Cancer Management and Research

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ORIGINAL RESEARCH

Human Equilibrative Nucleoside Transporter 1: Novel Biomarker and Prognostic Indicator for Patients with Gemcitabine-Treated Pancreatic Cancer

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Aim: This article aimed to find appropriate pancreatic cancer (PC) patients to treat with Gemcitabine with better survival outcomes by detecting hENT1 levels.

Methods: We collected surgical pathological tissues from PC patients who received radical surgery in our hospital from September 2004 to December 2014. A total of 375 PC tissues and paired adjacent nontumor tissues were employed for the construction of 4 tissue microarrays (TMAs). The quality of the 4 TMAs was examined by HE staining. We performed immunohistochemistry analysis to evaluate hENT1 expression in the TMAs. Moreover, we detected hENT1 expression level and proved the role of hENT1 in cell proliferation, drug resistance, migration and invasion in vivo and vitro.

Results: The results indicated that low hENT1 expression indicated a significantly poor outcome in PC patients, including shortened DFS (21.6±2.8 months versus 36.9±4.0 months, p<0.001) and OS (33.6±3.9 versus 39.6±3.9, p=0.004). Meanwhile, patients in stage I/II of TNM stage had a longer OS (40.2±3.4 versus 15.4±1.7, p=0.002) and DFS (31.0±3.1 versus 12.4±1.9, p=0.016) than patients in stage III/IV. Patients in M0 stage had a longer OS (39.7±3.4 versus 16.2±1.9, p=0.026) and DFS(30.7±3.0 versus 11.8±2.2, p=0.031) than patients in M1 stage, and patients with tumors not invading the capsule had a better DFS than those with tumor invasion into the capsule (30.8±3.0 versus 12.6±2.3, p=0.053). Patients with preoperative CA19-9 values ≤467 U/mL have longer DFS than that of patients who had preoperative CA19-9 values >467 U/mL (37.9±4.1 versus 22.9±4.0, p=0.04). In the subgroup analysis, a high hENT1 expression level was related to a longer OS(39.4±4.0 versus 31.5±3.9, p=0.001) and DFS(35.7±4.0 versus 20.6±2.7; p<0.0001) in the Gemcitabine subgroup.

Conclusion: PC patients with high hENT1 expression have a better survival outcomes when receiving Gemcitabine. hENT1 expression can be a great prognostic indicator for PC patients to receive Gemcitabine treatment.

Keywords: pancreatic cancer, human equilibrative nucleoside transporter 1, hENT1, gemcitabine chemoresistance, prognostic indicator, survival improvement

Introduction

Pancreatic cancer (PC) is a highly malignant disease with a 5-year survival rate of only 13%.[1] PC is a devastating malignancy with significant challenges and poor prognosis. Recent research has uncovered many new insights that are crucial for understanding the pathogenesis, clinical manifestations, and treatment modalities of this disease.[2] Recent research has explored diagnostic and therapeutic approaches for pancreatic cancer,[3,4] In terms of treatment, novel therapies such as FOLFIRINOX or targeted therapy and immunotherapy are gradually altering the survival prospects

Received: 28 March 2024
Accepted: 23 May 2024
Published: 21 June 2024

of PC patients. Additionally, the advent of personalized treatment strategies, targeting specific mutations or phenotypes of the tumor, provides patients with more precise and effective therapeutic options. However, PC remains an immensely challenging disease, with treatment still facing numerous difficulties and limitations. The development of drug resistance, the complexity of the tumor microenvironment, and the challenges of early diagnosis remain key issues awaiting resolution in current research and clinical practice. Therefore, future research efforts should continue to delve into the biological characteristics of PC and develop innovative treatment strategies to improve patient survival rates and quality of life.[5]

Radical surgical resection is the most effective method for PC treatment, and patients who have no contraindications were suggested to receive complementary chemotherapy after surgery.[6] Gemcitabine is the first-line adjuvant chemotherapy drug for postoperative PC patients. However, the therapeutic effect differs among different patients for the existence of primary and acquired chemo-resistance.[7] Thus, finding a novel effective biomarker and prognostic indicator to identify patients who are sensitive to Gemcitabine and to predict the outcome of patients is imperative.

Human equilibrative nucleoside transporter 1 (hENT1), which was reported to facilitate cross-membrane transport of nucleosides and nucleoside-derived drugs, plays an important role in cancer chemotherapy.[8] The relationship between hENT1 expression and PC survival is controversial. Most notably, elevated hENT1 expression is regarded as a diagnostic and therapeutic biomarker for PC patients treated with Gemcitabine,[9–11] High hENT1 expression was also associated with better survival in patients with other types of cancer who received Gemcitabine treatment, such as cholangiocarcinoma,[12,13] leiomyosarcoma and angiosarcoma.[14] However, some studies reached the opposite conclusion that hENT1 expression had no relationship with PC patient outcome,[15,16] Thus, the prognostic value of hENT1 in PC patients needs to be further verified by larger sample size studies. This article aimed to find appropriate PC patients to treat with Gemcitabine with better survival outcomes by detecting hENT1 levels.

Methods

Tissue Sample Collection

We collected the surgical pathological tissue of pancreatic cancer patients who received radical surgery in Peking Union Medical College Hospital from September 2004 to December 2014 continuously. The eligible criteria for patients included 1) R0 surgical resection. 2) A postoperative pathology diagnosis of pancreatic ductal adenocarcinoma. 3) No Gemcitabine-based neoadjuvant chemotherapy and/or radiotherapy before surgery. 4) Surgical specimens suitable for immunohistochemistry. In addition, those included patients started adjuvant chemotherapy within 8 weeks after surgery with regular follow-up monitoring by CA-199/CT/Ultrasonography, and their clinical and pathological data were complete. We excluded patients who had severe basic diseases or serious complications during the perioperative period that could affect the survival analysis results and those who had poor compliance and could not be followed regularly. Patients were excluded who died in the first 30 days postoperatively. We finally collected 375 samples for further analysis. This study was approved by the Ethics Committee of Peking Union Medical College Hospital.

