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Vimentin-positive circulating tumor cells as a biomarker for diagnosis and treatment monitoring in patients with pancreatic cancer

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**Abstract**

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**Title**

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**Keywords:** Pancreatic ductal adenocarcinoma, CTCs, Epithelial-mesenchymal transition, Biomarker

**Abbreviations**

CTC: Circulating tumor cells; PDAC: Pancreatic ductal adenocarcinoma; EpCAM: Epithelial cell adhesion molecule; EMT: Epithelial-mesenchymal transition; IPMN: Intraductal papillary mucinous neoplasm; CK: Cytokeratin; CA19-9: Carbohydrate antigen 19-9; CAF: Cancer-associated fibroblasts, TGF-β: Transforming growth factor beta.

## 1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy with poor prognosis [1, 2]. Only a small proportion of patients have resectable disease because of the occurrence of metastasis and vascular invasion. Even in patients who undergo curative resection, early recurrence, either locally or in distant organs, is common [3, 4]. This suggested that current criteria are not appropriate to select patients who might benefit from surgery. For patients with unresectable disease who receive chemotherapy, useful biomarkers to predict the drug response are lacking. Although carbohydrate antigen 19-9 (CA19-9) has been used frequently in clinical practice, it has been suggested that it does not confer additional value in guiding treatment. Besides, CA19-9 is not appropriate in those patients that are genetically incapable of producing the Lewis antigen [5]. Thus, more reliable biomarkers that can aid decision-making during diagnosis and treatment are urgently needed [6].

Circulating tumor cells (CTCs) refer to malignant cells that are shed from the primary tumor into the vasculature, which are believed to be the direct source of distant

metastasis [7]. Accumulating evidence confirms that CTCs have promise in a wide range of applications, including diagnosis, tumor staging, disease monitoring, and identification of therapeutic targets [8-10]. In breast cancer, CTC counting has been included in the latest version of the National Comprehensive Cancer Network guidelines and is defined as a prognostic factor in the American Joint Committee on Cancer staging. Although the evidence is not so strong in pancreatic cancer, a report has suggested that CTCs could be a useful biomarker in this malignancy [11].

To date, the majority of studies have used epithelial cell adhesion molecule (EpCAM), an epithelial cell marker, to identify CTCs. As the first and only FDA-approved CTC-detection platform, CellSearch® is based on an immunomagnetic method to capture $EpCAM^+$ CTCs. In a study evaluating the efficiency of CellSearch® for 964 patients across 12 different types of metastatic cancers, PDAC patients had the lowest detection rate and CTC counts [12]. Similarly, other studies adopting the CellSearch® pipeline reported low detection rates of CTCs in PDAC (6–30%) [13-15]. In contrast, enrichment of $EpCAM^+$ CTCs using microfluidics approaches, such as the $^{Hb}$CTC (herringbone)-Chip, showed favorable detection sensitivity. In addition, a few studies

reported better detection efficiency of isolated CTCs using size-based filtration compared with that achieved using CellSearch® [16]. In addition, EpCAM-based identification of CTCs has been challenged for years because it does not fit logically with the theory of epithelial-mesenchymal transition (EMT), which is believed to be an essential step of tumor metastasis [17, 18]. Theoretically, CTCs are more likely to have a mesenchymal phenotype because cells that have undergone EMT have enhanced capacities of migration and invasion. Hence, using EpCAM might underestimate the number of CTCs by missing $EpCAM^-$ CTCs, which probably have a mesenchymal phenotype and are critical for distant metastasis. Clinical sample analysis confirmed that EMT in primary tumors was associated with increased risk of metastasis [19]. A lineage tracing study in a genetically engineering mouse model suggested that pancreatic tumor cells are disseminated via EMT as early as the pre-cancerous stage [20]. These results supported the view that detection of CTCs with a mesenchymal phenotype using suitable markers in patients with PDAC is not only possible, but also important. Although a small number of studies have examined the mesenchymal phenotype of CTCs, in these studies, the initial CTC

enrichment relied on epithelial markers [21-23]. Thus, the accurate status of CTCs with a mesenchymal phenotype remains enigmatic.

Vimentin is a typical marker that is overexpressed during EMT. Vimentin can be located on the tumor cell surface in sarcoma, breast, and colon cancer [24]. This raises the possibility that cell-surface vimentin could be a potential marker to identify mesenchymal CTCs. In the present study, we applied a microfluidic chip-based platform to enrich and identify cell-surface vimentin-positive CTCs in pancreatic cancer, and analyzed their clinical significance.

