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# Diagnostic and prognostic significance of tissue lncRNA HOTAIR and hexokinase 2 mRNA expression in patients with pancreatic ductal adenocarcinoma

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## Abstract

**Background** Pancreatic adenocarcinoma, recognized for its aggressive behavior and frequent late-stage diagnosis, imposes significant challenges in early detection and prognosis. This study aimed to evaluate the diagnostic and prognostic potential by measuring the expression levels of long non-coding RNA HOTAIR and the glycolytic enzyme hexokinase 2 (HK2) mRNA in both tumorous and adjacent non-tumorous pancreatic tissue samples ( $n = 25$  each) using RT-qPCR. Results

Both lncRNA HOTAIR and HK2 expression levels significantly increased in tumorous pancreatic tissues compared to non-tumorous tissue ( $P = 0.001$ ). However, their levels in stage T2 and T3 showed no statistically significant difference ( $P = 0.01$ ). lncRNA HOTAIR and HK2 expression levels positively correlated with each other ( $P = 0.001$ ;  $r = 0.95$ ); however, no significant associations were found with serum tumor markers CA19-9 and CEA ( $P = 0.01$ ;  $r = 0.05$ ;  $p = 0.1$ ,  $r = 0.2$ ). ROC analysis demonstrated the significant abilities of both lncRNA HOTAIR and HK2 expression levels to discriminate between tumorous and non-tumorous pancreatic tissues (AUC = 0.92 and 0.84, respectively) with 96% and 88% sensitivity, and 72% and 40% specificity, respectively, at optimal cut-off values of 1.12 and 0.84 relative expression units. Patients with elevated lncRNA HOTAIR and HK2 expression had shorter median survival (8 and 7 months, respectively), increasing the risk of adverse outcomes or recurrence 4–4.8 times (HR = 4.08,  $p = 0.07$ ; HR = 4.8,  $p = 0.01$ ), thus emphasizing their prognostic potential in pancreatic cancer.

**Conclusion** lncRNA HOTAIR and HK2 expression levels exhibit diagnostic potential in pancreatic tumors. Elevated levels of both markers correlate strongly with adverse outcomes, underscoring their prognostic value.

**Keywords** Pancreatic ductal adenocarcinoma (PDAC), HOX transcript antisense RNA (lncRNA HOTAIR), Hexokinase 2 (HK2), Polymerase chain reaction (PCR)

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## Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive tumor associated with a grim 5-year survival rate of less than 10%. Its incidence is expected to rise globally, posing a significant clinical challenge [1, 2]. Challenges such as late-stage diagnosis, limited treatment options, and a high tendency for metastasis emphasize the urgent need for innovative approaches to improve both early detection and prognosis prediction [3, 4]. Despite being a traditional biomarker for diagnosing and monitoring PDAC, serum carbohydrate antigen 19-9 (CA19-9) falls short in terms of sensitivity and specificity, particularly for early detection and prognosis assessment. Compounding this issue, a substantial subset of PDAC patients does not exhibit CA19-9 expression, further complicating prognostic evaluations [5]. Recent advances in molecular biology have shed light on the critical roles of long non-coding RNAs (lncRNAs) in various cancers, including pancreatic adenocarcinoma [6–12]. These versatile RNA molecules, exceeding 200 nucleotides in length, actively participate in diverse cellular processes, including gene regulation, chromatin remodeling, and epigenetic modifications [13]. Dysregulation of lncRNAs is intricately linked to numerous diseases, with a particular focus on cancer, owing to their multifaceted contributions to tumorigenesis, progression, and metastasis [14, 15]. Among these lncRNAs, HOX transcript antisense RNA (HOTAIR) stands out as a crucial player in cancer biology. lncRNA HOTAIR does not encode proteins but exerts significant regulatory control over various cellular processes. Its involvement in gene regulation is well-documented, and it is closely associated with the development and progression of several cancer types [16, 17]. In breast cancer, lncRNA HOTAIR plays a pivotal role in regulating gene expression, promoting tumor invasiveness, and driving metastasis. It holds promise as both a prognostic marker and a potential therapeutic target [18]. Similarly, in prostate cancer, the overexpression of PCAT-1 (prostate cancer-associated transcript 1), another lncRNA, enhances cell proliferation while suppressing apoptosis, intensifying disease aggressiveness [19]. lncRNA HOTAIR and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) are implicated in non-small cell lung cancer (NSCLC), where they orchestrate epithelial-mesenchymal transition (EMT), a critical step in metastasis [17, 20]. In colorectal and ovarian cancers, HOTAIR dysregulation is linked to tumor growth and chemoresistance [21, 22]. In the context of pancreatic adenocarcinoma, various lncRNAs, including lncRNA HOTAIR, H19 imprinted maternally expressed transcript (H19), plasmacytoma variant translocation 1 (PVT1), and MALAT1, exhibit abnormal expression patterns that intersect with disease progression and patient

survival, suggesting their potential as diagnostic and prognostic biomarkers [23, 24]. Investigating the roles and mechanisms of these lncRNAs may yield valuable insights into the complex biology of pancreatic adenocarcinoma and unveil innovative avenues for diagnosis and prognosis assessment. Hexokinase 2 (HK2), a pivotal enzyme in the glycolytic pathway, plays a central role in the energy production and glucose metabolism of cancer cells. Aberrant overexpression of HK2 is observed in various cancer types, including pancreatic, liver, and gastric carcinomas, where heightened glycolysis provides the escalated metabolic demands of rapidly dividing cancer cells. Recent studies suggest that HK2 extends its functions beyond glycolysis, impacting cancer cell survival, invasion, and metastasis [16]. Its interaction with mitochondria further consolidates cancer cell resistance to apoptosis, a hallmark of malignancy. By facilitating the production of energy and metabolic intermediates necessary for rapid proliferation and migration, HK2 emerges as a critical driver of tumor growth and metastasis [16]. Given its central role in cancer metabolism and its distinctive expression in cancer cells compared to normal counterparts, HK2 emerges as a promising therapeutic target. Inhibiting HK2 holds the potential to disrupt cancer cell metabolism and sensitize them to treatment [25, 26]. Examining the interplay between lncRNA HOTAIR and HK2 in the context of pancreatic adenocarcinoma holds the promise of forging novel pathways for cancer diagnosis and prognosis prediction. One of the proposed mechanisms for lncRNA HOTAIR's oncogenic actions involves chromatin structure alteration and the modulation of lactate and ATP production through the glycolytic enzyme HK2 [13, 18, 27]. Considering these considerations, the specific aim of the present study is to investigate the diagnostic and prognostic potential of the lncRNA HOTAIR and its interactions with HK2 in the context of pancreatic adenocarcinoma. The overarching goal is to advance early detection methodologies and refine prognostic models, ultimately contributing to improved patient outcomes in this highly aggressive form of cancer.

