Article

*Discovery Medicine* 2026; 38(214): 3730–3740
https://doi.org/10.24976/Discov.Med.202638214.283

# Relationship Between HIF-1α and IL-6 Expression and Clinicopathological Features, Perineural Invasion, and Prognosis in Pancreatic Cancer

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Submitted: 5 August 2026 Revised: 8 September 2026 Accepted: 20 September 2026 Published: 25 November 2026

**Background: Pancreatic cancer has an extremely poor prognosis, and hypoxia and inflammation in the tumor microenvironment are closely associated with its progression. Hypoxia-inducible factor-1α (HIF-1α) and interleukin-6 (IL-6) are key molecules involved in these processes; however, their clinical and prognostic significance in pancreatic cancer, especially regarding perineural invasion, remains incompletely understood. This study aimed to investigate the relationship between the expression of HIF-1α and IL-6 in pancreatic cancer tissues and clinicopathological features, perineural invasion, and prognosis.**

**Methods: Clinical data from 120 pancreatic cancer patients who underwent surgical resection at Huadu District People's Hospital of Guangzhou from March 2021 to March 2026 were retrospectively analyzed. Immunohistochemistry was used to detect the expression levels of HIF-1α and IL-6 in tumor tissues, and their relationships with clinicopathological parameters were analyzed. The Kaplan-Meier method was used to plot survival curves, with differences between groups compared using the log-rank test. Univariate Cox regression models were used to analyze prognosis-related factors.**

**Results: High HIF-1α expression was associated with larger tumor size ($p = 0.006$), poor differentiation ($p < 0.001$), carbohydrate antigen 19-9 (CA19-9) >35 U/mL ($p < 0.001$), total bilirubin (TBIL) >17.1 μmol/L ($p = 0.014$), vascular invasion ($p = 0.002$), and perineural invasion ($p < 0.001$). High IL-6 expression was associated with larger tumor size ($p < 0.001$), poor differentiation ($p < 0.001$), CA19-9 >35 U/mL ($p < 0.001$), carcinoembryonic antigen (CEA) >5 ng/mL ($p = 0.006$), lymph node metastasis ($p = 0.007$), and perineural invasion ($p < 0.001$). Both high HIF-1α and high IL-6 expression were associated with poorer overall survival (log-rank $p < 0.001$ for both). Univariate Cox regression identified tumor size >3 cm (hazard ratio (HR) = 2.182, $p = 0.033$), poor differentiation (HR = 4.609, $p < 0.001$), CA19-9 >35 U/mL (HR = 2.252, $p = 0.030$), lymph node metastasis (HR = 2.954, $p = 0.003$), vascular invasion (HR = 2.109, $p = 0.041$), perineural invasion (HR = 5.274, $p < 0.001$), low HIF-1α expression (HR = 0.231, $p < 0.001$), and low IL-6 expression (HR = 0.175, $p < 0.001$) as significant prognostic correlates.**

**Conclusion: HIF-1α and IL-6 are potential prognostic markers that may provide adjunctive information for risk stratification in pancreatic cancer, but their clinical utility requires further validation in larger, prospective cohorts with multivariate adjustment.**

**Keywords:** pancreatic cancer; HIF-1α; IL-6; perineural invasion

## Introduction

Pancreatic cancer is widely recognized as one of the most lethal malignancies of the digestive tract. The 2024 American Cancer Society statistics report shows that the 5-year survival rate of pancreatic cancer is only about 13%, making it the malignancy with the poorest prognosis [1]. Early pancreatic cancer often goes unnoticed, resulting in nearly 80% of patients presenting with advanced-stage or metastatic disease by the time curative resection is no longer feasible [2]. Despite curative resection, postoperative recurrence and metastasis are common, with a 5-year survival rate of only 20%–30% [3]. Therefore, identifying biomarkers that can accurately predict the prognosis of patients with pancreatic cancer is of significant clinical importance for guiding personalized treatment and improving patient survival.

Hypoxia and inflammatory responses within the tumor microenvironment play crucial roles in the occurrence, progression, and metastasis of pancreatic cancer [4,5]. Hypoxia-inducible factor-1α (HIF-1α) is a core transcription factor [6,7]. Under normoxic conditions, HIF-1α protein is rapidly degraded via the ubiquitin-proteasome pathway; under hypoxic conditions, HIF-1α stability increases, allowing it to translocate to the nucleus, form a heterodimer

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with hypoxia-inducible factor 1 subunit beta (HIF-1β), and activate the transcription of downstream target genes [8]. A previous study has shown that HIF-1α is overexpressed in various solid tumors and is associated with tumor aggressiveness and poor prognosis [9]. In pancreatic cancer, HIF-1α promotes tumor angiogenesis and extracellular matrix degradation by activating downstream molecules like vascular endothelial growth factor and matrix metalloproteinases, thereby driving local invasion. This suggests HIF-1α is a potential prognostic marker for pancreatic cancer.

Interleukin-6 (IL-6) is an inflammatory cytokine [10]. IL-6 binds to membrane-bound or soluble IL-6 receptors and signal transducer glycoprotein 130 to form a hexameric complex [11]. In pancreatic cancer patients, serum IL-6 levels are significantly elevated and associated with tumor burden and poor prognosis [12]. Chen et al. [13] demonstrated that blocking IL-6/glycoprotein 130 signaling inhibits the survival of human pancreatic cancer cells, further confirming the driving role of the IL-6 signaling pathway in the malignant progression of pancreatic cancer.

