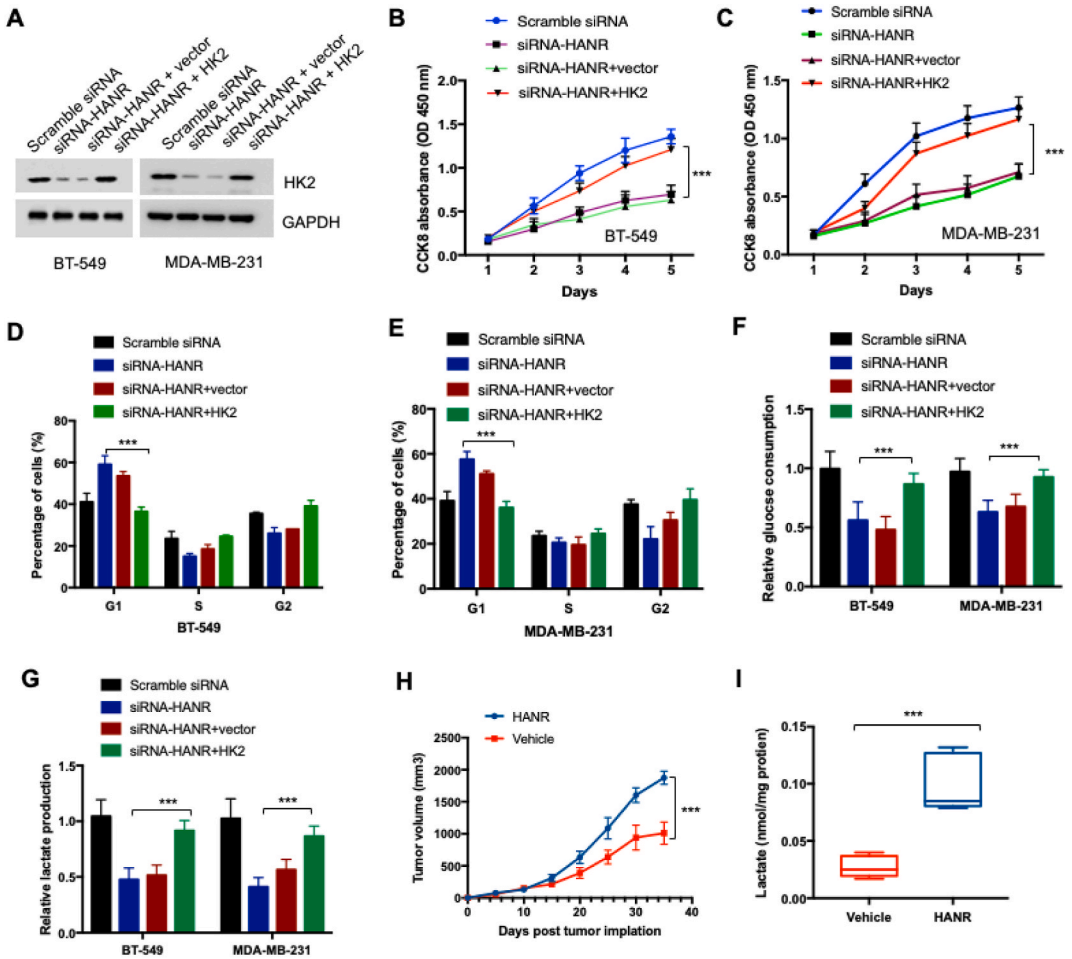

Fig. 5. HANR depletion inhibited TNBC via targeting HK2. (A) HK2 expression via recovered by transfecting pcDNA3.1-HK2. The original gel blots were provided as supplementary materials. (B, C) HK2 overexpression rescued the proliferation defects of TNBC cells induced by siRNA-HANR. (D, E) The stopped cell cycle of TNBC cells was abolished after HK2 transfection. (F, G) The suppressive effects of siRNA-HANR on TNBC glucose metabolism were recovered followed by HK2 transfection. (H) MDA-MB-231 expressing vehicle or HANR were subcutaneously injected into the nude mice and tumor volume was measured twice a week. (I) Lactate levels in tumors were detected and normalized to protein concentration. ***$P < 0.001$.

between HANR overexpression and TNBC aggressiveness.