## Patients and methods

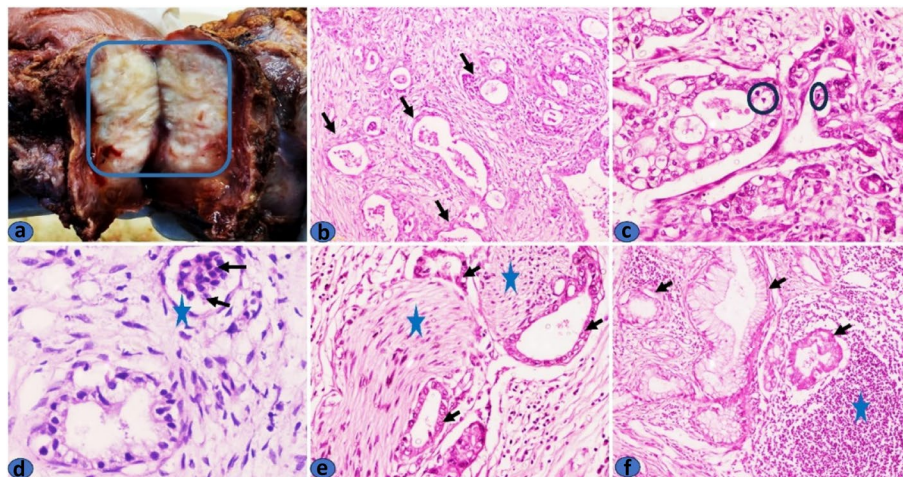
A case-control retrospective study involved 25 patients who had previously been diagnosed with PDAC. The diagnosis was confirmed through a combination of imaging modalities and pathological assessments. Surgical procedures, specifically Whipple's operation, were conducted at the Hepatobiliary and Pancreatic Surgery Department of Menofia University's National Liver Institute in Egypt. Frozen blocks of both the tumor and adjacent non-tumorous tissue were obtained from the Biorepository bank at the National Liver Institute's

Pathology Department. Demographic data, including age, gender, and BMI, along with relevant clinical information, were retrieved from medical records. Histopathological reports confirming the PDAC diagnosis were thoroughly reviewed. Additional clinical data, such as serum tumor markers CA19-9, CEA, and AFP, as well as hematological and biochemical tests assessing the patients' general status, tumor clinical and pathological stages, and post-surgical survival and recurrence data, were meticulously recorded, or tracked. Notably, patients with other malignancies were excluded from the study. Following the surgical procedures, all patients were diligently monitored for a duration of 36 months. The study was conducted from July 2020 to July 2023 and received ethical approval from the National Liver Institute Ethics Committee (NLIIRB protocol number 00447/2023). A comprehensive written informed consent was obtained from all enrolled patients.

#### Specimen collection and procedure

Blood samples were taken before any surgical procedure. Seven mL of venous blood samples were collected and divided as follows: 4 ml were added to a plain vacutainer tube, left 15 min for coagulation, and subsequent centrifugation at 3000 rpm for 5 min then the sera was aliquoted into two vials; one was used for measurement of liver and kidney function tests, electrolytes, C-reactive protein (CRP), lactate, lactate dehydrogenase using Cobas c501 Auto analyzer (Roche-Germany) and,

the other one was used for virological screen (hepatitis C virus (HCV) antibodies, hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HbcAb) antibodies) and tumor markers assay (alpha-fetoprotein (AFP), CA 19-9, and carcinoembryonic antigen (CEA)) using Cobas e 601 Auto analyzer (Roche-Germany). One ml was placed into a tube containing Ethylenediaminetetraacetic acid (EDTA) for complete blood count assay by Sysmex XT-1800i automated hematology analyzer (Sysmex, Japan). The remaining two ml were placed into a sodium citrate tube for prothrombin time, activated partial thromboplastin time (pt), and international normalized ratio (INR) assays by The Sysmex CS-1600 Automated hemostasis testing (Sysmex Corporation, Kobe, Japan). Pancreatic tissue samples were taken from all cases and categorized into two subgroups: tumorous and adjacent non-tumorous pancreatic tissue. As part of the management of the patients, the corresponding formalin-fixed, paraffin-embedded (FFPE) tissue blocks were obtained from the Pathology Department's archive where Hematoxylin and Eosin-stained slides were retrieved and examined for confirmation of the diagnosis. PDAC cases were graded into well-differentiated, moderately differentiated, and poorly differentiated grades according to the World Health Organization guidelines (WHO) [28]. Figure 1 displays gross and microscopic images of a pancreatic ductal adenocarcinoma. The pathologic stage was assessed by the tumor-node-metastasis (TNM) classification system as follows: T1, tumor restricted to the



**Fig. 1** Gross and microscopic images of pancreatic ductal adenocarcinoma. **a** A macroscopic appearance of PDAC shows a whitish, firm, infiltrative mass (blue box) in a background of unremarkable pancreatic tissue. **b** Microscopic findings of well-differentiated PDAC show irregular, malformed glands (arrows) embedded in a desmoplastic stroma, (H&E  $\times$  100). **c** Microscopic findings of moderate-differentiated PDAC show a cribriform pattern with a moderate degree of anaplasia and mitoses (circles), (H&E  $\times$  200). **d** Microscopic findings of PDAC show lymphovascular emboli (arrows), blood vessel wall is highlighted by an asterisk (H&E  $\times$  400). **e** Microscopic findings of PDAC show perineural invasion (arrows), nerves are highlighted by asterisks, (H&E  $\times$  200). **f** Microscopic findings of PDAC show metastasis to regional lymph node (arrows), residual nodal tissue is highlighted by asterisks, (H&E  $\times$  200)



pancreas with a maximum diameter less than 2 cm; T2, tumor confined to the pancreas with 2–4 cm in diameter; T3, tumor confined to the pancreas with a diameter above 4 cm; and T4, celiac axis or the superior mesenteric artery were included [13]. Additionally, lymph vascular invasion (LVI), perineural invasion, and surgical margins were similarly evaluated [29].

