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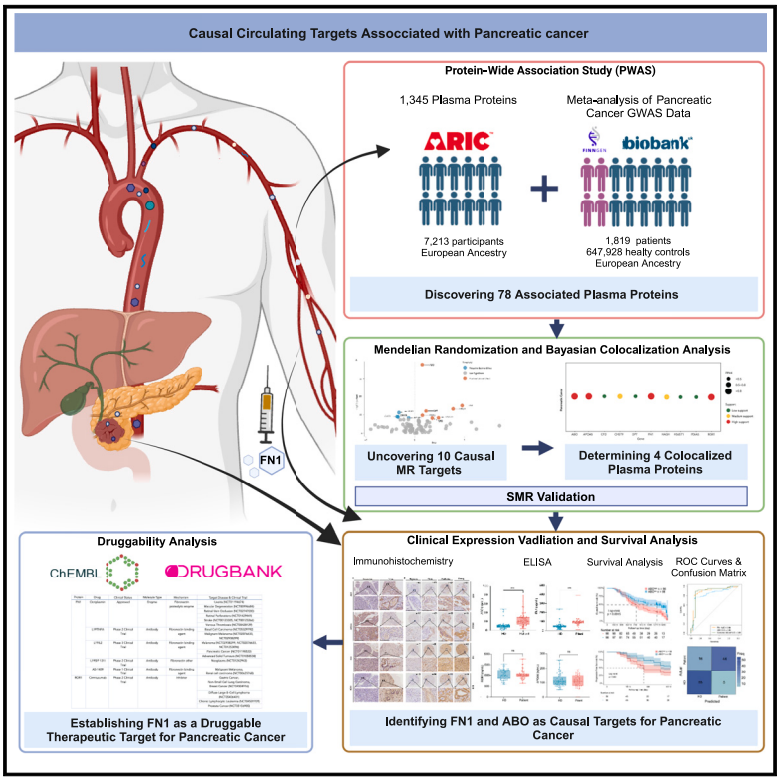
Unveiling circulating targets in pancreatic cancer: Insights from proteogenomic evidence and clinical cohorts

Graphical abstract



Highlights

- A pipeline identifies pancreatic cancer risk factors through proteogenomics
- FN1 is identified as a promising novel target and biomarker
- ROR1 shows opposing roles, requiring caution in ROR1-targeted therapies

Authors

Haokang Feng, Zhixue Chen, Jianang Li, ..., Xu Han, Hua Su, Lei Zhang

Correspondence

liu.liang@zs-hospital.sh.cn (L.L.), han.xu1@zs-hospital.sh.cn (X.H.), suhua@fudan.edu.cn (H.S.), zhang.lei@zs-hospital.sh.cn (L.Z.)

In brief

Health sciences; Clinical finding; Proteomics

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Unveiling circulating targets in pancreatic cancer: Insights from proteogenomic evidence and clinical cohorts

Haokang Feng,[1,2,3,11] Zhixue Chen,[4,11] Jianang Li,[1,2,3] Jiale Feng,[1,2,3] Fei Yang,[5,6] Fansheng Meng,[7] Hanlin Yin,[1,2,3] Yuquan Guo,[1,2,3] Huaxiang Xu,[1,2,3] Yuxin Liu,[8] Runjie Liu,[8] Wenhui Lou,[1,2,3,9] Liang Liu,[1,2,3,*] Xu Han,[1,2,3,*] Hua Su,[10,*] and Lei Zhang[1,2,3,12,*]

[1]Department of Pancreatic Surgery, Zhongshan Hospital, Fudan University, Shanghai 200032, China
[2]Cancer Center, Zhongshan Hospital, Fudan University, Shanghai 200032, China
[3]Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai 200032, China
[4]Department of Gastroenterology and Hepatology, Shanghai Institute of Liver Diseases, Zhongshan Hospital, Fudan University, Shanghai 200032, China
[5]Innovative Institute of Tumor Immunity and Medicine (ITIM), Hefei, Anhui, China
[6]Anhui Province Key Laboratory of Tumor Immune Microenvironment and Immunotherapy, Hefei, Anhui, China
[7]Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai 200032, China
[8]Department of Basic Medicine and Institute of Liver Diseases, Shan Xi Medical University, Taiyuan 030000, China
[9]The Shanghai Geriatrics Medical Center, Zhongshan Hospital MinHang MeiLong Branch, Fudan University, Shanghai 201104, China
[10]Institutes of Biomedical Sciences, Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai 200032, China
[11]These authors contributed equally
[12]Lead contact
*Correspondence: liu.liang@zs-hospital.sh.cn (L.L.), han.xu1@zs-hospital.sh.cn (X.H.), suhua@fudan.edu.cn (H.S.), zhang.lei@zs-hospital.sh.cn (L.Z.)
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SUMMARY

Pancreatic cancer (PC), characterized by the absence of effective biomarkers and therapies, remains highly fatal. Data regarding the correlations between PC risk and individual plasma proteome known for minimally invasive biomarkers are scarce. Here, we analyzed 1,345 human plasma proteins using proteome-wide association studies, identifying 78 proteins significantly associated with PC risk. Of these, four proteins (ROR1, FN1, APOA5, and ABO) showed the most substantial causal link to PC, confirmed through Mendelian randomization and colocalization analyses. Data from two clinical cohorts further demonstrated that FN1 and ABO were notably overexpressed in both blood and tumor samples from PC patients, compared to healthy controls or para-tumor tissues. Additionally, elevated FN1 and ABO levels correlated with shorter median survival in patients. Multiple drugs targeting FN1 or ROR1 are available or in clinical trials. These findings suggest that plasma protein FN1 associated with PC holds potential as both prognostic biomarkers and therapeutic targets.