Perineural invasion is a unique invasion pathway characteristic of pancreatic cancer and a key prognostic factor [14]. The pancreas has a rich nerve supply, and pancreatic cancer has a much higher propensity for perineural invasion than other gastrointestinal tumors. A previous study has found that perineural invasion is relatively common in pancreatic cancer and is an important pathological feature distinguishing it from other digestive tract tumors [15]. Perineural invasion not only reflects the local invasive capacity of the tumor but may also represent a pathway for tumor cells to spread distantly along the nerve sheath. However, the association between HIF-1α and IL-6 and perineural invasion lacks systematic investigation. Previous studies suggest that HIF-1α promotes directional migration of tumor cells along nerves by inducing the expression of nerve growth factor and its receptors, while IL-6 fosters an inflammatory microenvironment conducive to perineural invasion by activating inflammatory signaling pathways and upregulating chemokine release [16,17]. However, these mechanistic hypotheses are primarily based on *in vitro* and animal studies, and direct evidence from pancreatic cancer patient tissues remains insufficient, requiring further validation in clinical cohort studies.

Based on this background, this study retrospectively collected clinical data from 120 patients who underwent pancreatic cancer surgery, detected HIF-1α and IL-6 expression levels, and systematically analyzed their associations with clinicopathological parameters, perineural invasion, and prognosis, aiming to provide a theoretical basis for individualized treatment and prognostic assessment of pancreatic cancer.

## Patients and Methods

### *Study Population*

This was a retrospective study. Patients with pancreatic cancer who underwent surgical resection at the Huadu District People's Hospital of Guangzhou between March 2021 and March 2026 were included. This study was approved by the Medical Ethics Committee of Huadu District People's Hospital of Guangzhou (Approval No.: 2026059Y). Due to the retrospective nature of the study, informed consent was waived. The study process adhered to the ethical principles of the Declaration of Helsinki. Inclusion criteria were: (1) Pathologically confirmed primary pancreatic ductal adenocarcinoma after surgery; (2) complete clinical and follow-up data; (3) no neoadjuvant chemoradiotherapy before surgery; (4) complete immunohistochemical results for HIF-1α and IL-6. Exclusion criteria were: (1) Coexisting other malignancies; (2) severe cardiovascular or cerebrovascular diseases, liver or renal insufficiency, or immunodeficiency diseases; (3) received radiotherapy, chemotherapy, or targeted therapy before surgery; (4) incomplete clinicopathological data or lost to follow-up. A total of 160 patients with pancreatic cancer who underwent surgical tumor resection were initially identified. After reviewing the medical records, 40 patients were excluded for the following reasons: 8 received neoadjuvant chemoradiotherapy before surgery, 6 had coexisting malignancies, 7 had severe cardiovascular or cerebrovascular diseases or hepatic/renal insufficiency, 4 had incomplete clinicopathological data, 15 had incomplete immunohistochemical results for HIF-1α and/or IL-6. Finally, 120 patients were included in this study.

### *Collection of Clinicopathological Data*

Clinicopathological data were retrospectively collected from the hospital's electronic medical record system. Data were independently extracted and verified by two researchers to ensure accuracy. Collected data included general information (age, sex, body mass index (BMI), blood type, smoking history, alcohol consumption history, and diabetes history), tumor pathological data (tumor size (≤3 cm/>3 cm), tumor location (head/neck vs. body/tail), differentiation degree (moderately_well differentiated/poorly differentiated), lymph node metastasis (yes/no), vascular invasion (yes/no), and perineural invasion (yes/no)), laboratory indicators (carbohydrate antigen 19-9 (CA19-9) (≤35 U/mL/>35 U/mL), carcinoembryonic antigen (CEA) (≤5 ng/mL/>5 ng/mL), and total bilirubin (TBIL) (≤17.1 μmol/L/>17.1 μmol/L)).

### *Detection of HIF-1α and IL-6 Expression*

Immunohistochemistry was used to detect the expression levels of HIF-1α and IL-6 proteins in surgically resected tumor tissues. Surgical specimens were fixed in 10% neutral formalin, routinely embedded in paraffin, and

cut into 4 μm serial sections. The sections were deparaffinized in xylene, rehydrated through graded ethanol, and subjected to antigen retrieval by microwave heating in citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% $H_2O_2$. Then, the sections were incubated with mouse anti-human HIF-1α monoclonal antibody (Proteintech, Cat#: 66730-1-Ig, dilution 1:200) and mouse anti-human IL-6 monoclonal antibody (Abcam, Cat#: ab9324, dilution 1:100) overnight at 4 °C. The next day, the sections were incubated with horseradish peroxidase (HRP)-labeled goat anti-mouse Immunoglobulin G (IgG) secondary antibody (Servicebio, Cat#: GB23301, dilution 1:200), which specifically recognizes the mouse-derived primary antibodies, for 30 minutes at 37 °C. Immunoreactivity was visualized using 3,3′-diaminobenzidine, and the sections were counterstained with hematoxylin. Known positive pancreatic cancer tissues were used as positive controls, and phosphate-buffered saline replacing the primary antibody was used as a negative control.