#### Quantitative measurement of tissue lncRNA HOTAIR and HK2 mRNA expressions by RT-qPCR

First, RNA extraction from formalin-fixed, paraffin-embedded tissue sections was performed using RNA purification from the formalin-fixed, paraffin-embedded kit (Rneasy FFPE Kit, Qiagen, Cat. No. 73504, Germany) according to the manufacturer's instructions. The purity and quality of RNA were determined by measuring its absorbance at 260 nm using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). The extracted RNA samples were transferred into a sterile Eppendorf tube, labeled, and stored at  $-20^{\circ}\text{C}$  until the time of the assay. The revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, Cat. No. K1622, Lithuania) was used according to the manufacturer's instructions for the reverse transcription step on ice with a total volume of 20  $\mu\text{L}$ , as follows: 1  $\mu\text{L}$  of reverse transcriptase enzyme, 4  $\mu\text{L}$  of reverse transcriptase buffer then 10  $\mu\text{L}$  of template RNA, and 5  $\mu\text{L}$  of nuclease-free water. The sample was then incubated in a 2720 thermal cycler, Applied Biosystems, life technology (Singapore), as follows: 10 min at  $42^{\circ}\text{C}$ , next 5 min at  $95^{\circ}\text{C}$  to deactivate the reverse transcriptase enzyme, and lastly, 5 min at  $4^{\circ}\text{C}$  for each cycle. Next, relative quantification (RQ) of *tissue lncRNA HOTAIR* and *HK2 mRNA* gene expression was performed using the following primers designed by Primer 3 Plus software (Midland, TX, USA): *lncRNA HOTAIR* forward primer: 5'-AAC GAT GTG TGT GTG CCT TGAT-3' and *HOTAIR* reverse primer, 5'-TGG TCC GAC AGG GTG AAT T-3'. *HK2* forward, 5'-GGG CAT CTT GAA ACA AG-3'; and reverse, 5'-GGT CTCAAG CCC TAAG-3'; and  $\beta$ -actin as a housekeeping gene forward, 5'-GAC CTC TATGCC AAC ACAGT-3'; and reverse, 5'-AGT ACT TGC GCTCAG GAG GA-3'. The total volume of the reaction was 20  $\mu\text{L}$  as follows: 10  $\mu\text{L}$  of Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific Inc., Cat. No. K0251, Lithuania), 1  $\mu\text{L}$  of Nuclease-free water, and 6  $\mu\text{L}$  of template cDNA in addition to 1.5  $\mu\text{L}$  of each primer (forward and reverse). Finally, *lncRNA HOTAIR*, *HK2*, and the reference gene  $\beta$ -actin were amplified using ABI7500 real-time PCR instrument (Applied Biosystems Thermo Fisher Scientific Inc., Life Technologies TM, CA, USA) software 2.0.1 by following thermocycling conditions:  $95^{\circ}\text{C}$  for 50 s, followed by 40 cycles of  $95^{\circ}\text{C}$  for 12 s and  $60^{\circ}\text{C}$  for 30 s. Supplementary Fig. S1 displays

a representative set of amplification curves for lncRNA HOTAIR, HK2, and  $\beta$ -actin Gene. The data were processed using the  $2^{-\Delta\Delta\text{Ct}}$  method and the expression levels of lncRNA HOTAIR and HK2 were normalized to the endogenous  $\beta$ -actin control levels [13, 30].

#### Statistical analysis

The data collected in this study underwent rigorous statistical analysis using SPSS version 23 on an IBM personal computer. Quantitative data were presented with mean, standard deviation, and range, while qualitative data were represented as numbers and percentages. To compare quantitative data, the study used the Student *t*-test for normally distributed data and the Mann-Whitney test for non-normally distributed data. The Kruskal-Wallis test was employed for non-normally distributed quantitative variables when comparing three or more groups. To explore correlations, Pearson's correlation was used for normally distributed data, while Spearman's correlation was applied for non-normally distributed data. ROC curves were created to establish optimal cut-off values that maximize both sensitivity and specificity, employing the Youden index (Youden index,  $J = \text{sensitivity} + \text{specificity} - 1$ ). Survival analysis was performed using Kaplan-Meier survival analysis when appropriate. Furthermore, multivariate Cox regression analysis was carried out to identify independent predictors of low survival. These robust statistical methods were systematically applied to ensure a comprehensive and precise analysis of the data.

## Results

### Demographic, clinical, and pathological characteristics of patients with PDAC

Table 1 summarizes the demographic and clinical characteristics of PDAC patients, encompassing details such as tumor location, size, post-operative tumor recurrence, and survival data. Additionally, the table presents pathological features, including pathological staging, nodal metastasis, extent of involvement with the superior mesenteric vein, as well as lymphovascular and perineural invasion.

### Biochemical and hematological profile of PDAC patients

Supplementary Table S1 provides a comprehensive overview of the patients' biochemical and hematological profiles, incorporating key statistical measures such as the range, mean values, standard deviation, as well as median and interquartile range. In the initial evaluation of each case as a prognostic indicator before surgery, tumor markers such as CEA, CA19-9, and AFP were employed. Furthermore, CBC and hematological indices and biochemical parameters encompassed liver and kidney

**Table 1** Demographic, pathological, and clinical characteristics of enrolled patients with pancreatic adenocarcinoma

Characteristic	Value (%)
Patient number	25 (100%)
Gender (males/females)	18 (72%)/7 (28%)
Age range (years)	36–76
Age, mean (± standard deviation)	57.16 ± 10.41
HBs–Ag positive cases	1 (4%)
Tumor location	
Head of the pancreas	21 (84%)
Body and tail of the pancreas	4 (16%)
Tumor size (greatest dimension, cm)	2.5–8.0
Superior mesenteric vein encasement	20 (80%)
Pathological stage	
T2	15 (60%)
T3	10 (40%)
Nodal metastasis	
N0	3 (12%)
N1	10 (40%)
N2	12 (48%)
Lympho-vascular invasion	11 (44%)
Perineural invasion	24 (96%)
Tumor recurrence	6 (24%)
Post-surgical survival (months)	2.0–36.0
Mean survival (± SD, months)	11.24 ± 8.57
One-year survival	13 (52%)

T2 tumor pathological stage 2 (greater than 2 cm but less than 4 cm) on gross examination. T3 tumor pathological stage 3 (greater than 4 cm but less than 5 cm) on gross examination. N0 No nodal involvement. N1 nodal metastasis, stage 1. N2: Nodal metastasis, stage 2

**Table 2** Comparison of lncRNA HOTAIR and HK2 relative expression levels in tumorous tissue versus adjacent non-tumorous tissues in patients with PDAC

	ANT (M ± SD)	PDAC (M ± SD)	P value
lncRNA HOTAIR	0.86 ± 0.85	35.95 ± 50.46	< 0.001
HK2	1.08 ± 0.69	22.06 ± 45.03	< 0.001

PDAC pancreatic ductal adenocarcinoma, ANT adjacent normal tissue, lncRNA long non-coding RNA, HOTAIR HOX transcript antisense RNA, HK2 hexokinase2, M mean, SD standard deviation

Significance level at P value < 0.05

function tests were utilized to assess the overall health status of the patients.