INTRODUCTION

Managing pancreatic cancer (PC) remains a significant challenge, with an overall survival (OS) rate of only 12%.[1] Most therapeutic agents targeting PC have demonstrated limited success in clinical trials, showing minimal impact on enhancing patient survival.[2] Even in cases where drugs show effectiveness, their benefits are often restricted to specific patient subgroups. Olaparib, approved by the Food and Drug Administration (FDA) for treating germline BRCA-mutated (gBRCAm) metastatic PC, is one such example.[3,4] Although olaparib provides a moderate improvement in progression-free survival (PFS), its clinical utility is constrained by the low prevalence of BRCA mutations, which occur in only 4%–7% of patients.[5] This highlights the pressing need for identifying new therapeutic targets to improve outcomes for a broader range of PC patients.

Circulating blood, which traverses tumor sites and all organs, offers a comprehensive insight into an individual's pathophysiological state,[6] providing a more integrated understanding of the pathological and molecular genetic landscape than isolated pancreatic tumor sampling. Circulating proteins, encompassing both functional proteins in the circulatory system and those released or secreted by tissues, have been extensively utilized to identify novel diagnostic and therapeutic biomarkers in cancer research.[7–10] However, isolating relevant low-abundance proteins from numerous circulating proteins remains a significant challenge.[11] Integrating high-throughput plasma proteomic sequencing with genetic association studies enables the precise identification of potential targets within this extensive protein pool. For instance, in 2022, the Atherosclerosis Risk in Communities (ARIC) study employed the SOMAscan proteomics assay to successfully measure 1,345 unique proteins in 9,084 European American (EA)

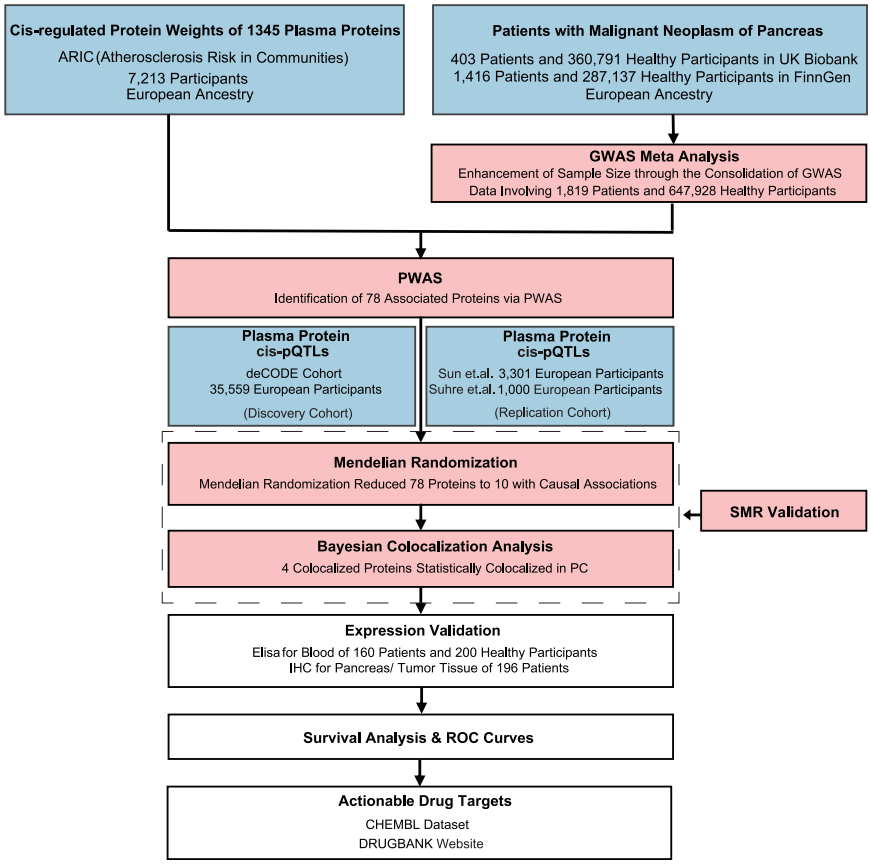

Figure 1. Overview of the study design

participants, presenting an extensive profile of plasma proteins.[12] Leveraging popular genetic association research methods such as protein-wide association study (PWAS) and Mendelian randomization (MR), it becomes a reality to link genes to PC phenotypes through protein functional variations[13] based on this extensive plasma protein spectrum. MR, by utilizing the random distribution of genetic variants, offers insights into genetically causal circulating proteins linked to PC.[14] Compared to previous smaller observational studies, the combination of PWAS and MR functions as a large-scale randomized clinical trial, yielding more robust evidence while reducing confounding variables.[15] Despite these advances, no current research has applied this methodology to investigate potential drug targets for PC.