TMA Construction and Immunohistochemistry Analysis

A total of 375 PC tissues and paired adjacent nontumor tissues were employed for the construction of 4 tissue microarrays (TMAs) using routine methods, namely, TMA1, TMA2, TMA3, and TMA4. The quality of 4 TMAs was reexamined by HE staining and in accordance with the design requirements. We performed immunohistochemistry analysis to evaluate hENT1 expression and excluded 16 samples because the tissue sections had detached from the slides. The hENT1 antibody was used to measure hENT1 expression in TMAs by IHC staining according to standard protocols. TMAs were first blocked with hydrogen peroxide and then incubated with an anti-hENT1 antibody (1:200, anti-SLC29A1 polyclonal antibody produced in rabbits from Sigma–Aldrich Company, USA). Subsequently, diaminobenzidine (DAB) was used for coloration, and hematoxylin was used for counterstaining. All immunohistochemistry results were determined by two independent pathologists on a double-blind basis. The staining intensities were graded as 0 (negative), 1 (low), 2 (medium), or 3 (high), while the staining extent was scored from 0 to 100%. The formula intensity score × percentage score × 100 was used to calculate the final IHC staining score, namely, the composite expression score

(CES), which ranged from 0 to 300. The average CES value of remaining 359 tumor tissues was 80.5 which was defined as the optimal cutoff value. A CES value >80.5 represents high hENT1 expression, while CES≤80.5 indicates low hENT1 expression. Immunohistochemical staining intensity is commonly scored on a scale ranging from 0 to 3. A score of 0 typically indicates the absence of staining or no detectable expression of the target antigen. A score of 1 indicates weak staining intensity, where the staining is faint and barely perceptible. A score of 2 suggests moderate staining intensity, characterized by staining that is more pronounced but not overwhelmingly strong. Finally, a score of 3 represents strong staining intensity, with intense and easily discernible staining of the target antigen. These intensity scores provide a qualitative assessment of the level of antigen expression within tissue samples, aiding in the interpretation of immunohistochemical results.

CCK8 Detects the Proliferation of Pancreatic Cancer Cells

The proliferation of pancreatic cancer cells was evaluated using the Cell Counting Kit-8 (CCK-8) assay kit reagent (Dojindo, Japan) according to the manufacturer's guidelines. Cells were seeded in a 96-well plate at a density of 1500 cells per well and incubated for 48 hours. Afterward, 10 μL of CCK-8 solution (5 mg/mL) was added to each well and incubated for an additional 2 hours. PC cell lines were planted in 96-well plates (1000 cells/well). CCK8 (10 μL/well) was added at 0, 24, 48, 72, and 96 hours, respectively, and incubated for 2.5 hours. T3M4-hENT1 overexpression, T3M4-control line cells; Panc-1-hENT1knockdown, Panc-1-control line cells were digested and re-planted in a 96-well plate (4000 cells/well), and the gradient was added after the cells adhered Concentration of Gemcitabine, after 48 hours of continuous intervention, the OD450 value was detected by CCK8 method, and the growth rate was calculated. We used a microplate reader to measure the absorbance at 450 nm (OD450), 630 nm as the reference wavelength (OD630), and the final absorbance (OD) = OD450-OD630. The cell growth curve was drawn with the time as the abscissa and the absorbance value as the ordinate.

In vivo Animal Experiments

After routine skin alcohol disinfection, 8×10^6 cells/200ul/mouse were subcutaneously inoculated into nude mice through the skin of the axilla. The long and short diameters of the tumors were measured regularly (twice a week), and the tumor volume was calculated [V(volume)=π/6(W1xW2xW2), W1 represents the largest tumor diameter, and W2 represents the smallest tumor diameter. After tumor formation (tumor volume was about 100 mm3), intraperitoneal Gemcitabine treatment was started, and the drug dose was intraperitoneal injection of 20 mg/kg, twice a week, and the mice were sacrificed on the 28th day. Each group consisted of a total of 4 nude mice. The T3M4 cell line stably over-expressing hENT1 was marked as the hENT1 OE group, and the control group was marked as the hENT1 OENC group; the Panc-1 cell line with stable low expression of hENT1 was marked as the hENT1 KD group, and the control group was marked as the hENT1 KDNC group.

Statistical Analyses

Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA), and the diagrams were sketched using Prism software (GraphPad, La Jolla, CA, USA). The Wilcoxon rank-sum test was used to compare the CES scores between normal and tumor tissues. Pearson's chi-square test was performed to evaluate the associations between hENT1 expression levels and clinicopathological features of patients. The clinical endpoints of patients were calculated using Kaplan–Meier analysis, and differences were compared using the Log rank test. Cox regression (proportional hazards model) was applied to determine the prognostic values of multivariate factors on patients' overall survival (OS) and disease-free survival (DFS). Hazard ratios (HRs) obtained by Cox regression analyses are reported as relative risks with corresponding 95% confidence intervals (CIs). Finally, a p value of < 0.05 was considered significant. The inclusion of various prognostic factors in our analysis, albeit unrelated to hENT1, serves the purpose of conducting a comprehensive multivariable analysis. By accounting for all relevant survival factors, we aim to minimize the confounding effects of other variables and provide a more robust assessment of the independent prognostic significance of hENT1. This approach allows us to elucidate whether hENT1 expression status has a unique and independent impact on patient outcomes, beyond the influence of other known prognostic factors.