Two pathologists independently evaluated staining. HIF-1α positivity was defined as brown-yellow granular staining localized to the nucleus. Cytoplasmic staining, when present, was recorded descriptively but was not included in the quantitative scoring system, as cytoplasmic HIF-1α does not represent the transcriptionally active form. IL-6 positivity was defined as cytoplasmic and/or membranous staining. Under low-power magnification, the entire section was scanned to identify three areas with the highest density of positive tumor cells (hotspots). Then, five high-power fields (×400) within the selected hotspot areas were evaluated for scoring. The percentage of positive cells was calculated based on the average proportion of stained tumor cells across these fields. Staining was scored based on a combined score of staining intensity and the percentage of positive cells. Staining intensity score: 0 (negative), 1 (weak), 2 (moderate), 3 (strong). Percentage of positive cells score: 0 (<5%), 1 (5%–25%), 2 (26%–50%), 3 (51%–75%), 4 (>75%). Total score = staining intensity score × percentage score. A total score ≥6 was considered indicative of high expression, whereas a total score <6 was considered indicative of low expression. The staining scoring system was based on the semi-quantitative immunoreactive score (IRS) described by Remmele and Stegner, which multiplies the staining intensity score by the percentage of positive cells [18]. This scoring approach has been widely adopted in immunohistochemical studies. The cut-off of ≥6 was selected because it corresponds to moderate-to-strong staining in more than 50% of tumor cells, or strong staining in 26–50% of tumor cells, representing a biologically meaningful threshold for "high" expression [19]. In cases of discrepancy between the two pathologists, the final score was determined through joint re-evaluation and discussion until a consensus was reached.

### *Follow-Up and Survival Analysis*

The starting point for follow-up was the date of surgery, and the endpoint was death or the last follow-up date. Follow-up data were retrospectively collected from inpatient and outpatient medical records. Survival status was verified through the hospital's electronic medical record system and the national death registry. Patients who were alive at the last follow-up were censored. Patients were followed up at 1 month after surgery, then every 3 months during the first 2 years after surgery and every 6 months thereafter. Loss to follow-up was defined as the inability to contact the patient or their family members after at least 3 consecutive telephone attempts, with no medical records available for more than 12 months. The follow-up cutoff date was set as March 30, 2026. The primary endpoint was overall survival (OS), defined as the time from the date of surgery to death from any cause or last follow-up, measured in months. For the 120 included patients, the median follow-up time was 15.2 months (95% confidence interval (CI): 13.7–21.4).

### *Statistical Methods*

Statistical analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA) and R 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were compared using the independent-samples *t*-test (normally distributed data, Mean ± standard deviation (SD)) or Mann-Whitney U test (non-normally distributed data, median). Categorical variables were compared using the chi-square test or Fisher's exact test when expected cell count <5, and are expressed as n (%). Survival curves were performed using the Kaplan-Meier method and compared using the log-rank test. Univariate Cox regression was used to identify prognostic factors, with results presented as hazard ratios (HRs) and 95% CIs. Multivariate Cox regression was not performed due to the limited number of death events (34 cases) to avoid overfitting. A two-sided $p < 0.05$ was considered statistically significant.

## Results

### *Baseline Characteristics*

This study included 120 patients who underwent pancreatic cancer surgery, with a mean age of 62.467 ± 7.910 years, including 56 males (46.667%) and 64 females (53.333%). Tumors were located in the head/neck in 76 cases (63.333%) and in the body/tail in 44 cases (36.667%). Tumor diameter was ≤3 cm in 49 cases (40.833%) and >3 cm in 71 cases (59.167%). Moderately_well-differentiated tumors were found in 82 cases (68.333%), and poorly differentiated tumors in 38 cases (31.667%). CA19-9 >35 U/mL was found in 78 cases (65.000%), and CEA >5 ng/mL in 28 cases (23.333%). Lymph node metastasis was present in 45 cases (37.500%), vascular invasion in 33 cases (27.500%), and perineural invasion in 55 cases (45.833%) (Table 1).

**Table 1. Baseline characteristics.**

| Variables | | Variables | |
|---|---|---|---|
| Age, Mean ± SD | 62.467 ± 7.910 | BMI, Mean ± SD | 25.809 ± 4.467 |
| Gender, n (%) | | CA19-9, n (%) | |
| Female | 64 (53.333) | ≤35 | 42 (35.000) |
| Male | 56 (46.667) | >35 | 78 (65.000) |
| Blood Type, n (%) | | CEA, n (%) | |
| Non-O | 75 (62.500) | ≤5 | 92 (76.667) |
| O | 45 (37.500) | >5 | 28 (23.333) |
| Smoking history, n (%) | | TBIL, n (%) | |
| No | 92 (76.667) | ≤17.1 | 76 (63.333) |
| Yes | 28 (23.333) | >17.1 | 44 (36.667) |
| Alcohol use, n (%) | | | |
| No | 101 (84.167) | | |
| Yes | 19 (15.833) | | |
| Diabetes, n (%) | | lymph node metastasis, n (%) | |
| No | 98 (81.667) | No | 75 (62.500) |
| Yes | 22 (18.333) | Yes | 45 (37.500) |
| Tumor size, n (%) | | Vascular invasion, n (%) | |
| ≤3 | 49 (40.833) | No | 87 (72.500) |
| >3 | 71 (59.167) | Yes | 33 (27.500) |
| Tumor location, n (%) | | Perineural invasion, n (%) | |
| Body_tail | 44 (36.667) | No | 65 (54.167) |
| Head_neck | 76 (63.333) | Yes | 55 (45.833) |
| Differentiation, n (%) | | | |
| Moderate_well | 82 (68.333) | | |
| Poor | 38 (31.667) | | |

SD, standard deviation; BMI, body mass index; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; TBIL, total bilirubin.

### *Association of HIF-1α Expression With Clinicopathological Features and Perineural Invasion*

Among the 120 patients, 67 showed high HIF-1α expression, and 53 showed low expression. The two groups did not differ significantly in age, sex, blood type, smoking history, alcohol use, diabetes, tumor location, CEA, or lymph node metastasis (all $p > 0.05$). High HIF-1α expression was associated with tumor size >3 cm ($p = 0.006$), poor differentiation ($p < 0.001$), CA19-9 >35 U/mL ($p < 0.001$), TBIL >17.1 μmol/L ($p = 0.014$), vascular invasion ($p = 0.002$), and perineural invasion ($p < 0.001$), indicating an association between HIF-1α overexpression and more aggressive tumor behavior (Table 2).