### Expression levels of lncRNA HOTAIR and HK2 in tumorous tissues

Table 2 demonstrates a significant and statistically marked increase in the tissue expression levels of lncRNA HOTAIR and HK2 in tumorous pancreatic tissues in

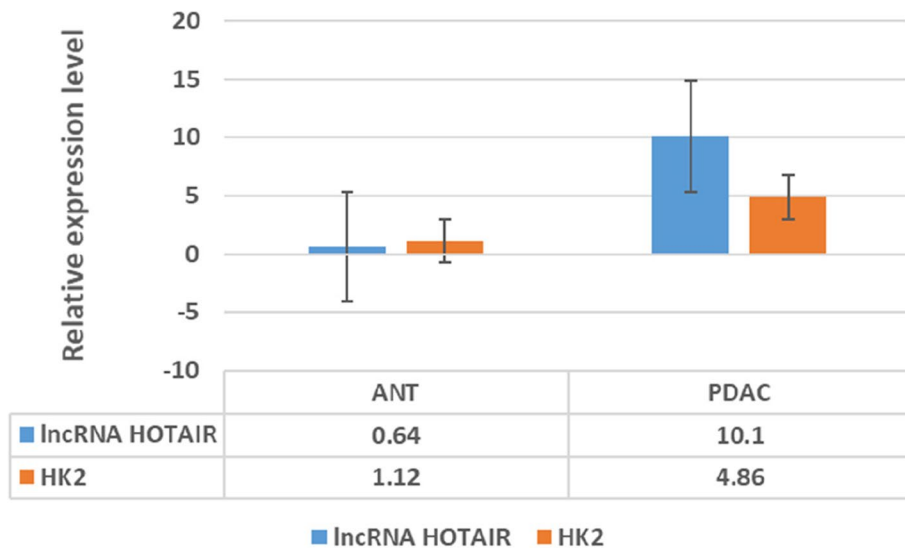
comparison to their corresponding adjacent non-tumorous tissues with *p* values of < 0.001 for both molecules. Figure 2 visually depicts the elevated median (IQR) expression level of lncRNA HOTAIR and HK2 in pancreatic tumor tissue when compared to adjacent normal tissue. The significantly elevated expression levels of both lncRNA HOTAIR and HK2 in PDAC compared to adjacent non-tumorous tissue underscore their potential as promising biomarkers for diagnostic evaluation.

### Expression of lncRNA HOTAIR and HK2 across pancreatic tumor stages

Table 3 presents the relative expression levels of lncRNA HOTAIR and HK2 in pancreatic tumor stages T2 and T3, in comparison to adjacent non-tumorous tissue (ANT), along with their corresponding statistical analysis results. Statistically significant higher median levels of both lncRNA HOTAIR and HK2 were observed in either stage T2 or stage T3 tumors than ANT (*p* = 0.005 and *p* = 0.001, respectively). However, no statistically significant difference was found in the median levels between stage T2 and stage T3 samples (*p* = 1.00). Figure 3 visually illustrates the elevated median (IQR) expression levels of lncRNA HOTAIR and HK2 in different stages of pancreatic ductal adenocarcinoma (PDAC) compared to the ANT.

### Correlations between lncRNA HOTAIR and HK2 expression levels and serum tumor markers CA19-9, CEA, and AFP

Table 4 displays correlations between lncRNA HOTAIR, HK2 expression, and the serum tumor markers, CA19-9, CEA, and AFP. A strong positive correlation was found between HOTAIR and HK2 expression (*r* = 0.943, *p* = 0.001), indicating potential co-regulation. CA19-9 showed a weak positive correlation with HOTAIR (*r* = 0.22, *p* = 0.27), but no significant correlation with HK2. CEA did not significantly correlate with HOTAIR (*r* = 0.06, *p* = 0.75), and there was a weak negative correlation with HK2 (*r* = − 0.34, *p* = 0.08), not reaching significance. AFP exhibited no significant correlation with lncRNA HOTAIR (*r* = − 0.02, *p* = 0.92) or HK2 (*r* = 0.02, *p* = 0.92) expression. These findings suggest a strong correlation between HOTAIR and HK2 expression, with limited or weak associations between these genes and the tumor markers AFP, CA19-9, and CEA in pancreatic cancer. Supplementary Table S2 presents correlation analysis of HOTAIR and HK2 expression with the biochemical, and hematological parameters. Weak and non-significant correlations were generally observed between HOTAIR and HK2 expression and most of the examined biochemical, and hematological parameters, as (indicated by the low correlation coefficients (*r*) and high *p* values), except for a significant negative correlation between

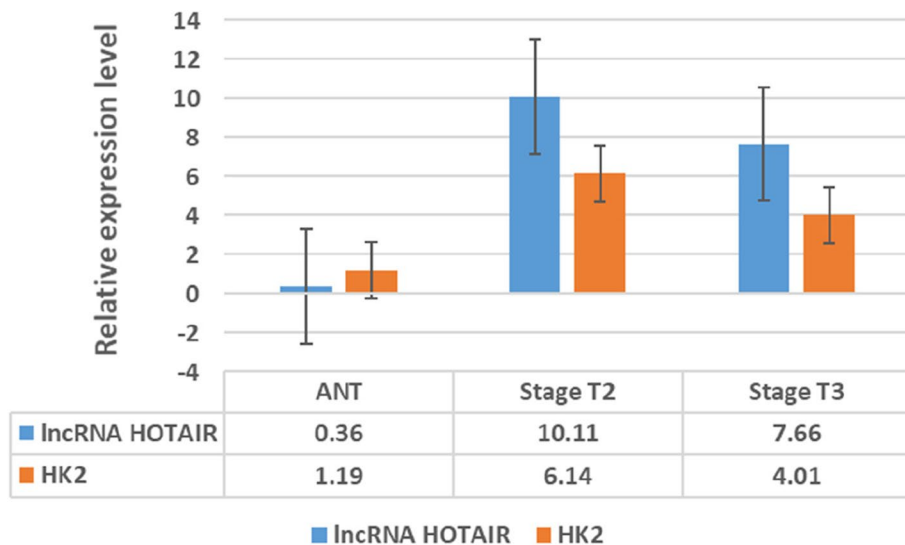


**Fig. 2** Column chart illustrating the median of the lncRNA HOTAIR and HK2 expression in pancreatic tumor and adjacent normal tissue. PDAC: pancreatic ductal adenocarcinoma, ANT: adjacent normal tissue lncRNA HOTAIR: HOX transcript antisense RNA, HK2: hexokinase2