## 2. Materials and Methods

### 2.1 Study design and participants

The study was conducted according to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria [25]. In total, 100 patients with PDAC, 16 patients with intraductal papillary mucinous neoplasm (IPMN), and 30 healthy individuals were enrolled. The included participants were all consecutive and diagnosed between December 2016 and November 2017. Written consent for the blood and tissue

sampling was obtained from each patient and healthy donor. All included patients with PDAC had negative history of malignancy, and were treatment-naïve before enrollment. Diagnosis of unresectable PDAC was supported by the pathological results of biopsy of either the primary tumor or metastatic lesions. Healthy individuals had no medical history of any malignant disease. The patient demographics and clinical information included age, gender, serum CA19-9 levels, tumor location, stage, and treatment choice. For patients who underwent surgical resection, other information, including tumor size, grade, perineural and perivascular invasion, and nodal and margin status were further collected. Assessment of chemotherapy response was performed via abdominal enhanced computed tomography and enhanced magnetic resonance imaging. According to the Response Evaluation Criteria in Solid Tumors version 1.1, measurement of the tumor response by imaging was qualitatively classified into four categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD).

Blood samples were obtained after the approval of the Institutional Review Board at the Second Affiliated Hospital of Zhejiang University, School of Medicine. Peripheral blood (5–10 ml) was harvested in EDTA-pretreated blood collection tubes. In a subset of

patients, portal venous blood was drawn intraoperatively before and after removal of pancreatic cancer. This work was conducted in accordance with the guidelines of the Declaration of Helsinki.

**2.2 CTCs enrichment and enumeration**

Blood samples were processed within 6 hours of collection. Peripheral blood mononuclear cells (PBMCs) were first acquired via density gradient centrifugation. The PBMC fraction was then washed and loaded into a CytoQuest™ CR system. Potential CTCs were captured using a vimentin or EpCAM immobilized microfluidic chip. Immunofluorescence staining was performed using fluorescein isothiocyanate (FITC) anti-Pan-cytokeratin (CK) and FITC anti-vimentin antibodies for EpCAM- and vimentin-captured cells, respectively. Phycoerythrin (PE) anti-CD45 antibodies were used to label white blood cells, while nuclei were marked using Hoechst staining. The chip slides were scanned using automated fluorescence microscope equipment (Nikon Ti-E). The imaging results were then analyzed independently by two experienced technicians. CTCs were defined as vimentin$^{+}$CD45$^{-}$ Hoechst$^{+}$ or Pan-CK$^{+}$CD45$^{-}$Hoechst$^{+}$.

### 2.3 Cell lines

Human pancreatic cell lines PANC-1, MIA PaCa-2, BxPC-3, and T3M4 were obtained from the ATCC (Manassas, VA, USA). All cell lines were authenticated using a short tandem repeat (STR) DNA test. A primary cancer-associated fibroblast (CAF) line was generated and preserved in our laboratory from a fresh sample for one patient with PDAC under written informed consent for research purposes.

### 2.4 Flow cytometry analysis

Cultured cells were detached using non-enzymatic dissociation buffer and stained with anti-vimentin antibodies for 30 minutes on ice. Cells were further labeled with secondary antibodies conjugated with the allophycocyanin (APC) fluorophore. The positive fraction of cells was gated based on the staining of the isotype control. The data were analyzed using FlowJo software.

### 2.5 Immunofluorescence staining

For primary pancreatic cancer tissues, paraffin-embedded, 5-μm thick sections were deparaffinized and rehydrated, and the antigen was retrieved at 98 °C for 10 minutes in 10 mM citrate buffer. The tissue sections were blocked with 1% bovine serum

albumin for 30 minutes at room temperature, and then incubated with primary antibodies overnight at 4 °C. The following primary antibodies were used: Anti-vimentin and anti-E-cadherin. Secondary antibody staining was performed the next day using anti-mouse or anti-rabbit Alexa Fluor-488 and Alexa-Fluor 555, respectively. Slides were then mounted using Vectashield Mounting Medium with 2-(4-amidinophenyl)-1H-indole-6-carboxamidine (DAPI) and a glass coverslip.