### *Clinicopathological and Perineural Invasion Correlates of IL-6 Expression*

There were 72 cases in the IL-6 high-expression group and 48 cases in the low-expression group. No statistically significant differences were found between the two groups regarding age, sex, blood type, alcohol consumption, diabetes, tumor location, TBIL, smoking history, or vascular invasion (all $p > 0.05$). High IL-6 expression was associated with tumor size >3 cm ($p < 0.001$), poor differentiation ($p < 0.001$), CA19-9 >35 U/mL ($p < 0.001$), CEA >5 ng/mL ($p = 0.006$), lymph node metastasis ($p = 0.007$), and perineural invasion ($p < 0.001$) (Table 3).

### *Survival Analysis*

Among the 120 patients, 34 deaths were recorded by the end of follow-up: 21 (HIF-1α high) vs. 13 (low), and 26 (IL-6 high) vs. 8 (low). The median OS for the entire cohort was 37.3 months (95% CI: 30-not reached; range: 0.7–56.2). Kaplan-Meier analysis demonstrated that high HIF-1α expression was associated with shorter OS (median: 18.10 months, 95% CI: 10.60-not reached) compared with low expression (median not reached; log-rank $p < 0.001$) (Fig. 1). Likewise, high IL-6 expression was associated with shorter OS (median: 18.20 months, 95% CI: 13.80–31.00) than low expression (median not reached; log-rank $p < 0.001$) (Fig. 2).

### *Prognostic Factor Analysis*

Univariate Cox regression analysis showed that tumor size >3 cm (HR = 2.182, $p = 0.033$), poor differentiation (HR = 4.609, $p < 0.001$), CA19-9 >35 U/mL (HR = 2.252, $p = 0.030$), lymph node metastasis (HR = 2.954, $p = 0.003$), vascular invasion (HR = 2.109, $p = 0.041$), perineural inva-

**Table 2. Association of HIF-1α expression with clinicopathological features and perineural invasion.**

| Variables | High (n = 67) | Low (n = 53) | Statistic | *p* |
|---|---|---|---|---|
| Age, Mean ± SD | 63.090 ± 8.043 | 61.679 ± 7.743 | $t = 0.970$ | 0.334 |
| BMI, Mean ± SD | 25.767 ± 4.645 | 25.862 ± 4.275 | $t = -0.115$ | 0.908 |
| Gender, n (%) | | | $\chi^2 = 0.698$ | 0.404 |
| Female | 38 (56.716) | 26 (49.057) | | |
| Male | 29 (43.284) | 27 (50.943) | | |
| Blood Type, n (%) | | | $\chi^2 = 0.507$ | 0.476 |
| Non-O | 40 (59.701) | 35 (66.038) | | |
| O | 27 (40.299) | 18 (33.962) | | |
| Smoking history, n (%) | | | $\chi^2 = 0.025$ | 0.873 |
| No | 51 (76.119) | 41 (77.358) | | |
| Yes | 16 (23.881) | 12 (22.642) | | |
| Alcohol use, n (%) | | | $\chi^2 = 0.039$ | 0.844 |
| No | 56 (83.582) | 45 (84.906) | | |
| Yes | 11 (16.418) | 8 (15.094) | | |
| Diabetes, n (%) | | | $\chi^2 = 0.018$ | 0.893 |
| No | 55 (82.090) | 43 (81.132) | | |
| Yes | 12 (17.910) | 10 (18.868) | | |
| Tumor Size, n (%) | | | $\chi^2 = 7.574$ | 0.006 |
| ≤3 | 20 (29.851) | 29 (54.717) | | |
| >3 | 47 (70.149) | 24 (45.283) | | |
| Tumor location, n (%) | | | $\chi^2 = 0.357$ | 0.550 |
| Body_tail | 23 (34.328) | 21 (39.623) | | |
| Head_neck | 44 (65.672) | 32 (60.377) | | |
| Differentiation, n (%) | | | $\chi^2 = 18.159$ | <0.001 |
| Moderate_well | 35 (52.239) | 47 (88.679) | | |
| Poor | 32 (47.761) | 6 (11.321) | | |
| CA19-9, n (%) | | | $\chi^2 = 13.265$ | <0.001 |
| ≤35 | 14 (20.896) | 28 (52.830) | | |
| >35 | 53 (79.104) | 25 (47.170) | | |
| CEA, n (%) | | | $\chi^2 = 2.141$ | 0.143 |
| ≤5 | 48 (71.642) | 44 (83.019) | | |
| >5 | 19 (28.358) | 9 (16.981) | | |
| TBIL, n (%) | | | $\chi^2 = 6.023$ | 0.014 |
| ≤17.1 | 36 (53.731) | 40 (75.472) | | |
| >17.1 | 31 (46.269) | 13 (24.528) | | |
| lymph node metastasis, n (%) | | | $\chi^2 = 2.453$ | 0.117 |
| No | 46 (68.657) | 29 (54.717) | | |
| Yes | 21 (31.343) | 24 (45.283) | | |
| Vascular invasion, n (%) | | | $\chi^2 = 9.726$ | 0.002 |
| No | 41 (61.194) | 46 (86.792) | | |
| Yes | 26 (38.806) | 7 (13.208) | | |
| Perineural invasion, n (%) | | | $\chi^2 = 17.355$ | <0.001 |
| No | 25 (37.313) | 40 (75.472) | | |
| Yes | 42 (62.687) | 13 (24.528) | | |

sion (HR = 5.274, $p < 0.001$), low HIF-1α expression (HR = 0.231, $p < 0.001$), and low IL-6 expression (HR = 0.175, $p < 0.001$) were associated with overall survival (all $p < 0.05$). Sex, age, BMI, smoking history, alcohol consumption, diabetes, tumor location, CEA, and TBIL showed no significant association with overall survival (all $p > 0.05$) (Table 4). Given the limited number of death events (34 cases) in this cohort, to ensure the stability and reliability of the statistical model, multivariate Cox regression analysis was not performed.