Results

hENT1 Expression is Downregulated in Pancreatic Cancer Tissues

A total of 375 PC tissues and paired adjacent nontumor tissues were employed while 16 samples were excluded because the tissue sections had detached from the slides. The IHC results of remaining 359 samples showed that the hENT1 protein was mainly localized in the membranes and/or the cytoplasm of cancer cells, as previously described (Figure 1a).[4] The average CES of hENT1 expression in tumor tissues was 80.5, and the average CES in non-tumor tissues was 89.5; thus, the hENT1 protein expression in tumor tissues was significantly lower than that in normal tissues (Figure 1b, 80.5±8.8 versus 89.5±8.9, p=0.005). Subsequently, we used the average CES value of 80.5 as the cutoff value to divide 359 patients into an hENT1 high-expression group (n=165) and a low-expression group (n=194).We then compared clinical pathological parameters between the high and low hENT1-expression groups. hENT1 expression was only related to the degree of tumor differentiation. Patients with high hENT1-expression tended to have highly differentiated tumors (Supplementary Table 1).

Low hENT1 Expression Correlates with a Poor Prognosis of Gemcitabine-Treated Patients

To explore the relationship between hENT1 expression and the prognosis of PC patients, we first analyzed the relationship between the hENT1 expression and disease-free survival (DFS) and overall survival (OS) in all patients using the Kaplan–Meier method. The results indicated that low hENT1 expression indicated a significantly poor outcome in PC patients, including shortened DFS (Figure 2a, 21.6±2.8 months versus 36.9±4.0 months, p<0.001) and OS (Figure 2b, 33.6±3.9 versus 39.6±3.9, p=0.004). Meanwhile, the results revealed that the level of CA19-9, the M stage, the TNM stage of patients and whether the tumor invaded into the pancreas capsule were also related to DFS. The DFS period of patients who had CA19-9 values ≤467 U/mL (the average value of all patients) was longer than that of patients who had preoperative CA19-9 values >467 U/mL (Figure 3f, 37.9±4.1 versus 22.9±4.0, p=0.04). Similarly, patients in stage I/II of TNM stage had a longer DFS than patients in stage III/IV (Figure 3a, 31.0±3.1 versus 12.4±1.9, p=0.016); for M stage, patients in M0 stage had a longer DFS than patients in

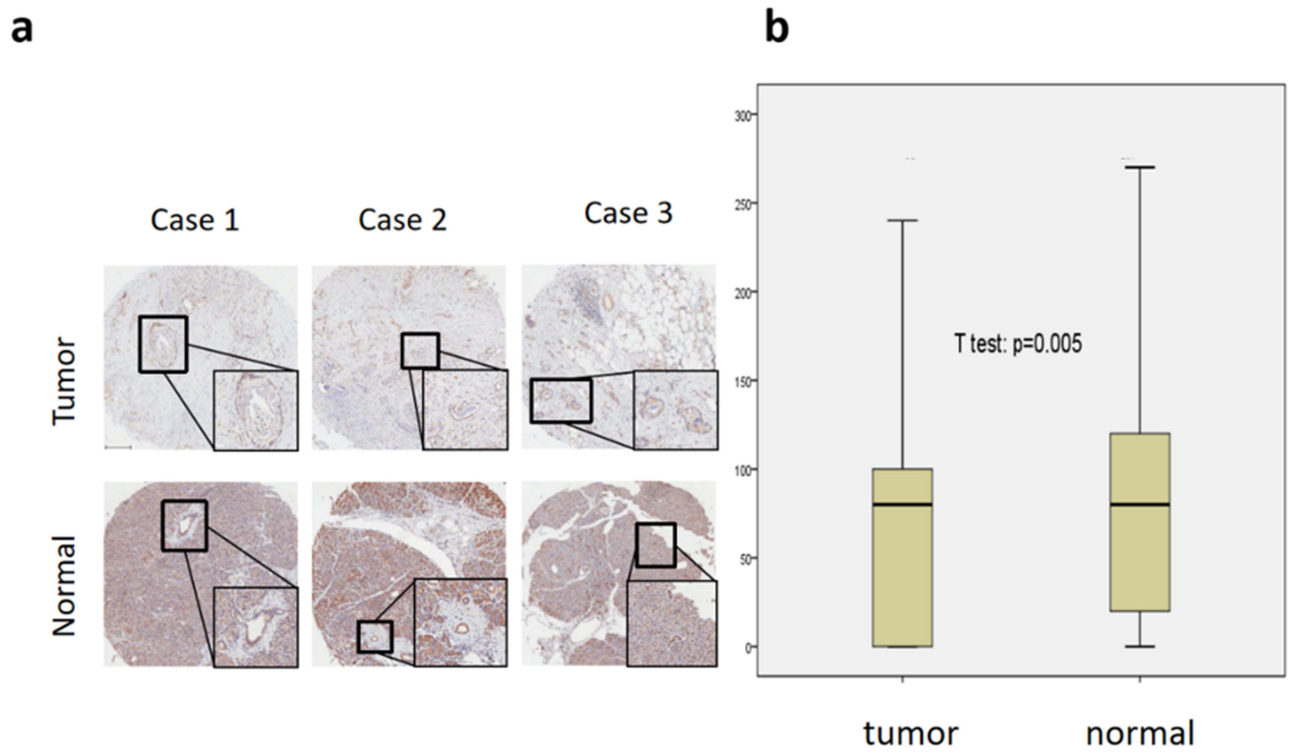

Figure 1 (**a**) hENT1 expression level in tumor and normal tissues. (**b**) hENT1 staining in tumor tissues was significantly lower than that in paired normal tissues (80.5±8.8, 95% CI 71.7–89.2 versus 89.5±8.9, 95% CI 80.6–98.4; p=0.005).