**2.6 Western blotting analysis**

Harvested cells were lysed using radioimmunoprecipitation assay (RIPA) lysis buffer with a protease inhibitor cocktail (Roche Molecular Systems Inc., Branchburg, NJ, USA). The total protein concentration was measured using a bicinchoninic acid assay. A total of 20 μg of the protein lysates were denatured, and separated using 10% Bis-Tris gels. The following primary antibodies were used: Anti-rabbit vimentin, anti-rabbit E-cadherin, anti-rabbit N-cadherin, and anti-rabbit glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

**2.7 Transwell migration assay**

PANC-1 cells were first labeled with anti-vimentin antibodies and then mouse IgG-binding magnetic-beads (Miltenyi Biotec) were added to the mixture. Vimentin$^{+}$ and vimentin$^{-}$ PANC-1 cells were then separated using a magnetic column (Miltenyi Biotec) according to the manufacturer's instructions. A Transwell migration assay was conducted using 24-well Transwell chambers with 8-μm pores on the membrane (Corning Inc., Corning, NY, USA). Briefly, PANC-1 cells in 200 μL of serum-free medium were loaded into the upper chamber. Then, 500 μL of medium supplemented with 10% fetal bovine serum was added into the lower chamber. After 24 hours, the cells on the underside of the membrane were stained, and cells in five random high-power fields were counted under a microscope.

**2.8 Statistical analysis**

Data are presented as the means ± the standard deviation (SD). A paired or unpaired Student *t* test was used for continuous variables, as appropriate. The *chi*-squared test was adopted for the comparison of categorical parameters. One-way analysis of variance (ANOVA) was performed to calculate the differences between multiple groups. Disease-free survival analysis was performed using Kaplan–Meir method. All statistical

analyses were performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA) and Prism 6.0 (Graphpad, La Jolla, CA, USA). A two-sided *p* value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1 Validation of vimentin as a surface marker for pancreatic cancer cells with a mesenchymal phenotype

Vimentin was detected in pancreatic cancer cell lines with a mesenchymal phenotype, including PANC-1 and MIA PaCa-2, but not in BxPC-3 and T3M4, which exhibit an epithelial phenotype (Figure 1A). Upon induction of EMT by hypoxia or transforming growth factor beta (TGF-β) treatment (10 ng/ml), further upregulation of mesenchymal markers vimentin and N-cadherin in PANC-1 cells was observed (Figure 1A). Flow cytometry analysis showed that vimentin was located on the cell membrane (Figure 1B). Importantly, the amount of vimentin on the cell surface was consistent with that of total vimentin, suggesting it could be an indicator of EMT in pancreatic cancer

cells (Figure 1B). The anti-vimentin antibody used in flow cytometry was identical to the one used for CTC capture.

To further confirm whether vimentin$^+$ pancreatic cancer cells have increased migratory capacity, a critical feature of EMT, we sorted PANC-1 cells with or without cell-surface vimentin staining and performed a Transwell assay. As expected, vimentin$^+$ cells exhibited a substantially higher migratory capacity than vimentin$^-$ cells (Figure 1C, $p < 0.001$). Similarly, a wound-healing assay showed enhanced closure of the wound in the presence of vimentin$^+$ PANC-1 cells compared with that in the presence of vimentin$^-$ PANC-1 cells (Supplementary Figure 1). Furthermore, we observed that vimentin expression was detected on PANC-1 cells under both permeabilized and non-permeabilized conditions, indicating its location in the cytoplasm and on the cell membrane. However, vimentin was barely detectable in non-permeabilized cancer-associated fibroblasts (CAFs), despite abundant expression being observed after permeabilization (Figure 1D). In addition, using the same anti-vimentin antibody, we detected cell membrane-located vimentin in human pancreatic cancer tissue (Figure 1E).

These data suggested that vimentin might serve as a surface marker for pancreatic cancer cells with a mesenchymal phenotype.

**3.2 Detection of vimentin$^+$ CTCs improves the diagnosis of pancreatic cancer**

We succeeded in detecting CTCs (vimentin$^+$CD45$^-$Hoechst$^+$ or Pan-CK$^+$CD45$^-$Hoechst$^+$) in patients with pancreatic cancer using antibody-coated microfluidic chips (Figure 2A). In the initial 20 cases with paired blood samples, we noticed that vimentin$^+$ CTCs were more frequently detected than EpCAM$^+$ CTCs (median, 3 *vs.* 0 cell/4 ml of blood) (Figure 2B, $p = 0.003$). To further validate the specificity of vimentin$^+$ CTCs as a biomarker for pancreatic cancer, we examined the number of vimentin$^+$ CTCs in 100 patients with PDAC, together with 30 healthy individuals and 16 patients with IPMN (Figure 2C). Overall, vimentin$^+$ CTCs were detected in 76% of patients with PDAC, but only in 2 out 30 healthy donors (Figure 2D). Besides, five patients (31.3%) with IPMN had vimentin$^+$ CTCs, and 10 CTCs were identified in one case. Using a cut-off value of two cells/4 ml of blood, 65% PDAC patients were positive for vimentin$^+$ CTCs , while the positivity rates for the patients with IPMN and the healthy controls were 25% and 0%, respectively (Figure 2E). The median

number of CTCs for all patients with PDAC was 3 (range: 0–23 in 4 ml of blood). Importantly, CTCs improved the diagnostic power of CA19-9. The combined analysis of CTCs and CA19-9 for PDAC diagnosis showed high accuracy, with an area under curve (AUC) of 0.968 (Figure 2F). Thus, vimentin$^+$ CTCs could be a potential diagnostic biomarker for pancreatic cancer.