## Discussion

In this study, we explored the clinicopathological and prognostic significance of HIF-1α and IL-6 expression in 120 patients with surgically resected pancreatic cancer. Our

**Table 3. Association of IL-6 expression with clinicopathological features and perineural invasion.**

| Variables | High (n = 72) | Low (n = 48) | Statistic | *p* |
|---|---|---|---|---|
| Age, Mean ± SD | 62.139 ± 7.988 | 62.958 ± 7.850 | $t = -0.554$ | 0.581 |
| BMI, Mean ± SD | 25.575 ± 4.352 | 26.160 ± 4.658 | $t = -0.701$ | 0.484 |
| Gender, n (%) | | | $\chi^2 = 0.357$ | 0.550 |
| Female | 40 (55.556) | 24 (50.000) | | |
| Male | 32 (44.444) | 24 (50.000) | | |
| Blood type, n (%) | | | $\chi^2 = 0.148$ | 0.700 |
| Non-O | 46 (63.889) | 29 (60.417) | | |
| O | 26 (36.111) | 19 (39.583) | | |
| Smoking history, n (%) | | | $\chi^2 = 2.803$ | 0.094 |
| No | 59 (81.944) | 33 (68.750) | | |
| Yes | 13 (18.056) | 15 (31.250) | | |
| Alcohol use, n (%) | | | $\chi^2 = 0.094$ | 0.759 |
| No | 60 (83.333) | 41 (85.417) | | |
| Yes | 12 (16.667) | 7 (14.583) | | |
| Diabetes, n (%) | | | $\chi^2 = 0.334$ | 0.563 |
| No | 60 (83.333) | 38 (79.167) | | |
| Yes | 12 (16.667) | 10 (20.833) | | |
| Tumor size, n (%) | | | $\chi^2 = 15.545$ | <0.001 |
| ≤3 | 19 (26.389) | 30 (62.500) | | |
| >3 | 53 (73.611) | 18 (37.500) | | |
| Tumor location, n (%) | | | $\chi^2 = 0.383$ | 0.536 |
| Body_tail | 28 (38.889) | 16 (33.333) | | |
| Head_neck | 44 (61.111) | 32 (66.667) | | |
| Differentiation, n (%) | | | $\chi^2 = 16.694$ | <0.001 |
| Moderate_well | 39 (54.167) | 43 (89.583) | | |
| Poor | 33 (45.833) | 5 (10.417) | | |
| CA19-9, n (%) | | | $\chi^2 = 22.717$ | <0.001 |
| ≤35 | 13 (18.056) | 29 (60.417) | | |
| >35 | 59 (81.944) | 19 (39.583) | | |
| CEA, n (%) | | | $\chi^2 = 7.461$ | 0.006 |
| ≤5 | 49 (68.056) | 43 (89.583) | | |
| >5 | 23 (31.944) | 5 (10.417) | | |
| TBIL, n (%) | | | $\chi^2 = 0.861$ | 0.353 |
| ≤17.1 | 48 (66.667) | 28 (58.333) | | |
| >17.1 | 24 (33.333) | 20 (41.667) | | |
| lymph node metastasis, n (%) | | | $\chi^2 = 7.259$ | 0.007 |
| No | 38 (52.778) | 37 (77.083) | | |
| Yes | 34 (47.222) | 11 (22.917) | | |
| Vascular invasion, n (%) | | | $\chi^2 = 0.111$ | 0.738 |
| No | 53 (73.611) | 34 (70.833) | | |
| Yes | 19 (26.389) | 14 (29.167) | | |
| Perineural invasion, n (%) | | | $\chi^2 = 20.140$ | <0.001 |
| No | 27 (37.500) | 38 (79.167) | | |
| Yes | 45 (62.500) | 10 (20.833) | | |

findings demonstrated that both markers were closely associated with aggressive tumor features, particularly perineural invasion. Notably, univariate Cox regression analysis suggested that low expression of either marker was associated with more favorable outcomes. These results highlight the potential role of HIF-1α and IL-6 as prognostic markers in pancreatic cancer.

This study found that high HIF-1α expression was associated with multiple aggressive pathological features, consistent with previous studies suggesting that targeting the hypoxic tumor microenvironment is an important therapeutic strategy for pancreatic cancer, with HIF-1α, as a key regulator of this microenvironment, playing a crucial role in tumor progression [20]. Similarly, other studies have identified HIF-1α as a potential prognostic marker in vari-