Here, a comprehensive genetic association study was conducted, utilizing PWAS and MR analyses to refine a pool of 1,345 circulating proteins derived from the ARIC study. This approach identified four proteins (ROR1, FN1, APOA5, and ABO) with a causal link to PC. To further assess the therapeutic relevance of these proteins, their expression levels were analyzed in two independent single-center retrospective cohorts. Notably, ABO and FN1 were significantly elevated in both blood and tumor tissues from PC patients, in contrast to blood samples from healthy individuals or adjacent non-cancerous tissues. Expression levels of ROR1 and APOA5 remained consistent across blood samples, though elevated in tumor tissues compared to adjacent non-tumorous regions. Furthermore, higher concentrations of ABO and FN1 in blood or tumor tissues correlated with reduced median survival in patients. Importantly, several FDA-approved drugs or those undergoing clinical trials target FN1. This analysis reveals a potential causal link between circulating proteins and PC risk, highlighting FN1 as a viable therapeutic target and a circulating prognostic marker for PC.

RESULTS

Study design

The study design was depicted in Figure 1. Initially, genome-wide association studies (GWASs) summary statistics were sourced from the UK Biobank (via Neale Lab) and the FinnGen consortium, encompassing data on 403 European individuals with PC and 360,791 controls from the UK Biobank, alongside 1,416 European patients and 287,137 controls from FinnGen. A subsequent GWAS meta-analysis was performed, utilizing these summary statistics. By integrating 1,345 plasma proteins from the ARIC study, which involved 7,213 participants, with the meta-analysis data, PWAS was employed to identify potential PC-associated proteins. Causally relevant proteins were then identified through a two-sample MR analysis using conditionally independent genetic variants, with Bayesian colocalization employed to mitigate linkage disequilibrium. Following this, Summary-data-based Mendelian randomization (SMR) analysis, along with its corresponding colocalization testing, was conducted to further validate the identified causal proteins. Two independent single-center cohorts were established to assess the potential of MR-identified causal proteins as biomarkers or therapeutic targets in clinical settings. The first cohort involved analyzing the protein levels in blood samples from 160 PC patients and 200 healthy volunteers, evaluating the correlation between protein blood expression and PFS in patients. Receiver operating characteristic (ROC) curve analysis and confusion matrices were employed to evaluate the clinical diagnostic performance of these differentially expressed proteins. In the second cohort, protein levels were compared between paired tumor and para-tumor tissues from 196 PC patients to explore their association with prognosis. Subsequently, the drug availability of the potential candidates was investigated via the CHEMBL dataset, DRUGBANK, and OpenTargets.

PWAS identifies 78 circulating proteins associated with PC

To identify proteins associated with PC, we leveraged the comprehensive dataset from the ARIC study, a large-scale