### 3.3 Vimentin$^+$ CTCs correlate with the tumor burden

We then evaluated the clinical significance of vimentin$^+$ CTCs in PDAC. Clinical parameters between patients with less than two CTCs were compared with those of patients with more than two CTCs (Table 1). There were no significant differences in age, sex, and CA19-9 level between the two groups. However, patients with two or more detectable vimentin$^+$ CTCs had more advanced stage disease and metastasis than those with one or no CTCs ($p$ = 0.015, $p$ = 0.005). In parallel, patients with metastasis had significantly more CTCs than those with localized disease (median: 5 *vs*. 3 CTCs/4 ml blood, $p$ = 0.018) (Figure 2G). In the 40 patients who underwent surgical resection, patients with higher numbers of vimentin$^+$ CTCs were associated with poor differentiation ($p$ = 0.031).

We collected peripheral blood samples before surgery and at seven days after surgery in 16 patients. Except for two patients in whom CTCs were not detected before surgery, the other 14 patients demonstrated reduced CTC counts after surgery (Figure 3A, $p = 0.003$). In particular, we failed to detect any vimentin$^{+}$ CTCs postoperatively in six of these patients. In the eight patients with detectable CTCs on postoperative day 7, three of them who had a peripheral blood sample available one month after surgery had undetectable CTCs. Notably, 10 patients had concomitant portal vein blood samples taken during surgery. The paired analysis showed that the CTC counts were higher in portal vein blood than peripheral blood (median: 6 *vs*. 2 CTCs/4 ml blood) (Figure 3B, $p < 0.05$).

Therefore, we further tested whether this biomarker could reflect the tumor response to chemotherapy. We collected serial blood samples before and after a course of modified FOLFIRINOX (oxaliplatin, leucovorin, irinotecan, and fluorouracil (5-FU)) or Gemcitabine plus Nab-Paclitaxel chemotherapy in 15 subjects (Table 2). In the 13 patients exhibiting a response (either tumor shrinkage or stable tumor burden), their CTC counts were all decreased or remained the same, except for one case. In contrast, the two

subjects defined as having progressive disease showed no reduction in CTC counts (Figure 3C). These data confirmed that the vimentin$^+$ CTC count might indicate the tumor burden and treatment response. In addition, patients with higher numbers of vimentin$^+$ CTCs had inferior recurrence-free survival after surgical resection (Figure 3D, Log-rank test: $p = 0.02$).

## 4. Discussion

In the present study, we reported the detection of vimentin$^+$ CTCs and its clinical relevance in pancreatic cancer. As a classic molecular marker of EMT, vimentin constitutes one of the most abundant proteins in the cytoplasm of mesenchymal tumor cells. Li and colleagues created an anti-vimentin antibody that could bind with the full-length vimentin antigen and found that vimentin was present on the tumor cell surface [24, 26]. Similarly, in pancreatic cancer, we confirmed that this antibody could recognize cell-surface vimentin.

Our study supported the view that the enriched vimentin$^+$ circulating cells are cancerous cells originating from pancreatic cancer. First, vimentin$^+$ circulating cells in

healthy individuals were rare. Second, the vimentin$^+$ circulating cell count in portal vein blood was significantly higher than that in peripheral blood. This result was consistent with the theoretical distribution of tumor cells in the vasculature for PDAC, reflecting the retention of CTCs in the liver [27]. A similar pattern of CTC distribution was seen in colon and lung cancers [28-30]. Third, patients who received curative tumor resection had remarkably reduced CTC counts after surgery. The good correlation with the tumor burden strongly indicated that vimentin$^+$ CTCs were derived from pancreatic cancer rather than non-specific recognition of non-cancerous cells.