**Table 4. Cox regression analysis results.**

| Variables | β | SE | Z | *p* | HR (95% CI) |
|---|---|---|---|---|---|
| Gender | | | | | |
| Female | | | | | 1.000 (Reference) |
| Male | 0.152 | 0.351 | 0.433 | 0.665 | 1.164 (0.585–2.316) |
| Blood type | | | | | |
| Non-O | | | | | 1.000 (Reference) |
| O | 0.440 | 0.346 | 1.274 | 0.203 | 1.553 (0.789–3.058) |
| Smoking history | | | | | |
| No | | | | | 1.000 (Reference) |
| Yes | –0.304 | 0.426 | –0.715 | 0.475 | 0.738 (0.320–1.699) |
| Alcohol use | | | | | |
| No | | | | | 1.000 (Reference) |
| Yes | 0.394 | 0.534 | 0.737 | 0.461 | 1.483 (0.520–4.226) |
| Diabetes | | | | | |
| No | | | | | 1.000 (Reference) |
| Yes | –0.319 | 0.485 | –0.658 | 0.511 | 0.727 (0.281–1.880) |
| Tumor size | | | | | |
| ≤3 | | | | | 1.000 (Reference) |
| >3 | 0.780 | 0.365 | 2.137 | 0.033 | 2.182 (1.067–4.465) |
| Tumor location | | | | | |
| Body_tail | | | | | 1.000 (Reference) |
| Head_neck | 0.100 | 0.360 | 0.276 | 0.782 | 1.105 (0.545–2.239) |
| Differentiation | | | | | |
| Moderate_well | | | | | 1.000 (Reference) |
| Poor | 1.528 | 0.366 | 4.180 | <0.001 | 4.609 (2.251–9.436) |
| CA19-9 | | | | | |
| ≤35 | | | | | 1.000 (Reference) |
| >35 | 0.812 | 0.373 | 2.173 | 0.030 | 2.252 (1.083–4.682) |
| CEA | | | | | |
| ≤5 | | | | | 1.000 (Reference) |
| >5 | 0.644 | 0.390 | 1.652 | 0.099 | 1.905 (0.887–4.091) |
| TBIL | | | | | |
| ≤17.1 | | | | | 1.000 (Reference) |
| >17.1 | –0.277 | 0.377 | –0.736 | 0.462 | 0.758 (0.362–1.585) |
| lymph node metastasis | | | | | |
| No | | | | | 1.000 (Reference) |
| Yes | 1.083 | 0.361 | 3.000 | 0.003 | 2.954 (1.456–5.995) |
| Vascular invasion | | | | | |
| No | | | | | 1.000 (Reference) |
| Yes | 0.746 | 0.365 | 2.043 | 0.041 | 2.109 (1.031–4.316) |
| Perineural invasion | | | | | |
| No | | | | | 1.000 (Reference) |
| Yes | 1.663 | 0.381 | 4.360 | <0.001 | 5.274 (2.497–11.137) |
| HIF-1α | | | | | |
| High | | | | | 1.000 (Reference) |
| Low | –1.465 | 0.385 | –3.806 | <0.001 | 0.231 (0.109–0.491) |
| IL-6 | | | | | |
| High | | | | | 1.000 (Reference) |
| Low | –1.743 | 0.429 | –4.065 | <0.001 | 0.175 (0.076–0.406) |
| Age | 0.010 | 0.022 | 0.478 | 0.633 | 1.011 (0.968–1.055) |
| BMI | 0.002 | 0.039 | 0.046 | 0.964 | 1.002 (0.927–1.082) |

SE, standard error.