**Table 3** Comparative analysis of lncRNA HOTAIR and HK2 expression in pathological stages T2, T3, and adjacent non-tumorous tissues

	ANT, n = 25 Median (IQR)	Stage T2, n = 15 Median (IQR)	Stage T3, n = 10 Median (IQR)	P value
lncRNA HOTAIR,	0.36 (1.64)	10.11(80.23)	7.66 (70.7)	a, b < 0.001, c > 0.05
HK2	1.19(1.21)	6.14(15.7)	4.01(34.4)	a, b < 0.001, c > 0.05

HK2 hexokinase 2, HOTAIR HOX transcript antisense RNA, IQR interquartile range, Significance level at P value < 0.05, TNM classification: T2 indicates that the cancer's greatest dimension measures between 2 cm and 4 cm, while T3 indicates that the cancer is larger than 4 cm but still confined within the pancreas. Post hoc test P value: a ANT vs T2, b ANT vs T3, c T2 vs T3



**Fig. 3** Column chart illustrating the median of HOTAIR and HK2 expression in different stage of PDAC and ANT. HOTAIR: HOX transcript antisense RNA, HK2: hexokinase2, PDAC: pancreatic ductal adenocarcinoma, ANT: adjacent normal tissue, StageT2: PDAC (2–4 cm), StageT3: PDAC > 4 cm)

**Table 4** Correlation of HOTAIR and HK2 expression levels in tumorous pancreatic tissue and serum tumor markers in PDAC patients

	lncRNA HOTAIR		HK 2	
	r	P value	r	P value
HOTAIR	1	1	0.943	0.001
CA19-9 U/ml	0.22	0.27	0	0.99
CEA, U/ml	0.06	0.75	- 0.34	0.08
AFP, ng/l	- 0.02	0.92	0.02	0.92

Significance level at P value < 0.05, r Pearson correlation, HK2 hexokinase 2, lncRNA HOTAIR HOX transcript antisense RNA, AFP alpha-fetoprotein, CA 19-9 carbohydrate antigen 19.9, CEA carcinoembryonic antigen

HOTAIR expression and coagulation factors PT and INR ( $r = - 0.39, p = 0.04; r = - 0.40, p = 0.04$ , respectively).

**lncRNA HOTAIR and HK2 exhibit high sensitivity in detecting pancreatic tumors**

To determine optimal clinical thresholds for distinguishing between tumorous and non-tumorous masses, ROC curve analysis was employed (Fig. 4). The selection of these thresholds based on the J index that determines the optimal cut-off value that defines the discriminatory power of the marker on the ROC curve and takes into account both sensitivity and specificity. ROC curves analysis revealed that both HOTAIR and HK2 demonstrated significant discriminatory potential, with AUC values of 0.92 and 0.84, respectively, signifying their robust ability

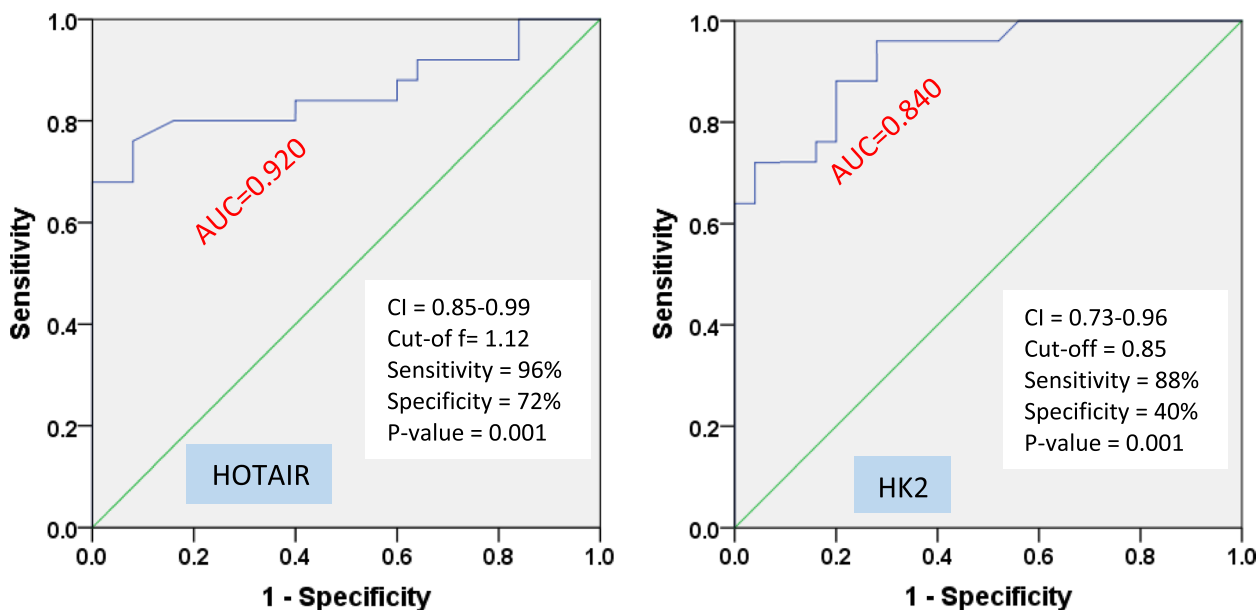
to differentiate tumors from ANT. Specifically, lncRNA HOTAIR exhibited a sensitivity of 96% and specificity of 72% at a cut-off point of 1.12 of relative expression unit (REU), while HK2 showed a sensitivity of 88% and specificity of 40% at a cut-off point of 0.85 REU.