The performance of CTC detection in PDAC diagnosis varies according to the different techniques applied. Generally, immunological capture methods, like CellSearch®, provide sensitivity generally below 50% [13, 14], while cell size-based enrichment techniques have a better diagnostic performance [16]. Sefrioui and colleagues determined the diagnostic value of CTCs in patients with PDAC across distinct tumor stages using a filtration method and morphological characterization [31]. The sensitivity and specificity for CTCs detection were 67% and 80%, respectively, which was concordant with other reports using similar strategies. When we used a cut-off value of

two cells/4 ml of blood, we obtained a sensitivity of 65% and a specificity of 100% for diagnosis of PDAC compared with that in healthy individuals. This remains inferior to CA19-9, which has a sensitivity of 79–81% and a specificity of 82–90%. Given the high specificity of vimentin$^{+}$ CTCs detection, further exploration of its value in discriminating pancreatic malignant tumors and benign disease is warranted. In addition, vimentin$^{+}$ cells were detectable in a fraction of patients with pancreatic cystic tumors, among whom four cases had more than two positive cells. Both subtypes of cystic neoplasm are defined as having a premalignant status, and a few studies have confirmed that epithelial CTCs could be found in blood of these patients [32, 33]. Importantly, using genetic engineered mouse models of pancreatic cancer, a study demonstrated that epithelial pancreatic cells entered into bloodstream in the early stage of pancreatic intraepithelial neoplasia, and maintained a mesenchymal phenotype in circulation [20]. This was in line with the current findings that vimentin$^{+}$ CTCs were present in patients with premalignant pancreatic cystic neoplasms. Encouragingly, the combination of CTCs and CA19-9 improved the specificity and sensitivity of PDAC diagnosis; however, this result requires further confirmation using other prospective cohorts.

There are currently no effective methods to stratify early recurrence risk or to guide neoadjuvant and adjuvant therapies in PDAC. CTCs are of particular interest as a method of liquid biopsy because they are believed to be the precursors of metastasis. Some previous studies demonstrated that CTCs levels had prognostic significance [34-36]. Poruk and colleagues reported that higher total CTC numbers, as measured using a size-based enrichment method, correlated with shortened survival in a group of patients undergoing curative resection [37]. In particular, vimentin$^{+}$ CTCs were negatively associated with progression-free survival. Our preliminary data suggested clinical applications of vimentin$^{+}$ CTCs. For early stage disease, the immediate reduction in the number of CTCs after resection suggested its potential in monitoring postoperative recurrence. Currently, longitudinal blood samples are routinely collected in our institute to determine whether CTCs could predict early tumor relapse. Furthermore, serial sample analysis for patients receiving chemotherapy indicated the relationship between the CTC count and the tumor response. EMT contributes to chemotherapy resistance in many types of cancer, including PDAC [38, 39]. Thus, future studies should focus on the significance of mesenchymal CTCs to predict the tumor response to chemotherapy.

## 5. Conclusions

In the present study, we demonstrated the feasibility of using cell-surface vimentin as a candidate biomarker to identify potential mesenchymal pancreatic CTCs. Vimentin$^{+}$ CTCs could be useful in diagnosis, monitoring the tumor burden, and to predict tumor recurrence. This strategy complements the current standard EpCAM-based CTCs detection, and warrants further validation in a larger PDAC cohort for its clinical significance.

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**Authors' contributions**

Conception and design: XL. Bai, TB. Liang, and T. Wei. Acquisition of data: T. Wei, XY. Zhang, JX. Wang, X. Li, JY. Chen, T. Ma, GG. Li, SL. Gao, JY. Lou, RS. Que, Y. Wang, TB. Liang, and XL. Bai. Analysis and interpretation of data: T. Wei, Q. Zhang, JQ. Yang, Q. Chen, XW. Dang, L. Zheng, TB. Liang, and XL. Bai. Writing of the manuscript: T. Wei, Q. Zhang, TB. Liang, and XL. Bai. Review of the manuscript: All authors

**Declarations of interest: none**

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**Table 1.** Clinicopathological characteristics of patients with ≥ 2 or < 2 vimentin$^+$ CTCs.

| Parameters | Total (n = 100) | ≥ 2 CTCs (n = 65) | < 2 CTCs (n = 35) | *p* value |
|---|---|---|---|---|
| *All patients (n=100)* | | | | |
| Age, median (range, years) | 64 (43–90) | 63 | 65 | |
| Gender | | | | 0.199 |
| Male | 60 | 36 | 24 | |
| Female | 40 | 29 | 11 | |
| Tumor location | | | | 0.377 |
| Proximal | 54 | 33 | 21 | |
| Distal | 46 | 32 | 14 | |
| AJCC staging | | | | **0.015** |
| I | 8 | 6 | 2 | |
| II | 31 | 17 | 14 | |
| III | 22 | 10 | 12 | |
| IV | 39 | 32 | 7 | |
| Metastasis | 39 | 32 | 7 | **0.005** |
| Liver | 28 | 24 | 4 | |
| Other | 13 | 10 | 3 | |
| CA19-9, median (range, U/ml) | 2439.8 (2–12000) | 2866.4 | 1647.6 | 0.145 |
| CA19-9 level | | | | 0.852 |
| Low (< 36 U/ml) | 19 | 12 | 7 | |
| High (> 36 U/ml) | 81 | 53 | 28 | |
| *Patients undergoing resection (n = 40)* | | | | |
| Tumor grade | | | | **0.031** |
| Well/moderate | 18 | 7 | 11 | |