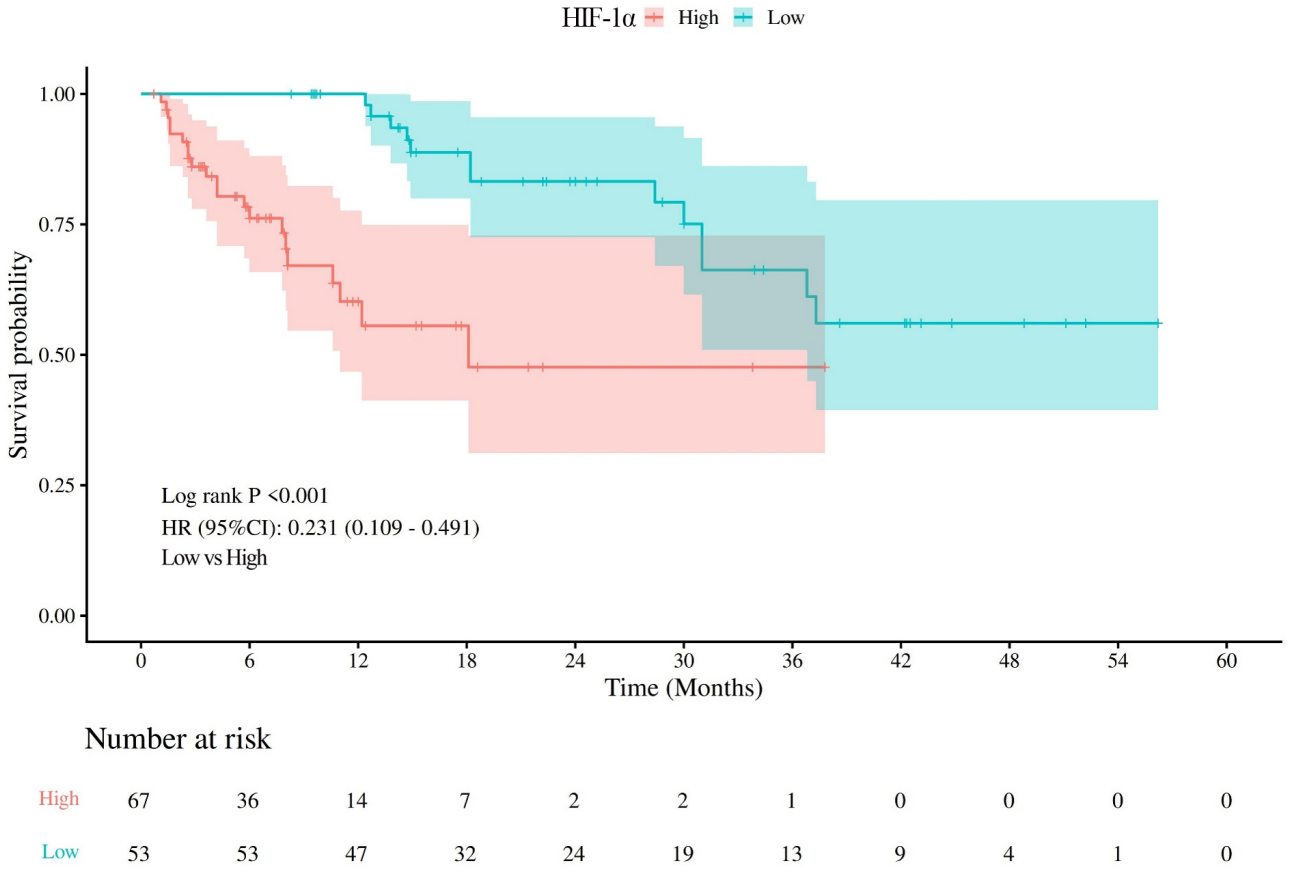



**Fig. 1. Kaplan-Meier survival curves stratified by HIF-1α expression (Low vs High)**. HIF-1α, hypoxia-inducible factor-1α.

ous solid tumors [21,22,23]. This study further linked HIF-1α to perineural invasion, a distinctive invasion pathway in pancreatic cancer. The positivity rate of perineural invasion was higher in the HIF-1α high-expression group than in the low-expression group, suggesting that HIF-1α may promote directional migration of tumor cells along nerves by inducing the expression of nerve growth factor and its receptors. Nevertheless, these associations were derived from univariate analyses and require further validation in multivariate-adjusted models. As a core pro-inflammatory cytokine, IL-6 plays multiple roles in the malignant progression of pancreatic cancer. This study found a prominent association between high IL-6 expression and perineural invasion, consistent with the findings of Suzuki et al. [17], which confirmed that the IL-6/glycoprotein 130 (gp130) axis promotes perineural invasion in pancreatic cancer. Studies suggest that IL-6 not only promotes tumor proliferation and inhibits apoptosis by activating the signal transducer and activator of transcription 3 (STAT3) signaling pathway [24], but also remodels the tumor microenvironment, creating an inflammatory milieu conducive to perineural invasion and metastasis [25]. Van Duijneveldt et al. [12] also highlighted the critical regulatory role of IL-6 family cytokines in pancreatic cancer. This study provides indirect clinical pathological evidence supporting these mechanisms; however, our survival analyses were unadjusted and require multivariate validation.

Perineural invasion is an important prognostic factor in pancreatic cancer. A recent study by Li et al. [14] revealed a novel mechanism by which cancer-associated fibroblasts drive perineural invasion in pancreatic cancer through a high-lactate microenvironment. The present study found that perineural invasion was associated with both HIF-1α and IL-6. This finding offers a new perspective on the molecular mechanisms of perineural invasion in pancreatic cancer.

The clinical significance of this study lies in the potential utility of HIF-1α and IL-6 as prognostic markers for pancreatic cancer, aiding in more refined prognostic risk stratification of patients. Currently, postoperative management of pancreatic cancer patients in clinical practice primarily relies on traditional pathological indicators such as lymph node status and surgical margin status. This study found that beyond traditional pathological features, the expression levels of HIF-1α and IL-6 were associated with tumor aggressiveness and patient survival, suggesting they could serve as effective complements to traditional pathological staging. For patients with high HIF-1α or IL-6 expression, even those with relatively early-stage disease may represent a high-risk group. Postoperative management