The Warburg effect has been considered as a hallmark of cancer [26,30–32]. The main characteristic of the Warburg effect is that cancer cells preferentially metabolize glucose through glycolysis instead of mitochondrial oxidative phosphorylation in anoxic or aerobic microenvironments [26,30–32]. The reprogramming of glucose metabolism is controlled by several key rate-limiting enzymes [33–35]. HK2, which catalyzes the first irreversible step in glycolysis by phosphorylating glucose to G6P and is regulated by oncogenic or tumor suppressive signals [33–35]. Up-regulation of HK2, which promotes tumorigenesis, has been reported in several types of cancers [36–38]. Inhibition of HK2 significantly suppressed the glycolysis and tumor cell growth. Increasing evidence has shown that that lncRNAs reprogramed cancer cell metabolism by targeting HK2. For example, a recent study reported that lncRNA PVT1 promotes gallbladder cancer progression by modulating the miR-143/HK2 axis [39]. HK2 is also shown to be regulated by lncRNA ZFAS1 in gliomas and affects the malignant behaviors of glioma cells [40]. It has been reported that the stability of HK2 was regulated via both autophagic degradation or ubiquitination-proteasome dependent degradation. In this study, an RNA pull-down assay coupled with mass spectrometry analyses indicated that HANR directly binds to HK2, inhibiting the proteasome-dependent degradation of HK2. Future study is needed to test the influence of HANR on the lys48-mediated polyubiquitination of HK2, which triggers the degradation of HK2. It would be interesting to examine whether HANR affects the stability of HK2 by modulating its binding with the E3 ubiquitin ligase. The majority of lncRNAs have been reported to modulate the expression of glycolytic enzymes by acting as miRNA sponges [15,17]. However, our findings demonstrated a unique mechanism through which HANR regulates HK2 through direct binding, broadening our understanding of the mechanism of cellular metabolism regulation by lncRNAs.

Screening for the novel interacting proteins is critical to explore the functions of lncRNAs. In addition to HK2, several other proteins have been identified as potential HANR binding partners. The interactions between HANR and other proteins predicted using mass

spectrometry require further validation to deeply understand the biological functions of HANR in TNBC. In this study, restoration of HK2 expression significantly recovered the defects in TNBC glycolysis induced by HANR knockdown. These data provide insight into the mechanism by which HANR impedes glycolysis in TNBC cells by targeting HK2. However, the correlation between HANR and HK2 expression in the clinical TNBC samples requires further investigation. It is necessary to explore the upstream mechanisms that are responsible for the up-regulation of HANR in TNBC to understand its function.

In conclusions, the present study demonstrated a higher expression of HANR in TNBC, which was correlated with the advanced progression of TNBC patients. More clinical samples should be enrolled to investigate the correlation of HANR expression with the prognosis of TNBC patients. Moreover, more analysis is necessary to determine whether HANR is an independent predictor of the postoperative recurrence and overall survival of TNBC patients. This mechanistic study uncovered that HANR directly binds and stabilizes HK2, thus promoting the glycolysis and proliferation of TNBC cells. Our findings suggest that HANR is a potential therapeutic target in TNBC. These conclusions were mainly obtained by *in vitro* assays, more *in vivo* studies, especially the patient derived xenograft models, are needed to confirm the regulatory mechanism of HANR/HK2 axis, which would enhance the robustness and translational relevance of the study. Recent study reported that nano transdermal delivery of fucoidan, an anticancer bioactive compound derived from *Sargassum* sp., showed local effects on the site of breast cancer cells using fucoidan nanoparticle polymer [41]. Secondary metabolites in Algae *Kappaphycus alvarezii* shows potential anti-cancer potency in breast cancer [42]. Technologies that can be applied for the specific targeting of HANR and efficient delivery into the tumor microenvironment are needed to achieve the therapeutic value of HANR in TNBC.

Conclusion

This study recovered the novel function of HANR in modulating the glycolysis of TNBC cells by regulating HK2 stability. Depletion of HANR inhibited the glucose metabolism and growth of TNBC cells. The increased expression of HANR in TNBC suggesting its potential clinical significance and a drug target for TNBC.

Ethics statement

The experiments using clinical tissues were approved by the Ethics Committee of Shanxi Province Cancer Hospital (#1815032). Written informed consents were obtained from all patients. The experimental protocol of the animal study was approved by the Ethics Committee of the Shanxi Province Cancer Hospital (IACUC#089535).

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Data availability

Data will be made available on request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Guohui Han: Writing – review & editing, Writing – original draft, Validation, Investigation, Data curation, Conceptualization. **Xiangdong Bai:** Writing – review & editing, Writing – original draft, Validation, Investigation, Data curation, Conceptualization. **Feng Li:** Writing – review & editing, Validation, Resources, Methodology. **Li Huang:** Validation, Resources, Methodology. **Yating Hao:** Validation, Resources, Methodology. **Weina Li:** Validation, Resources, Methodology. **Peng Bu:** Validation, Resources, Methodology. **Huanhu Zhang:** Validation, Resources, Methodology. **Xinxin Liu:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Data curation, Conceptualization. **Jun Xie:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23827.

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