**Impact of lncRNA HOTAIR and HK2 expression on survival outcomes in PDAC patients**

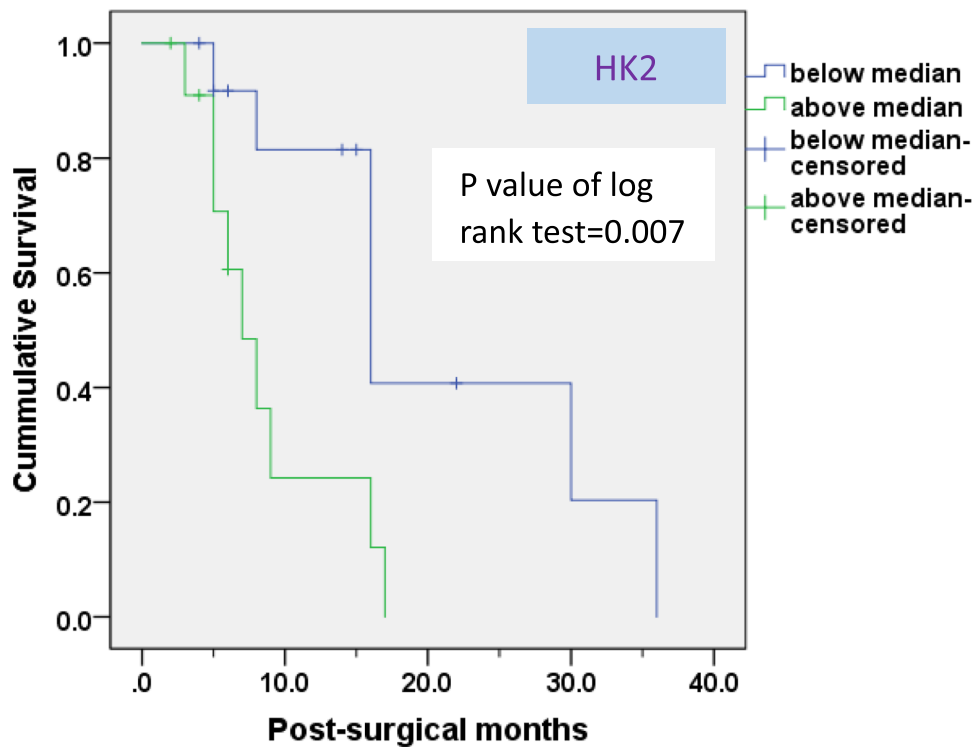
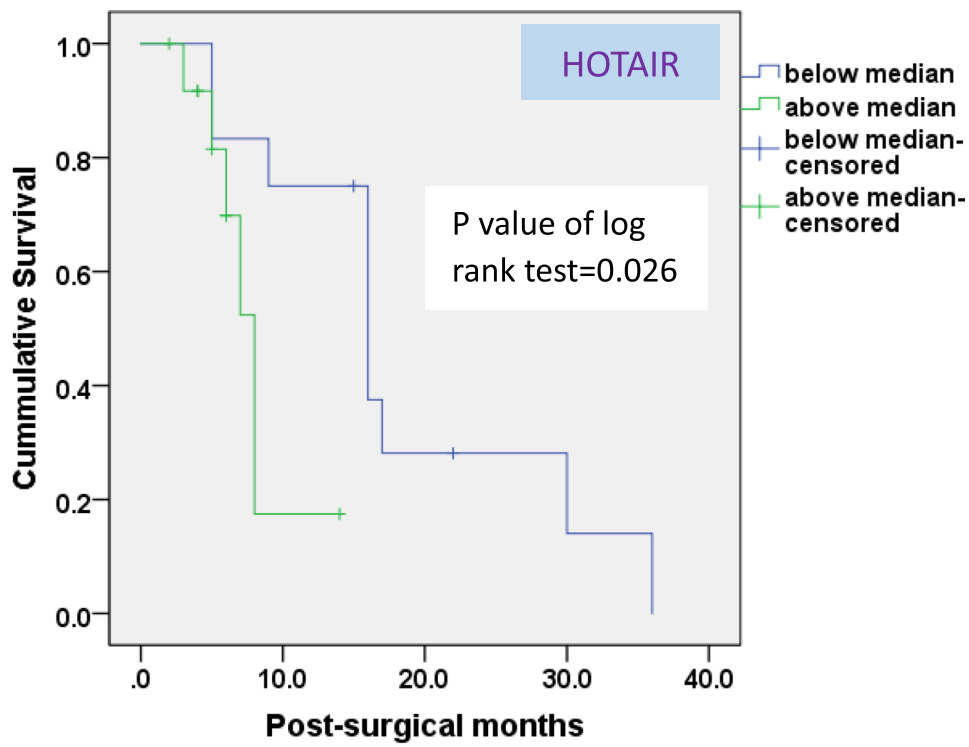
Patients were categorized into high- and low-expression groups based on the median expression levels of tissue lncRNA HOTAIR and HK2. Survival curves, constructed using the Kaplan-Meier method, revealed that patients with high relative expression levels of lncRNA HOTAIR and HK2 (above median) had median survival times of 8 and 7 months respectively, while patients with low relative expression levels of both lncRNA HOTAIR and HK2 exhibited a similar median survival time of 16 months. The statistical significance of these observations was confirmed through the log-rank test, which yielded significant results for both HOTAIR ( $p = 0.026$ ) and HK2 ( $p = 0.007$ ) (Fig. 5). These findings underscore the potential utility of lncRNA HOTAIR and HK2 as prognostic indicators for survival outcomes in PDAC patients, highlighting their importance in clinical decision-making and patient management.

**Prognostic significance of lncRNA HOTAIR and HK2 expression in PDAC**

Table 5 displayed the prognostic analysis of lncRNA HOTAIR and HK2 expression in PDAC patients.