| | | | | |
|---|---|---|---|---|
| Poor | 22 | 16 | 6 | |
| Perineural invasion | | | | 0.397 |
| Positive | 35 | 21 | 14 | |
| Negative | 5 | 2 | 3 | |
| Microvascular invasion | | | | 0.385 |
| Positive | 22 | 14 | 8 | |
| Negative | 18 | 9 | 9 | |
| Lymph nodes status | | | | 0.272 |
| Positive | 26 | 13 | 13 | |
| Negative | 14 | 10 | 4 | |
| Tumor size, mean (cm) | 3.2 | 3.4 | 3.0 | 0.304 |

CTC, circulating tumor cells; CA19-9, carbohydrate antigen 19-9; AJCC, American Joint Committee on Cancer.

**Table 2.** Dynamics of vimentin$^{+}$ CTC counts and CA19-9 level in patients receiving chemotherapy.

| Patient No. | Chemotherapy regimen | CTC counts | | CA19-9 (U/ml) | | Therapy response |
|---|---|---|---|---|---|---|
| | | Before therapy | After therapy | Before therapy | After therapy | |
| 1 | mFOLFIRINOX | 9 | 6 | 12000 | 240.0 | PR |
| 2 | mFOLFIRINOX | 7 | 1 | 133.5 | 2.5 | PR |
| 3 | mFOLFIRINOX | 6 | 4 | 347.3 | 88.2 | PR |
| 4 | mFOLFIRINOX | 1 | 0 | 11562.5 | 503.0 | SD |
| 5 | mFOLFIRINOX | 6 | 0 | 218.7 | 55.2 | SD |
| 6 | mFOLFIRINOX | 17 | 5 | 2.0 | 2.0 | SD |
| 7 | mFOLFIRINOX | 3 | 6 | 12000 | 12000 | SD |
| 8 | mFOLFIRINOX | 3 | 1 | 12000 | 12000 | SD |
| 9 | mFOLFIRINOX | 8 | 0 | 1395.8 | 242.9 | SD |
| 10 | mFOLFIRINOX | 3 | 2 | 797.0 | 1824.9 | SD |
| 11 | mFOLFIRINOX | 0 | 0 | 288.7 | 1451.9 | PD |
| 12 | Gem + Nab-Paclitaxel | 3 | 2 | 8667.6 | 530.0 | PR |
| 13 | Gem + Nab-Paclitaxel | 5 | 0 | 3479.6 | 2394.3 | SD |
| 14 | Gem + Nab-Paclitaxel | 0 | 0 | 3871.5 | 1940.9 | SD |
| 15 | Gem + Nab-Paclitaxel | 2 | 4 | 2.0 | 2.0 | PD |

CTC, circulating tumor cells; CA19-9, carbohydrate antigen 19-9; mFOLFIRINOX, modified regimen of oxaliplatin, leucovorin, irinotecan, and fluorouracil (5-FU); Gem, Gemcitabine.