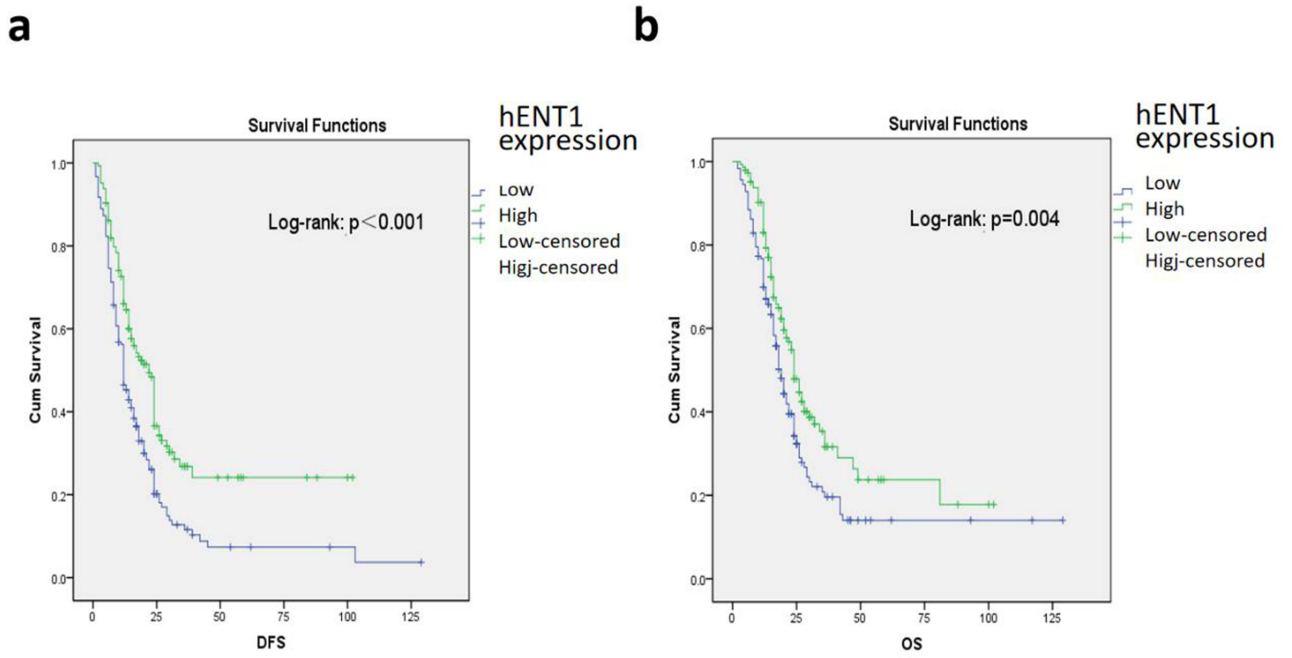

Figure 2 (**a**) In all patients, high hENT1 expression correlated with longer DFS (p<0.001). (**b**) In all patients, high hENT1 expression correlated with longer OS (p=0.004).