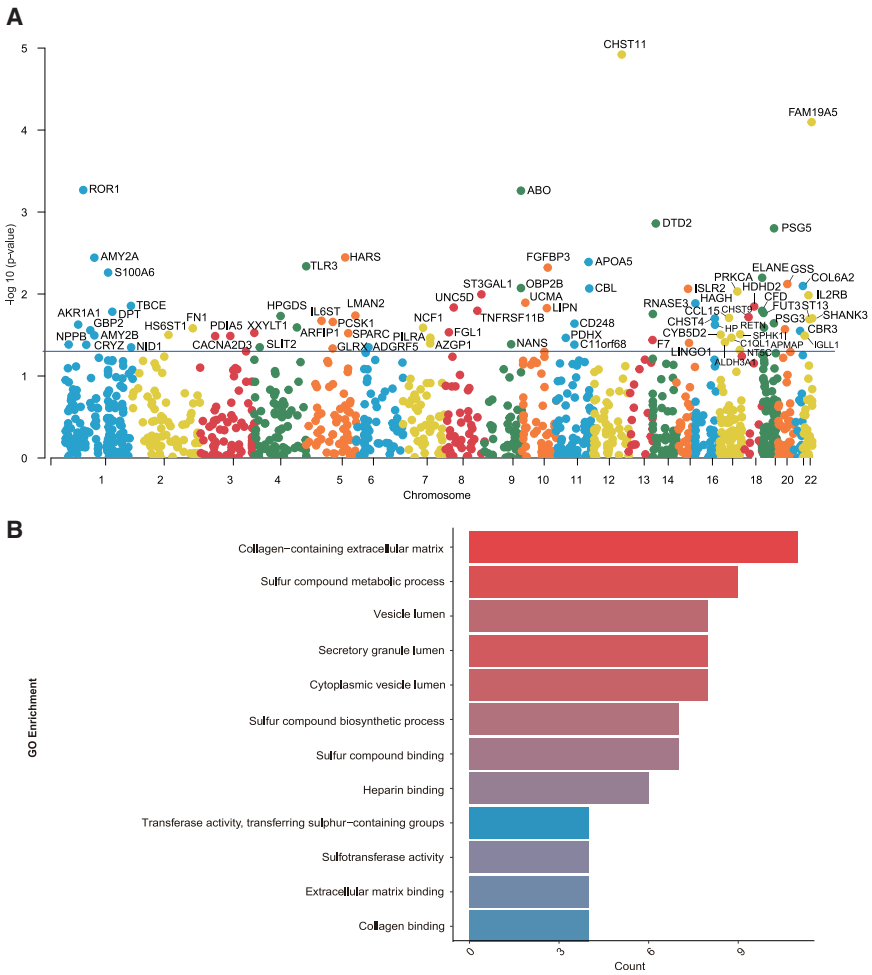

Figure 2. PWAS identifies 78 circulating proteins associated with PC

(A) The Manhattan plot presents the results of a PWAS analysis, integrating PC *meta*-GWAS with data from the ARIC study. Each dot represents an individual association test between a circulating target and PC, with the x axis denoting chromosomal location and the y axis showing -log10 (P). Dots positioned below the blue threshold indicate non-significant associations ($p > 0.05$), while those above denote significant associations ($p < 0.05$). (B) GO analysis highlights 78 circulating proteins associated with PC, as identified through PWAS.

prospective cohort that has proven invaluable for investigating various diseases beyond its initial cardiovascular focus.[16] While originally designed to study cardiovascular disease risk factors, ARIC's extensive and well-characterized dataset, comprising 7,213 participants and measurements of 1,345 plasma proteins, has been widely utilized in research spanning multiple diseases including cancer, diabetes, and chronic kidney disease.[16] Utilizing this rich resource, we performed PWAS based on integrating the GWAS meta-analysis and elastic-net-based algorithms produced accurately predicted proteins in the EA population from the ARIC study via using the FUSION pipeline. As a result, 78 circulating proteins demonstrated significant associations with PC ($p < 0.05$) (Table S1; Figure 2A). Subsequent gene ontology (GO) enrichment analysis of these proteins highlighted their involvement in diverse biological processes, particularly protein secretion pathways, including the collagen-containing extracellular matrix, sulfur metabolism, biosynthesis, and binding activities, vesicle lumen, secretory granule lumen, cytoplasmic vesicle lumen, sulfotransferase activity, heparin-binding, sulfur group transfer, extracellular matrix binding, and collagen binding (Figure 2B).

Ten proteins are identified as suspected causal PC risk targets via MR analysis

To explore causal relationships between circulating proteins and PC, a comprehensive analysis integrating both MR and GWAS was performed. The deCODE proteomic dataset was first utilized to identify conditionally independent *cis*-pQTLs for each circulating protein in a European cohort of 35,559 participants. In total, 4,002 *cis*-pQTLs were identified, covering 74 of the 78 proteins highlighted in the prior PWAS analysis. These *cis*-pQTLs exhibited robust statistical significance (F-statistic >10). Subsequently, the *cis*-pQTLs were applied as instrumental variables (IVs) in a two-sample MR analysis, employing *meta*-GWAS data for PC. This methodology identified 36 proteins with a statistically significant causal link to PC risk ($p < 0.05$). Following the application of Bonferroni correction ($p < 0.05/74 = 6.7 \times 10^{-4}$), only 11 proteins exhibited statistically significant differences (Figure 3; Table S2). To further evaluate the reliability of the primary MR analyses, assessments of heterogeneity and horizontal pleiotropy were conducted. The results revealed no evidence of heterogeneity (Q_*P* > 0.05, Table S2) or horizontal pleiotropy ($p > 0.05$) among the 11 proteins causally linked to PC, with the exception of OBP2B, which did not meet the criteria for heterogeneity ($Q = 0.034$) or horizontal pleiotropy ($p = 0.013$).

PC risk proteins ABO, FN1, and ROR1 exhibit colocalization with pQTL

To evaluate the potential influence of a shared SNP on both PC risk and protein expression levels, the 10 MR-identified protein targets underwent colocalization analysis. Using prior probabilities ($p1 = 1 \times 10^{-4}$, $p2 = 1 \times 10^{-4}$, $p12 = 5 \times 10^{-5}$) and posterior probabilities (PPH4 > 80%), four proteins (ROR1, FN1, APOA5, and ABO) exhibited significant genetic colocalization (PPH4 > 80%) (Figures 4A and S3). Data from the INTERVAL dataset and the KORA F4 study were employed to validate the replicability ($p < 0.05$) of three plasma targets (ABO, FN1, and ROR1) via the inverse-variance weighted (IVW) method, while APOA5 did not