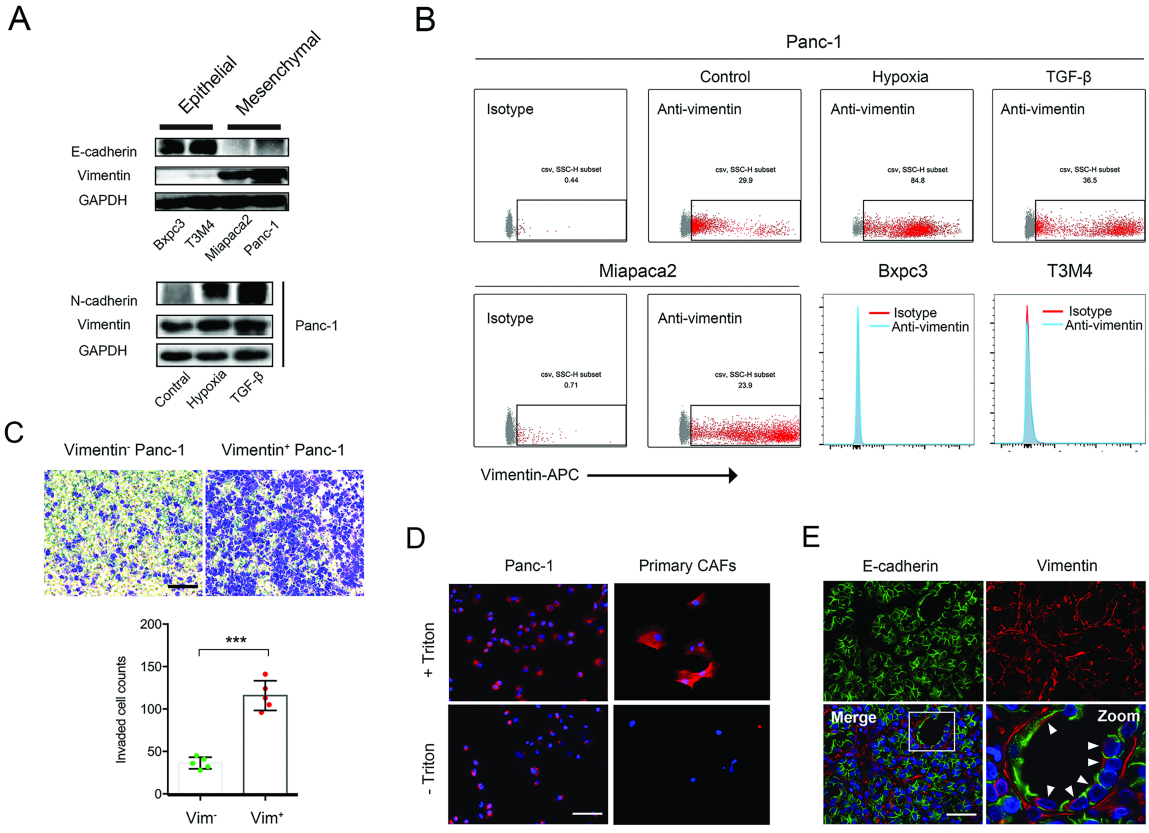
**Figure legends**

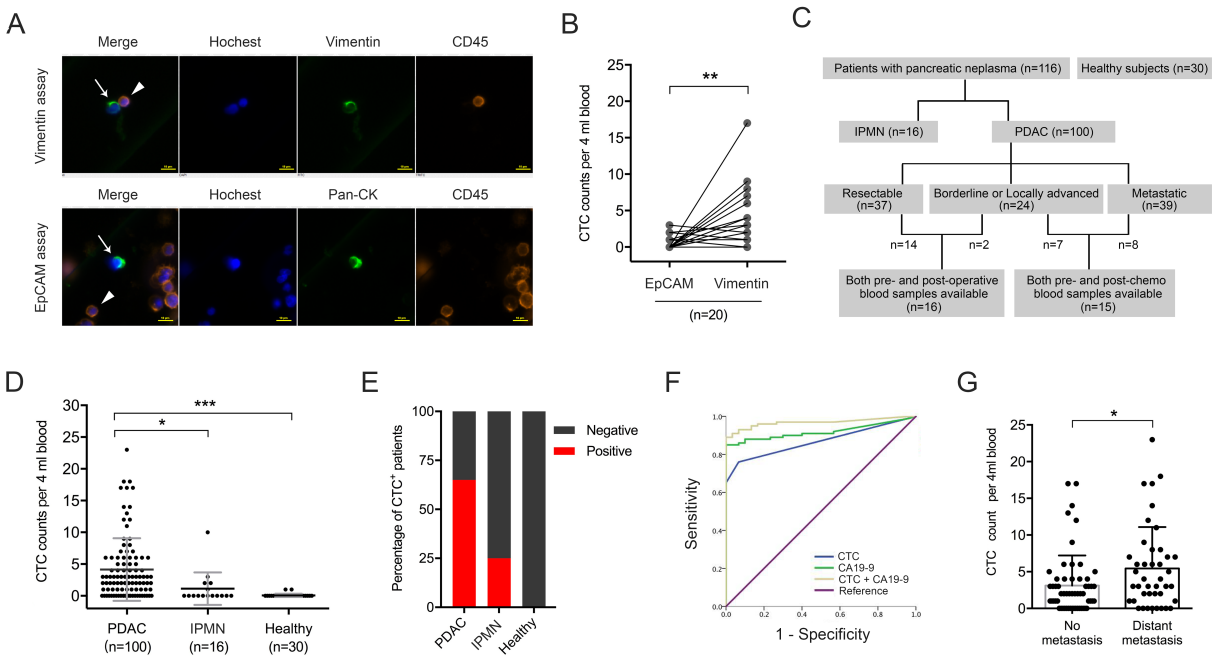
**Figure 1.** Validation of the cell-surface vimentin as a marker for mesenchymal pancreatic tumor cells. Immunoblotting (A) and flow cytometry (B) analysis for vimentin in epithelial and mesenchymal pancreatic cell lines. Comparison of the migration for sorted vimentin$^{+}$ and vimentin$^{-}$ PANC-1 cells (C), the scale bar represents 50 μm. Three biological replicates were performed. Immunofluorescence staining for vimentin in PANC-1 and primary cancer-associated fibroblasts (CAFs) (D); the scale bar represents 25 μm. Immunofluorescence staining for vimentin and E-cadherin in a pancreatic cancer tissue (E); the scale bar represents 50 μm. $^{***}$ $p < 0.001$.