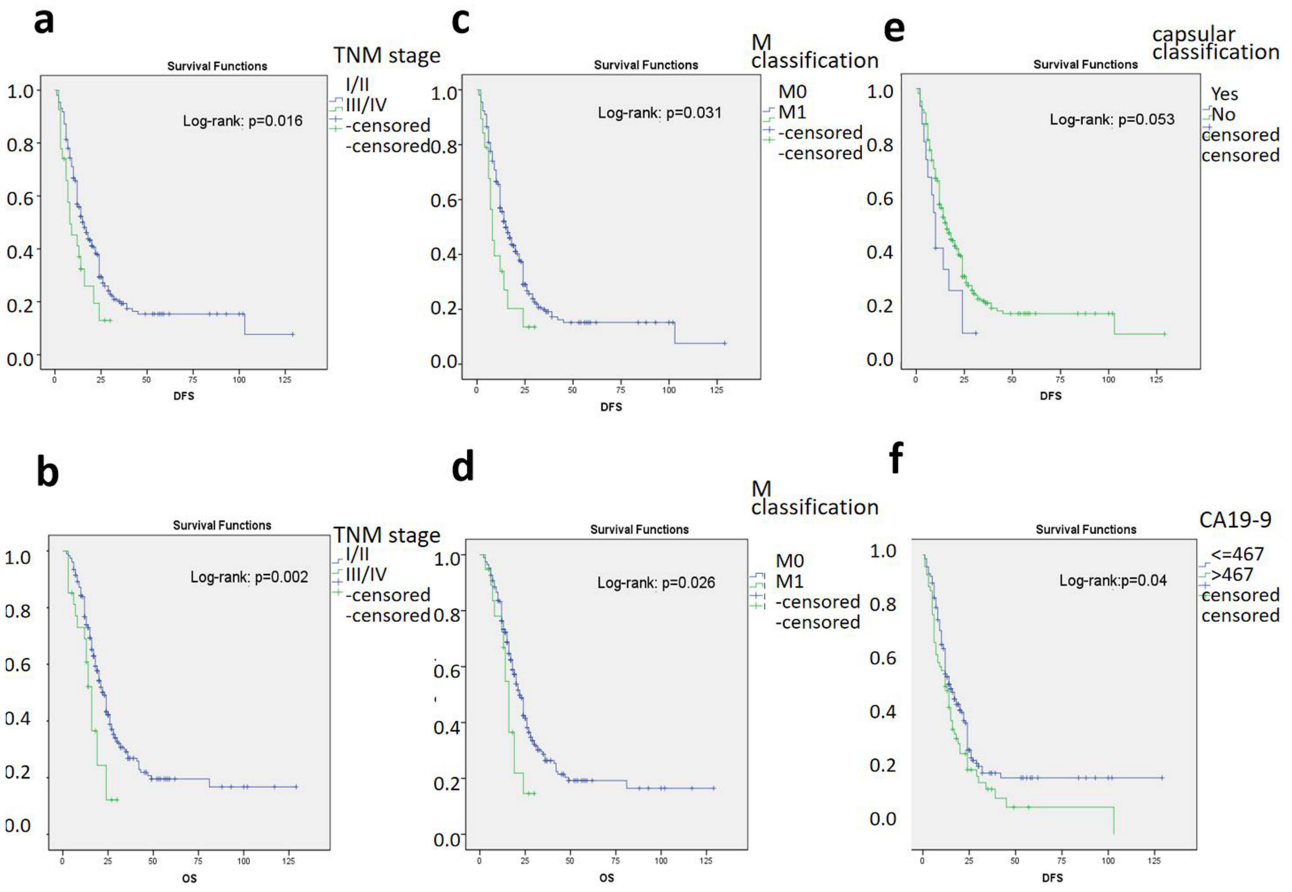

Figure 3 Several clinical parameters significantly related to patient prognosis. (**a** and **b**) Patients in stage I/II TNM tended to have longer disease-free survival (DFS) and overall survival (OS) than those in stage III/IV (p=0.016 and p=0.002). (**c** and **d**) Patients with an M0 classification tended to have longer DFS and OS than those with an M1 classification (p=0.031 and p=0.026). (**e**) Patients without capsular invasion tended to have longer DFS than those with capsular invasion (p=0.053). (**f**) Patients in the low CA19-9 group (<467 U/mL) tended to have longer DFS than those in the high CA19-9 group (≥467 U/mL) (p=0.04).

M1 stage (Figure 3c, 30.7±3.0 versus 11.8±2.2, p=0.031), and patients with tumors not invading the capsule had a better DFS than those with tumor invasion into the capsule (Figure 3e, 30.8±3.0 versus 12.6±2.3, p=0.053). Moreover, the M stage and the TNM stage of patients also had a significant correlation with overall survival (OS). M0 stage patients had a longer OS than did M1 stage patients (Figure 3d, 39.7±3.4 versus 16.2±1.9, p=0.026), and patients in stage I/II/of TNM stage had a better OS than those in stage III/IV (Figure 3b, 40.2±3.4 versus 15.4±1.7, p=0.002).

To further explore the connection between the hENT1 level and Gemcitabine treatment efficacy, we analyzed the relationship between hENT1 expression and DFS/OS in two cohorts (patients in one cohort received Gemcitabine treatment after surgery (OS: 299 patients; DFS:298 patients), while the other group did not receive chemotherapy (28 patients)). The findings suggested that a high hENT1 expression level was related to a longer DFS especially in the Gemcitabine subgroup(Figure 4a, 35.7±4.0 versus 20.6±2.7; p<0.0001). Similarly, patients with high hENT1 expression in the Gemcitabine-treated group showed longer OS than those with low expression (Figure 4b, 39.4±4.0 versus 31.5±3.9, p=0.001). In contrast, no significant difference in DFS or OS was found in the non Gemcitabine-treated group between hENT1 expression and patient prognosis (Figure 4c and d, p=0.413 and p=0.152).

A Low hENT1 Expression Level is an Independent Indicator for the Poor Prognosis of PC Patients

Through survival analysis, we found that M stage, TNM stage, presence or absence of tumor capsular invasion, preoperative CA19-9 value and hENT1 expression level were associated with the prognosis of PC patients. Therefore, we added these factors into multivariate analysis by Cox regression to further evaluate their prognostic value in PC patients. The results showed that a low