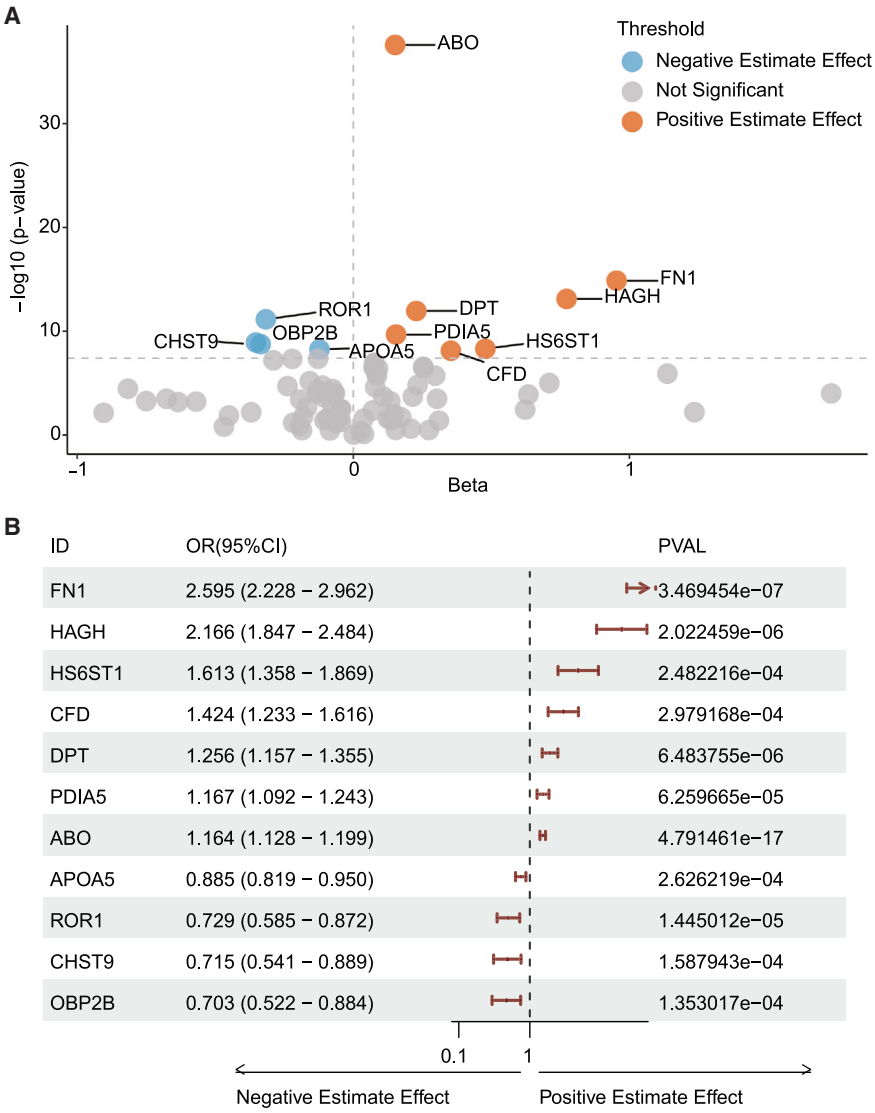

Figure 3. MR for associations between circulating proteins and PC risk

(A) Genetic variants from the pQTL in the deCODE dataset were employed for MR analysis. In the volcano plot, the x axis displays the beta values, while the y axis represents -log10 (P). Dots positioned above the horizontal dashed line indicate significant associations ($p < 6.7 \times 10^{-4}$ after Bonferroni correction). Red dots denote risk factors for PC, and blue dots indicate protective factors.

(B) The forest plot presents the odds ratios and 95% confidence intervals for PC and 11 circulating targets following Bonferroni correction. However, OBP2B did not meet the thresholds for pleiotropy and heterogeneity in subsequent tests. The dots represent the OR and the horizontal lines extending from each dot represent the 95% confidence intervals.

demonstrate consistent results (Beta = −0.162, $p = 0.244$) (Table 1). To further strengthen our findings, we conducted an additional summary-data-based MR (SMR) analysis with its corresponding colocalization testing. Consistent with our previous results, only these four proteins (ROR1, FN1, APOA5, and ABO) passed both the SMR significance threshold and colocalization analysis (Figure 4B; Table S3).

Elevated ABO and FN1 levels in peripheral blood and tumors are associated with reduced survival

A retrospective clinical cohort study was conducted, comprising 160 PC patients and 200 healthy controls enrolled between January and September 2022 at Zhongshan Hospital. Initial analysis of baseline characteristics revealed significant imbalances between the two groups in terms of age, gender, and diabetes status (all $p < 0.001$; Table S4). To address these disparities and minimize potential selection bias, we performed propensity score matching (PSM) to balance all baseline variables, resulting in 60 matched pairs of patients and controls (Table S4). Analysis of peripheral blood samples from this matched cohort using enzyme-linked immunosorbent assay (ELISA) demonstrated significantly elevated levels of ABO and FN1 proteins in PC patients compared to healthy controls, while ROR1 and APOA5 expression showed no significant differences between groups