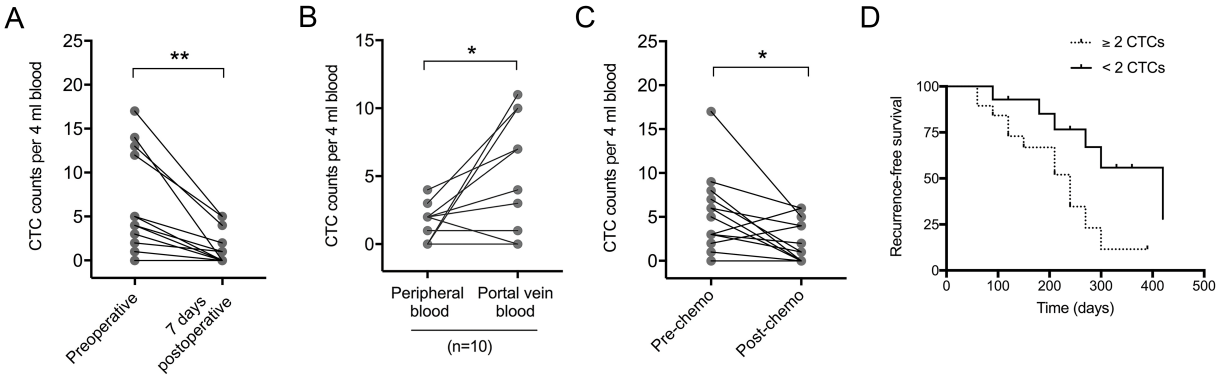
**Figure 2.** Vimentin$^{+}$ circulating tumor cells (CTCs) constitute a potential biomarker in pancreatic cancer. The identification of vimentin antibody or epithelial cell adhesion molecule (EpCAM) antibody-enriched CTCs (A); arrows indicate CTCs, and arrowheads indicate white blood cells. The paired comparison of CTC detection using vimentin versus the EpCAM assay (B). Flowchart of enrolled patients and blood sample analysis (C). The detection of vimentin$^{+}$ CTCs in patients with pancreatic ductal adenocarcinoma

(PDAC), intraductal papillary mucinous neoplasm (IPMN), and in healthy subjects (D). A cut-off value of two cells/4 ml of blood was used to discriminate positive and negative CTC detection (E). The diagnostic performance of vimentin$^{+}$ CTCs, carbohydrate antigen 19-9 (CA19-9), and combined vimentin$^{+}$ CTCs with CA19-9 was shown by a receiver operating characteristic (ROC) curve (F). Comparison of CTC counts for patents with or without distant metastasis (G). $^{*}$ $p < 0.05$, $^{**}$ $p < 0.01$, $^{***}$ $p < 0.001$.

**Figure 3.** Vimentin$^{+}$ circulating tumor cells (CTCs) reveal the tumor burden and chemotherapy response. Comparison of vimentin$^{+}$ CTC counts in blood samples from patients pre- and post-surgery (A), pre- and post-chemotherapy (B), and in peripheral and portal vein blood samples (C). Recurrence-free survival analysis based on the number of vimentin$^{+}$ CTCs (D). $^{*}$ $p < 0.05$, $^{**}$ $p < 0.01$.







**Highlights**

- Vimentin is expressed on the surface of pancreatic tumor cells with mesenchymal phenotype
- Vimentin$^{+}$ CTCs distinguish PDAC patients and healthy individuals, and confer favorable diagnosis performance when combined with CA19-9
- Vimentin$^{+}$ CTCs correlate with tumor burden and prognosis