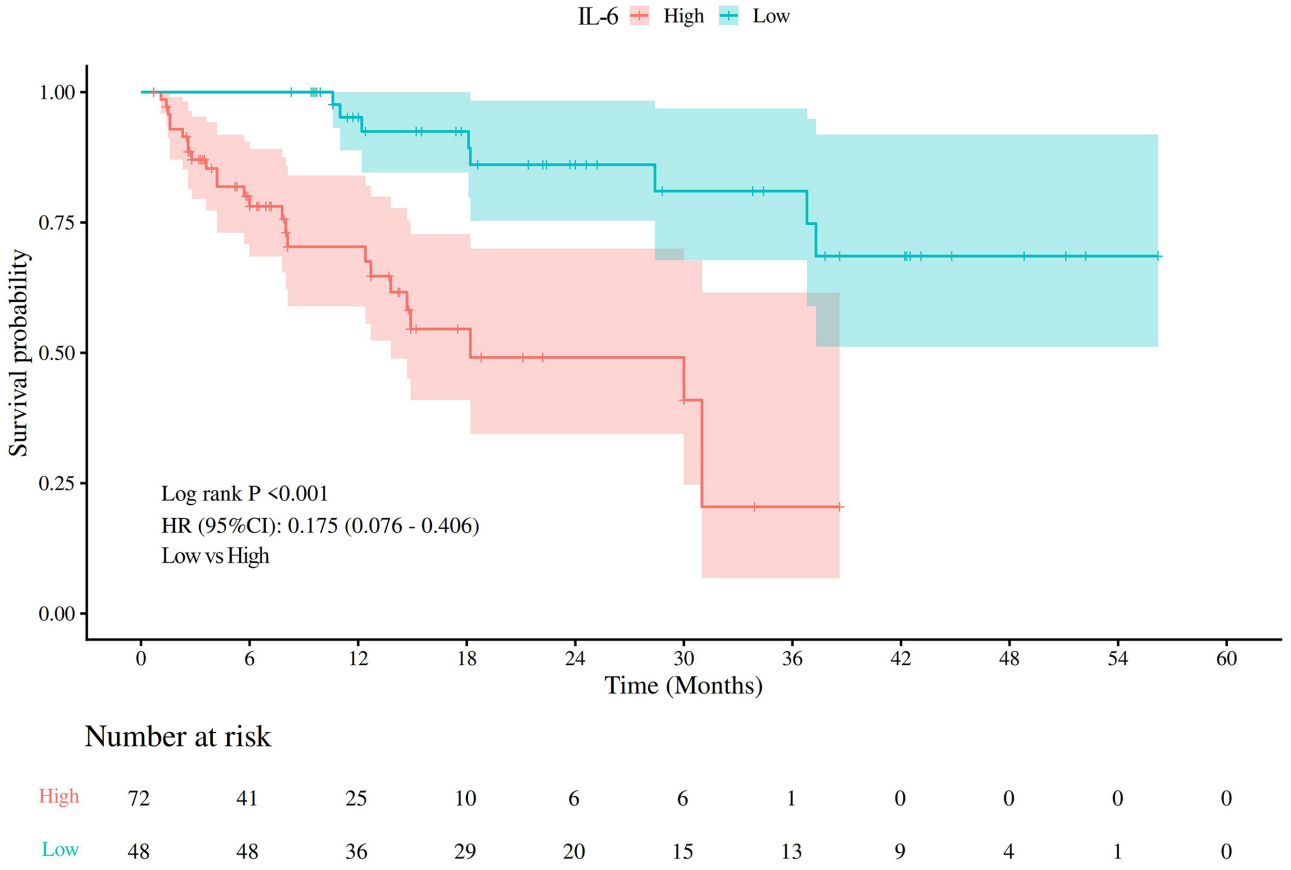



**Fig. 2. Kaplan-Meier survival curves for pancreatic cancer patients stratified by IL-6 expression (Low vs High)**. IL-6, interleukin-6.

should involve closer follow-up, more proactive evaluation of the necessity for adjuvant therapy, and earlier identification of potential signs of recurrence or metastasis.

This study has several limitations: First, it is a single-center retrospective design with a limited sample size, which may introduce selection bias. Second, the interpretation of immunohistochemistry results is inherently subjective; although two pathologists performed independent evaluations, this may still affect the reproducibility of the results. Third, information on postoperative adjuvant chemotherapy and radiotherapy was not included, which may have a confounding effect on survival analysis. Fourth, the assessment of perineural invasion relied on routine pathology reports without systematic evaluation of multiple tissue sections, potentially underestimating its true incidence. Fifth, due to the limited number of death events (34 cases), multivariate Cox regression analysis was not performed to avoid overfitting; consequently, the observed associations may be confounded by aggressive tumor features such as lymph node metastasis, vascular invasion, and perineural invasion. Therefore, our findings should be interpreted as prognostic associations rather than independent prognostic values, and whether HIF-1α and IL-6 merely reflect tumor aggressiveness or independently modulate prognosis remains to be determined. Future multicenter, prospective studies incorporating molecular subtyping and treatment information are needed for further validation. Sixth, although we specifically scored IL-6 expression only in morphologically identifiable tumor cells, we acknowledge that IL-6 can also be produced by tumor-associated immune cells (e.g., tumor-associated macrophages), cancer-associated fibroblasts, and other stromal components within the tumor microenvironment. These non-tumor sources of IL-6 may contribute to the local inflammatory milieu and potentially influence perineural invasion and tumor progression, independent of tumor-cell-derived IL-6. Given the retrospective nature of this study and the use of routine immunohistochemistry, we were unable to perform double immunofluorescence staining or spatial profiling to precisely determine the cellular origins of IL-6. Future studies using multiplex immunohistochemistry or single-cell-based approaches are warranted to delineate the distinct roles of tumor-cell-derived versus stroma-derived IL-6 in pancreatic cancer progression and perineural invasion.

## Conclusion

HIF-1α and IL-6 are potential prognostic markers that may provide adjunctive information for postoperative risk stratification in pancreatic cancer patients, but their clinical utility requires further validation in larger, prospective, multicenter studies with multivariate adjustment.

## Availability of Data and Materials

The datasets generated or analysed during the current study are available from the corresponding authors on reasonable request.

## Author Contributions

Conception and design: TFJ, YZ, LQC; Administrative support: TFJ; Provision of study materials or patients: TFJ, ZMZ; Collection and assembly of data: TFJ, ZMZ, YZ; Data analysis and interpretation: TFJ, LQC. YZ, LQC, and ZMZ drafted the manuscript, and TFJ critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity.

## Ethics Approval and Consent to Participate

This study was approved by the Medical Ethics Committee of Huadu District People's Hospital of Guangzhou (Approval No.: 2026059Y). Due to the retrospective nature of the study, informed consent was waived. The study process adhered to the ethical principles of the Declaration of Helsinki.

## Acknowledgment

Not applicable.

## Funding

This project was supported by the Huadu District Basic and Applied Basic Research Joint Funding Project (District-Hospital Collaboration), Guangzhou (No.24HDQYLH010), the Open Funds of Guangxi Key Laboratory of Tumor Immunology and Microenvironmental Regulation (No.2026KF012) and the Key Medical Disciplines and Specialities Program of Guangzhou [2025-2027] (no fund number assigned).

## Conflict of Interest

The authors declare no conflict of interest.

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