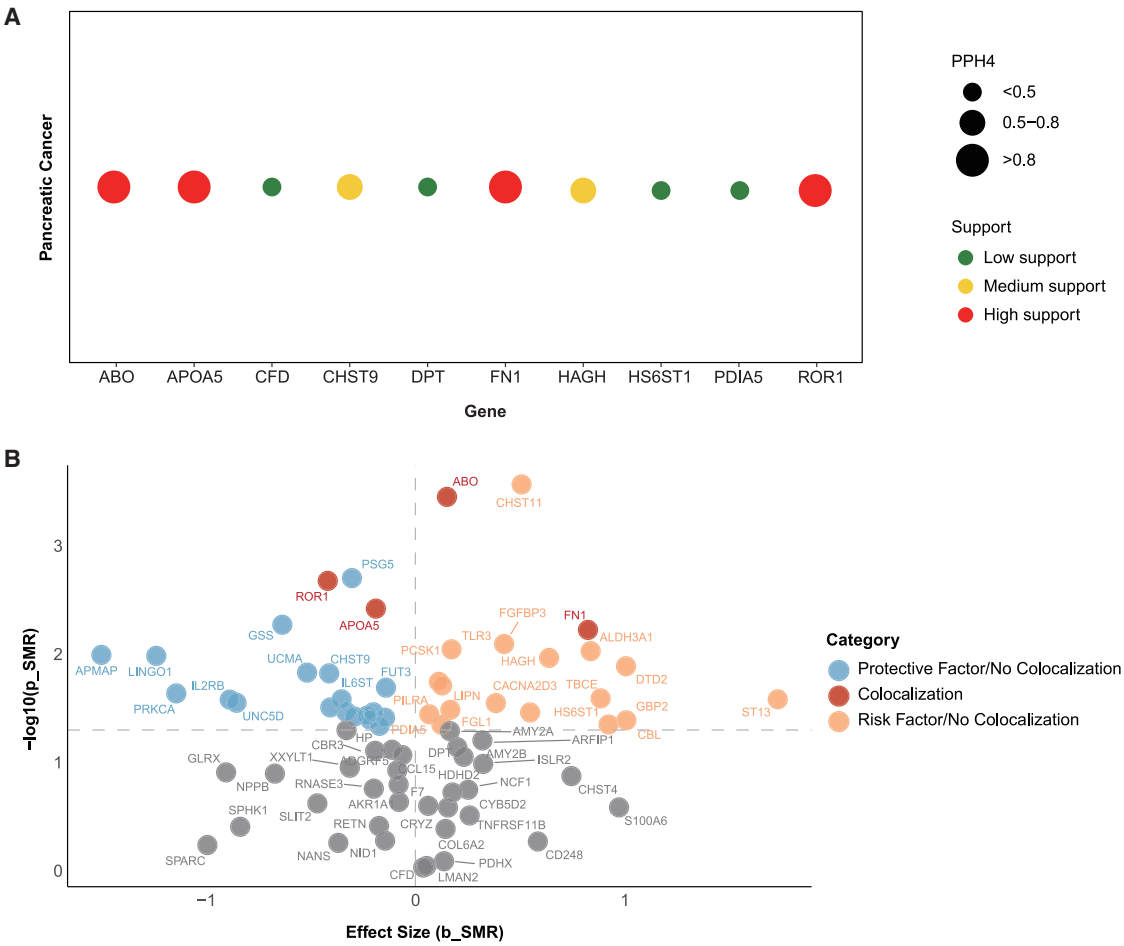

Figure 4. Regional association plot illustrating the colocalization analysis of four proteins causally associated with PC risk

(A) The circle size reflects the colocalization probability for H4 (PPH4), where values exceeding 0.8 indicate strong colocalization evidence between the two signals. The color gradient represents the strength of this evidence, with red signifying the highest level of support.

(B) Volcano plot showing results from SMR analysis using genetic variants from the deCODE pQTL dataset. The x axis represents the effect size (b_SMR), and the y axis shows the -log10(p_SMR). The horizontal dashed line indicates the significance threshold ($p < 0.05$). Red dots represent proteins showing significant associations with strong genetic colocalization for PC risk, while blue and brown dots indicate protective and risk factors without strong colocalization evidence, respectively.

(Figures 5A–5D). To account for potential confounding effects of demographic factors on biomarker levels, we conducted subgroup analyses as a sensitivity analysis. The matched cohort was stratified by three demographic factors: age (≥ 65 years), gender (male), and diabetes status (yes). These analyses confirmed that the expression patterns of ABO and FN1 remained consistent across all subgroups (interaction $p > 0.05$) (Figures S4). To further evaluate the diagnostic utility of these differentially expressed proteins, we performed ROC curve analyses and constructed confusion matrices. The area under the ROC curve (AUC) analysis revealed strong discriminatory power for both individual markers, with FN1 and ABO achieving AUC

Table 1. The MR results of four associated circulating targets in the discovery and replication cohorts

	PWAS		MR (IVW)				Colocalization
Protein	Z	P	Beta (D)	P (D)	Beta (R)	P (R)	PPH4
ABO	3.456	0.0005	0.152	4.79E-17	0.177	0.011	0.864
APOA5	−2.872	0.004	−0.122	2.63E-04	−0.162	0.244	0.854
FN1	2.223	0.026	0.954	3.47E-07	0.424	0.005	0.808
ROR1	−3.46	0.0005	−0.317	1.45E-05	−0.286	0.0001	0.900

Z, z score; P, *p* value; D, discovery cohort; R, replication cohort.