**Fig. 4** ROC analysis of lncRNA HOTAIR and HK2 for tumor vs. non-tumor pancreatic tissues. AUC: area under the curve; CI: confidence interval; Cut-off: cut-off point. P value < 0.05 indicates significant test



**Fig. 5** Kaplan-Meier plot for overall survival analysis in PDAC patients. The patients are categorized into two groups based on HOTAIR and HK2 expression levels, using the median value as the dividing point. This graph illustrates the impact of these expressions on the overall survival rates of PDAC patients. HK2: hexokinase 2, HOTAIR: HOX transcript antisense RNA



**Table 5** Survival analysis: cox regression of demographic and clinicopathological factors in PDAC patients

	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Age > 60	0.7 (0.2–2.1)	0.6		
Gender	0.5 (0.19–1.7)	0.3		
Tumor size ≥ 4	0.5 (0.18–1.7)	0.3		
LVI	1.7 (0.5–5.03)	0.3		
TNM classification				
N1	1.3 (0.2–7.5)	0.7		
N2	1.05 (0.2–5.3)	0.9		
Pathological stage	0.4 (0.14–1.25)	0.12	3.4 (1.009–11.62)	0.04
HK2 high expression (above median)	3.7 (1.24–11.4)	0.01	4.8 (1.4–16.59)	0.01
lncRNA HOTAIR high expression (above median)	4.5 (1.04–19.5)	0.04	4.08 (0.8–19.35)	0.07

Statistical analysis of demographic data and clinicopathological features: Univariable and Multivariable Cox regression results. *CI* confidence interval, *HR* hazard ratio, significance level at *P* value < 0.05, *LVI* lymph vascular invasion, *HK2* hexokinase 2, *lncRNA HOTAIR* HOX transcript antisense RNA, *TNM* classification *N1* indicates 1 to 3 lymph nodes with cancer cells, *N2* indicates cancer in 4 or more lymph nodes

Univariate analysis showed no significant associations between patient outcomes and age ( $HR = 0.7$ ,  $p = 0.6$ ), gender ( $HR = 0.5$ ,  $p = 0.3$ ), tumor size ( $HR = 0.5$ ,  $p = 0.3$ ), LVI ( $HR = 1.7$ ,  $p = 0.3$ ), or TNM classification ( $N1$ :  $HR = 1.3$ ,  $p = 0.7$ ;  $N2$ :  $HR = 1.05$ ,  $p = 0.9$ ). In multivariate analysis, the pathological stage showed a significant impact on patient outcomes ( $HR = 3.4$ ,  $p = 0.04$ ). Patients with high HK2 expression (above the median) exhibited an HR of 4.8 (95% CI 1.4–16.59) with a *P* value of 0.01, indicating a significant association with adverse outcomes. High HOTAIR expression (above the median) had an HR of 4.08 (95% CI 0.8–19.35) with a *P* value of 0.07, suggesting a potential association with patient outcomes, although not statistically significant at the 0.05 level. These results emphasize the clinical relevance of HK2 and HOTAIR as potential prognostic markers in PDAC, providing valuable insights for patient risk assessment and tailored treatment strategies.

## Discussion

Despite a multitude of clinical trials and continued research endeavors, pancreatic cancer continues to pose a difficult challenge in the realm of oncology, primarily due to its elusive nature when it comes to early-stage diagnosis [31, 32]. This study investigated the roles of lncRNA HOTAIR and the glycolytic enzyme HK2 in PDAC. The study assessed their expression in pancreatic tissues, exploring their associations with

clinicopathological aspects and patient outcomes, with the aim of enhancing diagnostic and prognostic understanding in PDAC. The majority of PDAC patients had tumors located in the pancreatic head, often presenting as solitary tumors, which is typical for this aggressive cancer. Pathological staging indicated a substantial number of cases in the T2 and T3 categories, highlighting the late-stage diagnosis frequently associated with PDAC. High rates of nodal metastasis were consistent with the aggressive nature of the disease, and the presence of lymphovascular and perineural invasion underscored the invasive characteristics of these tumors [2, 3, 28]. A significant finding in this study is the marked upregulation of lncRNA HOTAIR and HK2 in tumorous pancreatic tissues compared to adjacent non-tumorous tissues, highlighting their potential roles in PDAC development and progression. This aligns with previous research by Jiang et al. [17], Ma et al. [13], Tang et al. [33], and Yang et al. [24], collectively suggesting lncRNA HOTAIR's clinical relevance as a biomarker in PDAC. Additionally, a strong positive correlation between the expression levels of these two molecules implies potential interactions or co-regulation within pancreatic cancer. Furthermore, their expression levels increased with higher primary tumor stages, suggesting that elevated HK2 levels may contribute to PDAC growth and progression, possibly due to metabolic changes related to energy and glucose uptake that support tumor cell survival [13, 16]. Notably, previous studies have documented lncRNA HOTAIR's ability to interact with and regulate HK2's biological functions, establishing lncRNA HOTAIR as an upstream regulator of HK2 in pancreatic adenocarcinoma and esophageal squamous cell carcinoma [13, 34–36]. The remarkable sensitivity demonstrated by both lncRNA HOTAIR and HK2 in distinguishing between tumorous and non-tumorous pancreatic tissues represents a significant breakthrough. Their robust discriminatory capabilities, as evidenced by the high AUC values in ROC curve analysis, suggest their potential as valuable diagnostic markers for pancreatic ductal adenocarcinoma (PDAC). Notably, lncRNA HOTAIR exhibited excellent sensitivity, reaching 96%, underscoring its clinical relevance in the early detection of pancreatic tumors. While HK2 also displayed substantial sensitivity at 72%, further investigations are warranted to fine-tune and optimize its diagnostic performance. These findings align with the study conducted by Yu Ma et al., who assessed the diagnostic utility of serum lncRNA HOTAIR and HK2 levels in pancreatic adenocarcinoma. Their results strongly indicate that serum lncRNA HOTAIR and HK2 levels hold promise as valuable biomarkers for the diagnosis of pancreatic adenocarcinoma [13, 37]. Similarly, Chen et al. investigated lncRNA HOTAIR expression in lung cancer