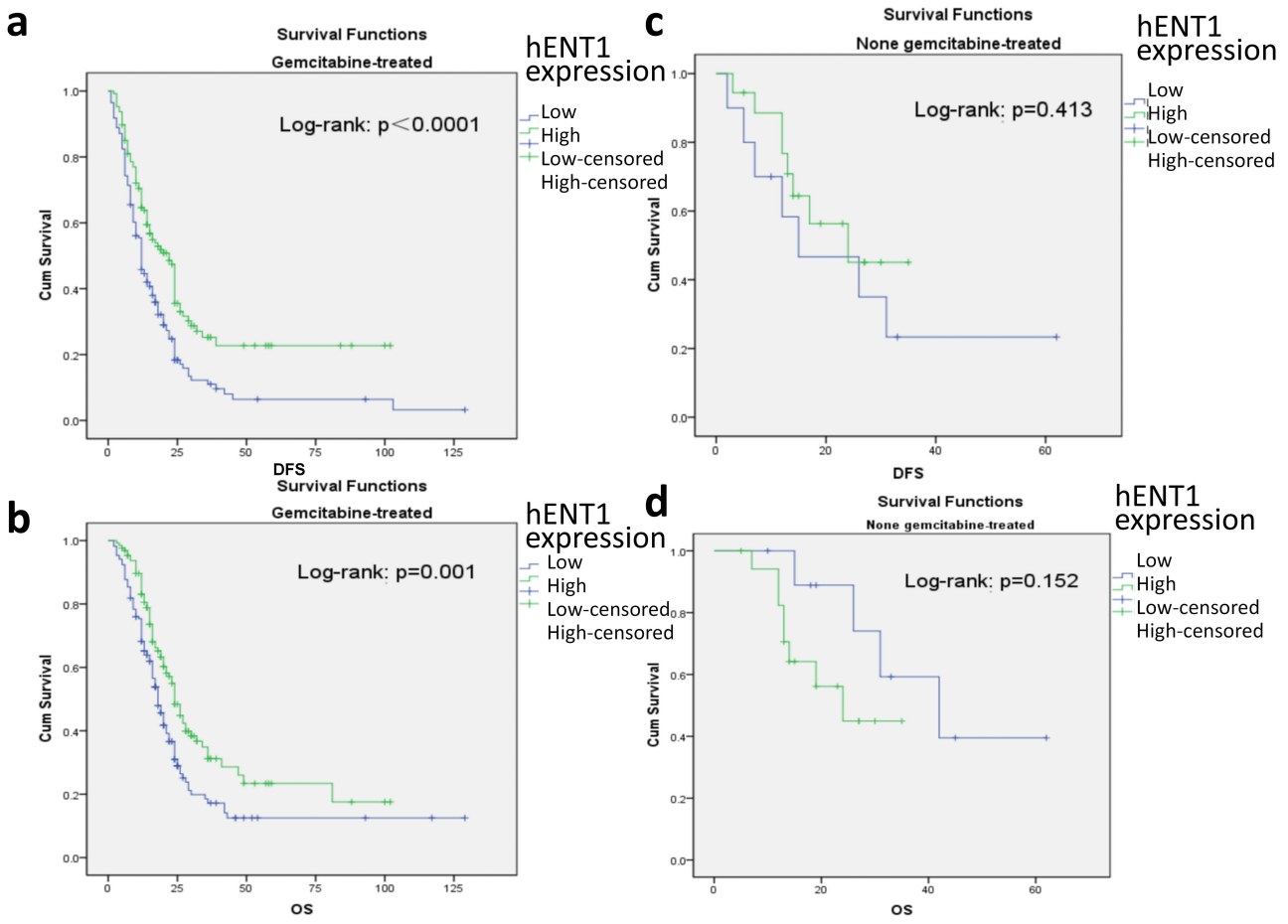

Figure 4 hENT1 expression level is related to patient prognosis in the Gemcitabine-treated group. (**a** and **b**) In the Gemcitabine-treated group, patients with high hENT1 expression tended to have obviously longer DFS and OS (p<0.0001 and p=0.001). (**c** and **d**) There was no significant correlation between hENT1 expression and prognosis in the untreated group (p=0.413 and p=0.152).

hENT1 expression level in tumor tissues was an independent risk factor for PC recurrence or metastasis because it was associated with shorter DFS in patients with pancreatic cancer (HR 0.53; 95% CI: 0.39–0.72; p<0.001). In addition, advanced TNM stage (HR 2.68; 95% CI: 1.09–6.58; P=0.031) and a low expression level of hENT1 (HR 0.60; 95% CI: 0.43–0.82; p=0.001) were independent risk factors for overall survival of PC (Supplementary Table 2). In addition, we also added these factors into multivariate analysis in the separate Gemcitabine-treated group and produced similar findings: advanced TNM stage predicted worse OS (HR 2.90; 95% CI: 1.06–7.92; p=0.038), and low hENT1 expression levels predicted worse OS (HR 0.60; 95% CI: 0.43–0.82; p=0.002) and DFS (HR 0.56; 95% CI: 0.41–0.77; p<0.001)(Supplementary Table 3). In conclusion, a low hENT1 expression level is an independent risk factor for patients' shortened DFS and OS.

hENT1 Increases Gemcitabine Chemosensitivity in vivo

The hENT1 KD group and hENT1 KDNC group did not develop tumors on the 28th day and 15th day, respectively, which precluded further experimentation. Consequently, four mice in both the hENT1 OE and hENT1 OENC groups successfully developed tumors and proceeded to the final experiment. Compared with the hENT1 OENC group, the hENT1 OE group could significantly increase the chemotherapy effect of Gemcitabine. ANOVA with repeated measures was statistically different (P<0.05)(Figure 5).