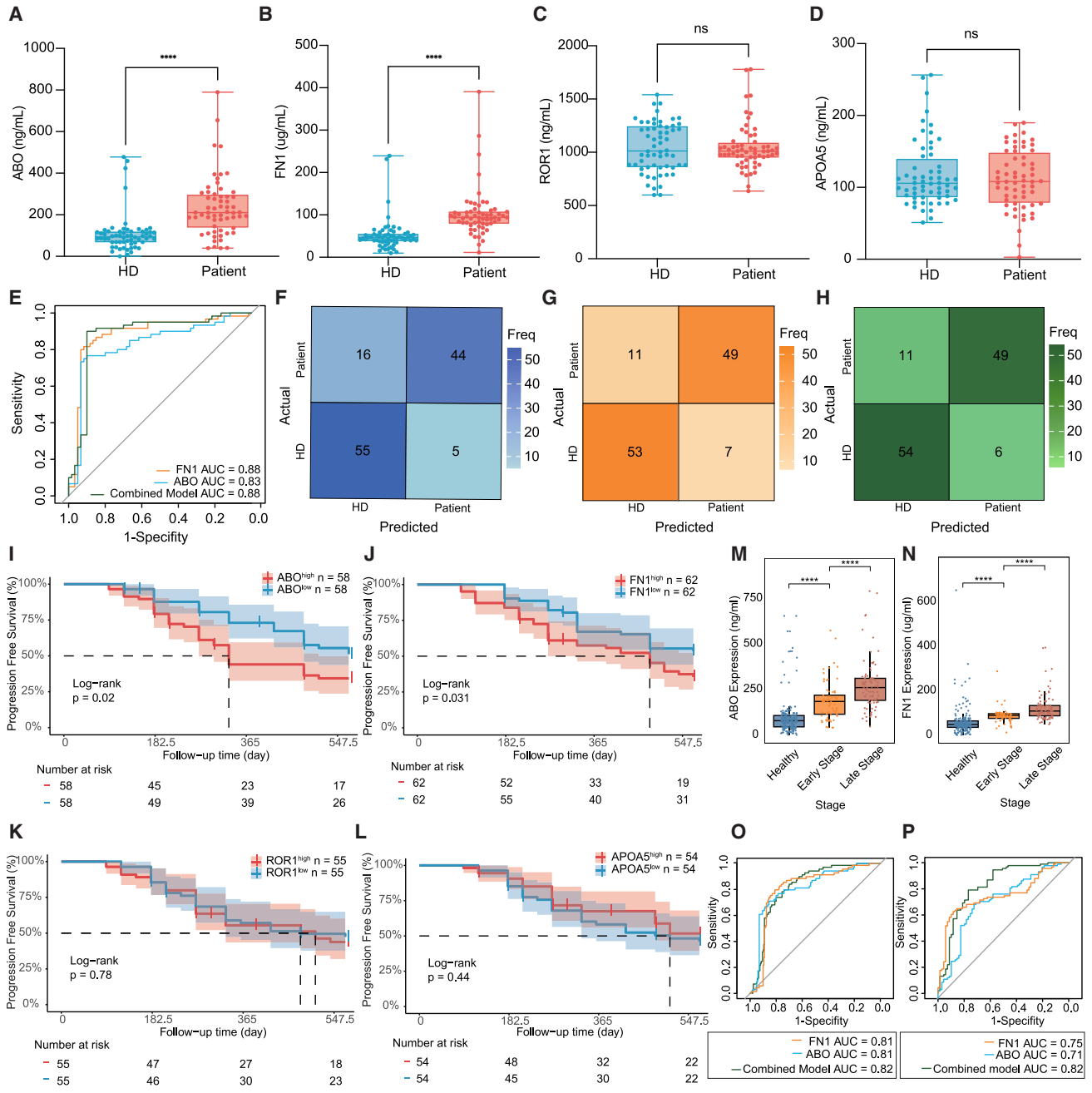

Figure 5. Blood expression levels of four causally associated proteins and their correlations with survival of the patients

(A–D) ELISA analysis comparing plasma concentrations of ABO, FN1, ROR1, and APOA5 proteins in 60 healthy donors (HD) and propensity-matched 60 PC patients demonstrates a significant distinction (****$p < 0.0001$, ns $p > 0.05$). Error bars represent the median and interquartile range. HD refers to healthy donors.