patients. Their findings revealed a significant upregulation of HOTAIR in lung cancer tissues compared to the control group, with a sensitivity of 74%, and a specificity of 77% [38]. These results underscore the potential utility of lncRNA HOTAIR as a diagnostic marker in lung cancer as well. Currently, the only approved serum marker for diagnosing pancreatic cancer is CA 19-9, but it is not PC-specific and can also increase in other cancers like colorectal, liver, lung, and ovarian cancers [39]. To improve the specificity and sensitivity of pancreatic cancer diagnosis, combining CA 19-9 with other investigated biomarkers in a multi-marker panel is a promising approach. The study examined links between lncRNA HOTAIR and HK2 expression levels and serum tumor markers CA19-9, CEA, and AFP frequently used in the clinical course of PDAC [31]. However, the study found weak and non-significant correlations between lncRNA HOTAIR, HK2 expression, and these tumor markers, these underscores the need for further research to comprehensively explore their interactions in the intricate molecular landscape of PDAC [40]. Interestingly, Xie et al. explored the potential of salivary lncRNA HOTAIR as a non-invasive biomarker for detecting resectable PDAC. They discovered that salivary lncRNA HOTAIR could effectively differentiate pancreatic cancer from healthy controls, particularly when serum CA19-9 levels were < 37 U/ml, achieving a sensitivity of 70% and a specificity of 87% [41]. This study concluded that differential expression of salivary lncRNA HOTAIR closely correlates with distal pancreatic cancer and suggests its potential as a non-invasive biomarker with superior sensitivity and specificity compared to serum CA19-9 for PC detection. It opens new possibilities for early pancreatic cancer detection and screening for other systemic diseases through non-invasive methods [41]. Exploring the relationships between lncRNA HOTAIR and HK2 expression levels and various histopathological features in PDAC patients revealed significant associations between either HOTAIR or HK2 expression and pathological stages T2 and T3. These findings are consistent with Liu et al., who observed a similar correlation between high HOTAIR expression and advanced pathological stages, lymph node metastasis, and poorer prognosis in NSCLC [42, 43]. However, no significant correlations were detected with other histopathological features such as lymph node status and margin status. These results emphasize the potential of lncRNA HOTAIR and HK2 as biomarkers linked to disease progression, aligning with its well-established roles in cancer metabolism and progression [25]. Given the limited associations found in this study, further research may be warranted to explore the specific histopathological contexts in which lncRNA HOTAIR and HK2 exert a significant influence. Kaplan-Meier survival analysis indicated

that elevated expression levels of both lncRNA HOTAIR and HK2 correlated with shorter median survival times, highlighting their potential prognostic significance. In univariate analysis, high expression of lncRNA HOTAIR and HK2 was significantly linked to decreased overall survival. Patients with tumors exhibiting high lncRNA HOTAIR and HK2 levels were 4.08 to 4.8 times more likely to experience adverse outcomes compared to those with lower expression. Conversely, factors such as age, gender, tumor size, LVI, and TNM classification did not demonstrate significant associations with survival outcomes. These results are consistent with previous studies by Ogawa et al., which reported similar reductions in overall survival for patients with high lncRNA HOTAIR or HK2 expression [44]. While previous studies have demonstrated a significant link between tumor aggressiveness and the expression of lncRNA HOTAIR and HK2 in various cancer types [13, 41, 44], our study focuses on the prognostic significance of lncRNA HOTAIR and HK2 expression levels in pancreatic tumor patients. We also explore the potential importance of assessing these markers. Elevated expression of lncRNA HOTAIR and HK2 in cancer tissue may indicate increased malignancy, possibly linked to enhanced aerobic glycolysis driven by the hypoxic microenvironment [45].

Limitations of this study include the relatively small sample size, which may limit the generalizability of the findings to a broader population of PDAC patients. Additionally, the cross-sectional nature of the data restricts the ability to establish causal relationships between variables. Further longitudinal research is needed to confirm these associations over time and assess the dynamic changes in lncRNA HOTAIR and HK2 expression. Additionally, while this study explored the associations of lncRNA HOTAIR and HK2 with various clinicopathological factors and tumor markers, the underlying mechanisms of their interactions in PDAC remain to be fully elucidated and should be investigated in future studies. Finally, the study's retrospective design may introduce biases that could impact the observed associations and survival outcomes.

## Conclusion

The current study emphasizes the prognostic significance of lncRNA HOTAIR and HK2 gene expression in pancreatic ductal adenocarcinoma (PDAC). Elevated levels of lncRNA HOTAIR and HK2 were associated with shorter survival and a fourfold increase in the risk of adverse outcomes among surgically treated patients, confirming their robust role as prognostic indicators. In addition to addressing challenges in early-stage pancreatic cancer diagnosis, the study's focus on prognostic accuracy, going beyond mere biomarker identification, establishes a foundation for future research aimed at deepening our understanding of pancreatic ductal adenocarcinoma.

## Abbreviations

PDAC	Pancreatic ductal adenocarcinoma
RNA	Ribonucleic acid
Lnc	Long non-coding
ncRNAs	Non-coding RNAs
mRNA	Messenger RNA
HOTAIR	HOX transcript antisense RNA
HK2	Hexokinase 2
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
CA19-9	Carbohydrate surface antigen
AFP	Alpha feto protein
CEA	Carcinoembryonic antigen
MALAT1	Metastasis-associated lung adenocarcinoma transcript 1
H19	H19 imprinted maternally expressed transcript
PVT1	Plasmacytoma variant translocation 1
NSCLC	Non-small cell lung cancer
EMT	Epithelial-mesenchymal transition
CRP	C-reactive protein
INR	International normalized ratio
aptt	Activated partial thromboplastin time
FFPE	Formalin-fixed, paraffin-embedded
TNM	Tumor-node-metastasis
PCR	Polymerase chain reaction
REU	Relative expression unit
SD	Standard deviation
OS	Overall survival
EMT	Epithelial-mesenchymal transition

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43066-023-00308-1>.

**Additional file 1: Supplementary Table S1.** Descriptive statistics for biochemical, and hematological parameters in pancreatic adenocarcinoma patients. **Supplementary Table S2.** Correlation of HOTAIR and HK2 Expression Levels with Biochemical and Hematological Parameters in Tumorous Pancreatic Tissue. **Supplementary Figure S1.** PCR Amplification curves depicting the real-time amplification profiles for lncRNA HOTAIR, HK2, and  $\beta$ -actin genes. The vertical axis displays delta Rn values, representing fluorescence signal normalized to the baseline. These delta Rn values offer insight into amplification dynamics, enabling precise quantification of target gene expression levels.

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## Authors' contributions

GS, SA, and DS were the major contributors in performing the practical section of this work and the interpretation of the laboratory data. SA also contributed to writing the manuscript and is a corresponding author. DS had performed the pathological section of the study. ES contributed to the interpretation of the clinical data and supervision of the final diagnosis of the cases. AK contributed to the study design and data analysis, edited the manuscript, and is a corresponding author. MO contributed to the conception, design, and revision of the data analysis and all the work. All authors have read and approved the final manuscript.

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## Availability of data and materials

All data generated or analyzed during this study are included in this article.

## Declarations

### Ethics approval and consent to participate

Approval was obtained from the Ethics Committee of the National Liver Institute, Menoufia University (00447/2023). Informed written consent was obtained from the participants before enrollment in the study.

### Competing interests

The authors declare that they have no competing interests.

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