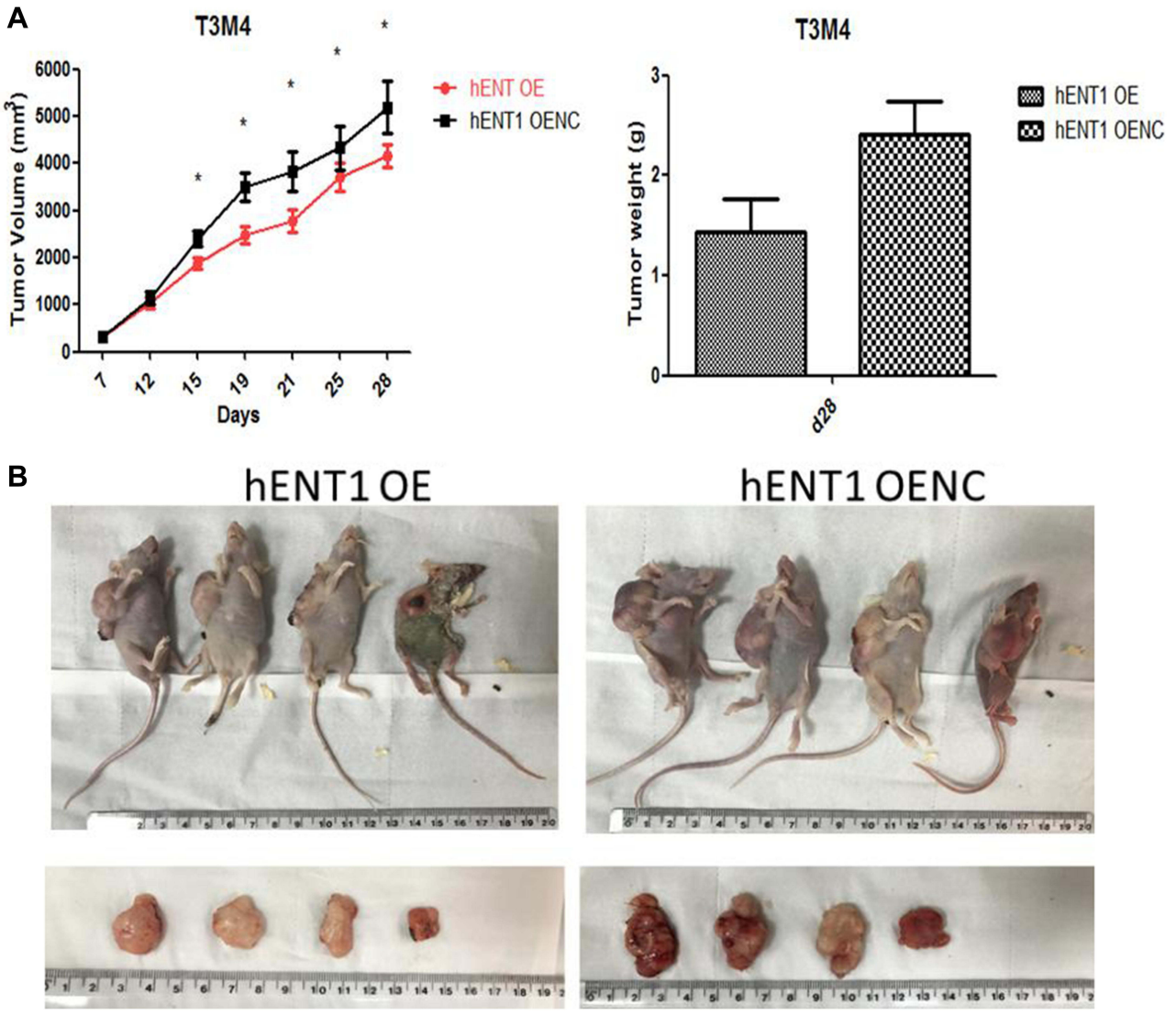

Figure 5 (**A**) In vivo experiments to explore the effect of hENT1 on gem sensitivity. The T3M4 cell line stably over-expressing hENT1 was marked as the hENT1 OE group, and the control group was marked as the hENT1 OENC group. (**B**) Compared with the hENT1 OENC group, the hENT1 OE group could significantly increase the chemotherapy effect of Gemcitabine. (*p<0.05).

Regulation of hENT1 on the Chemosensitivity of Pancreatic Cancer Cells to Gemcitabine

Compared with T3M4-control strain, T3M4-hENT1 over-expression significantly increased the chemosensitivity of Gemcitabine ($P<0.05$) (Supplementary Figure 1). Conversely, Panc-1-hENT1 knockdown significantly reduced the cytostatic rate of Gemcitabine compared with Panc-1-control strain ($P<0.05$)(Supplementary Figure 1).

Discussion

At present, PC is the vital cause of cancer-related death in China and the USA, respectively. The inherent and acquired resistance to Gemcitabine is stunting its therapeutic effect. PC exhibits a profound malignancy characterized by its aggressive nature, rapid progression, and high metastatic potential. One of the most striking features contributing to its malignancy is the often asymptomatic or nonspecific symptoms in its early stages, leading to delayed diagnosis and advanced disease at presentation. By the time symptoms manifest, the tumor has often invaded surrounding tissues and metastasized to distant organs, severely limiting treatment options and prognosis. Moreover, PC is notorious for its resistance to conventional therapies, including chemotherapy and radiation. The dense stromal environment surrounding pancreatic tumors acts as a physical barrier, hindering drug delivery and promoting therapeutic resistance. Additionally, the molecular heterogeneity of pancreatic cancer poses significant challenges in developing targeted therapies, as tumors may harbor multiple genetic alterations driving tumor growth and survival.

Furthermore, PC is associated with a dismal prognosis, with one of the lowest five-year survival rates among all cancer types. Even with aggressive treatment approaches, including surgery, chemotherapy, and radiation therapy, the majority of patients experience disease recurrence and succumb to the illness within a short period after diagnosis. This emphasizes the urgent need for novel therapeutic strategies that can effectively target the underlying molecular mechanisms driving pancreatic cancer progression and metastasis.

In conclusion, the malignancy of PC stems from its aggressive behavior, therapeutic resistance, and poor prognosis. Addressing these challenges requires a comprehensive understanding of the intricate molecular and cellular processes underlying pancreatic carcinogenesis, as well as the development of innovative treatment modalities aimed at improving patient outcomes and survival rates.

For PC, Gemcitabine is the main treatment method and has been proven to enter into tumor cells mainly through transportation by hENT1 to undergo a series of metabolic transformations and then exert its anticancer effect. However, the expression and prognostic value of hENT1 have shown discrepancies between different studies. Bird et al performed a meta-analysis containing 770 patients (405 hENT1-negative, 365 hENT1-positive) and concluded that high hENT1 expression was significantly associated with prolonged DFS (HR 0.58, 95% CI: 0.42 to 0.79) and OS (HR 0.52, 95% CI: 0.38 to 0.72). This result existed in patients receiving adjuvant Gemcitabine but not in those receiving fluoropyrimidine-based adjuvant therapy.[10] Similarly, a systematic review by Stina et al found that patients with high hENT1 expression had significantly longer OS in all included studies that evaluated this outcome measurement.[9] In contrast, Poplin et al detected no difference in OS in the low hENT1 subgroup or overall, with hazard ratios (HRs) of 0.994 (95% CI: 0.746 to 1.326) and 1.072 (95% CI: 0.856 to 1.344), respectively, which suggested that hENT1 expression did not predict Gemcitabine-treated patient outcomes.[15] This discrepancy may be due to the diversity of sample size and study characteristics. Moreover, the lack of a unified and clear cutoff value to define high/low hENT1 expression levels must be taken into consideration.

Here, we performed IHC to assess hENT1 expression in 359 tumor tissues and adjacent normal tissues from surgical specimens of PC patients and analyzed the relationships between hENT1 expression levels and several clinicopathological features. We found that tumor tissues tended to have a relatively low hENT1 expression level. Our TMA analysis revealed that hENT1 protein had a low expression level in PC tumor tissues compared with adjacent normal tissues, which means hENT1 expression is downregulated in PC. In addition, we evaluated the prognostic value of hENT1 and concluded that low hENT1 expression was an independent risk factor for Gemcitabine-treated patients. In subgroup analysis, those patients received Gemcitabine treatment after surgery who had low hENT1 expression levels tended to have worse outcomes (shortened DFS and OS) than those with high hENT1 expression. In our results, over-expression of

hENT1 group could significantly increase the chemotherapy effect of Gemcitabine. The results indicated that the hENT1 expression level might be an indicator for Gemcitabine treatment after radical surgery.

The limitations of this research are as follows. First, our cohort is highly selective, along with a greater overall prognosis than the average level, but our grouping is balanced so that the groups are comparable. Second, the distribution of CES scores for normal and tumor samples did not conform to a normal distribution. However, nonparametric rank sum tests also reported significant differences between the two groups. Moreover, setting a cutoff based on a mean when most samples cluster around the mean may make the result lack sufficient representativeness. However, the cutoff value is still meaningful and informative. Furthermore, while discussing conflicting findings regarding hENT1 expression and PC patient outcomes, it is crucial to acknowledge certain limitations that may contribute to these disparities. Firstly, variations in study design, such as differences in sample size, inclusion criteria, and follow-up durations, can lead to inconsistent results across studies. Secondly, the heterogeneity of patient populations, including demographic characteristics, disease stage, treatment history, and genetic variability, may also influence the observed outcomes. Additionally, discrepancies in analytical methods, including variations in immunohistochemical techniques, scoring systems, and data interpretation, could contribute to divergent findings among studies. Furthermore, factors such as sample processing protocols, tissue preservation methods, and interobserver variability in assessing hENT1 expression levels may introduce further complexities and potential biases into the analysis.

Conclusion

Our findings highlight the prognostic significance of hENT1 expression in PC patients undergoing Gemcitabine treatment. Low hENT1 expression correlates with poor prognosis, whereas high hENT1 expression suggests improved outcomes with Gemcitabine therapy. Looking ahead, our research lays the foundation for personalized treatment approaches in PC by leveraging hENT1 expression levels to identify patients who are likely to benefit from Gemcitabine treatment. By integrating hENT1 assessment into clinical practice, we can enhance treatment efficacy and optimize patient care. Moreover, future investigations should explore innovative therapeutic strategies aimed at improving treatment outcomes in PC. One promising avenue is the exploration of combination therapies targeting nucleoside transporters. By elucidating the role of nucleoside transporters in drug uptake and intracellular metabolism, we can develop synergistic treatment approaches that enhance the effectiveness of existing therapies like Gemcitabine. These efforts hold great promise for advancing the field of pancreatic cancer treatment and improving patient survival rates.

In conclusion, our study underscores the importance of hENT1 as a prognostic biomarker and emphasizes the need for continued research to translate these findings into clinical practice. By harnessing the potential of combination therapies targeting nucleoside transporters, we can usher in a new era of personalized medicine for pancreatic cancer patients, ultimately leading to improved treatment outcomes and enhanced quality of life.

Abbreviations

PC, Pancreatic cancer; hENT1, human equilibrative nucleoside transporter 1; IHC, immunohistochemistry; TMAs, tissue microarrays.

Ethics Statement

All experiments were performed in compliance with the guidelines of the Institute of Laboratory Animals Science. All the data involved in the analysis of this paper has been collected from patients after signing the informed consent, which was audited by ethical committee of Peking UnionMedical College Hospital before the research began. The ethical batch number is I-23PJ1887. The pancreatic cancer cell lines were bought from American Type Culture Collection. Clinical samples in this study complies with the Declaration of Helsinki. All experiments were performed following PUMCH and national guidelines and regulations.

Acknowledgements

We would like to thank all laboratory members for their critical discussion of this manuscript.

Novelty & Impact Statements

This work further investigates the expression and prognostic features of human equilibrative nucleoside transporter 1 (hENT1) in pancreatic cancer patients extensively. Given the current situation that Gemcitabine is the preferred post-operative chemotherapy drug, using hENT1 as an index to distinguish those who are sensitive to Gemcitabine and predict the long-term prognosis of patients is very promising.

Author Contributions

Jianchun Xiao was responsible for the ethical review and the acquisition authorization of tissue specimens. Fangyu Zhao was responsible for the data analysis and processing of this article. Wenhao Luo was responsible for polishing the language, correcting grammatical errors, and submitting the article. Gang Yang was responsible for the animal experiment part of this article, including the entire process of the animal experiments, and the collection of tissue specimens, the tissue microarray, the immunohistochemical part and the in vitro experiment part. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the National High Level Hospital Clinical Research Funding (2022-PUMCH-A-056).

Disclosure

The authors declare no potential conflicts of interest in this work.

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