(E–H) Diagnostic performance of ABO and FN1 for PC. ROC curve for FN1, ABO, and their combined model in predicting PC (E), Prediction confusion matrix for ABO (F), Prediction confusion matrix for FN1 (G), Prediction confusion matrix for the combination of ABO and FN1(H).

(I–L) Progression-free survival analysis in PC patients stratified by median blood levels of the aforementioned proteins. The number at risk denotes the individuals still free from progression at the indicated time point. The shaded areas around the curves represent 95% confidence intervals.

(M–P) Expression and diagnostic performance of ABO and FN1 across different stages of PC. Boxplots showing ABO (M) and FN1 (N) expression levels across healthy participants, early-stage, and late-stage PC patients. ROC curves illustrating the diagnostic performance of ABO, FN1, and their combined signature for distinguishing early-stage PC from healthy participants (O). ROC curves illustrating the diagnostic performance of ABO, FN1, and their combined signature for distinguishing late-stage PC from early-stage PC (P). Error bars represent the median and interquartile range.

values of 0.88 and 0.83, respectively (Figure 5E). A multivariate logistic regression model combining both markers maintained robust diagnostic performance with an AUC of 0.88 (Figure 5E). Confusion matrix analysis revealed impressive diagnostic metrics for individual markers. ABO demonstrated high accuracy (0.825), sensitivity (0.9167), and specificity (0.7333) (Figure 5F), while FN1 showed comparable performance with accuracy of 0.85, sensitivity of 0.8833, and specificity of 0.8167 (Figure 5G). The combined marker model further enhanced diagnostic performance, achieving an accuracy of 0.8583, sensitivity of 0.9, and specificity of 0.8167 (Figure 5H). These comprehensive results strongly support the potential of FN1 and ABO as robust diagnostic biomarkers for PC, demonstrating both high sensitivity and specificity.

Tumor protein expression was stratified into high and low groups based on median values from the 160 PC patients, with PSM applied to correct baseline variable imbalances (Table S5). Survival analysis revealed that elevated ABO or FN1 expression significantly correlated with shorter progression-free survival (PFS), while ROR1 and APOA5 levels showed no such association (Figures 5I–5L), indicating ABO and FN1 as potential circulating prognostic markers for PC. Analysis of biomarker expression patterns across disease stages was performed using the complete cohort of 160 PC patients and 200 healthy participants. Based on the AJCC staging system, patients were stratified into early stages (stages 0, IA, IB, and IIA; n = 69) without lymph node metastasis or vascular invasion, and late stages (stages IIB, III, and IV; n = 91) with metastatic involvement. FN1 and ABO were consistently detectable in peripheral blood throughout all disease stages, with significantly elevated levels observed in early-stage PC patients compared to healthy controls. Both markers demonstrated a progressive increase correlating with disease advancement (Figures 5M and 5N). ROC curve analysis revealed robust early-stage diagnostic capability for both biomarkers, with individual AUC values of 0.81 when distinguishing between healthy individuals and early-stage patients. The combination of both markers yielded an AUC of 0.82 (Figure 5O). In discriminating between early and late-stage disease, individual markers showed moderate performance (AUC = 0.75 for FN1 and 0.71 for ABO), while their combination demonstrated enhanced diagnostic accuracy for metastatic disease (AUC = 0.82; Figure 5P).

In a second cohort comprising 196 surgically resected human PC tumors and their matched para-tumor tissues collected between March 2014 and September 2017, IHC analysis revealed elevated protein levels of ABO (151/196), FN1 (162/196), and ROR1 (170/196) in PC tumors, while APOA5 (133/196) was decreased in comparison to para-tumor tissues (Figure 6A). Expression levels of ABO, APOA5, FN1, and ROR1 in PC tumors were subsequently categorized into four tiers-negative, weak, moderate, and strong-based on IHC scores. Negative and weak classifications were combined into the low-expression group, while moderate and strong were grouped as high-expression (Figure 6B). PSM was not applied as the baseline variables were already well-balanced (Table S6). Patients with ABO^{high} or $FN1^{high}$ tumors exhibited significantly reduced median survival compared to those with lower expression levels (Figures 6C and 6D). In contrast, no survival differences were observed between the high and low groups of ROR1 or APOA5 (Figures 6E and 6F). These outcomes align with those from the initial retrospective clinical cohort study involving blood samples, supporting ABO and FN1 as potential therapeutic targets and prognostic biomarkers.

Identification of actionable druggable risk targets

To identify potential repurposed drugs for PC therapy, ABO, FN1, ROR1, and APOA5 were evaluated as therapeutic targets using the ChEMBL, DrugBank, and OpenTargets datasets. While no clinical trial drugs were identified for ABO and APOA5, five compounds were found to target FN1, including ocriplasmin, which has FDA approval for symptomatic vitreomacular adhesion (sVMA) therapy,[17] with other compounds currently in clinical trials for various cancers. Additionally, the anti-ROR1 antibody cirmtuzumab was being tested in clinical trials for hematologic malignancies[18,19] and multiple solid tumors (NCT04504916). These data highlight FN1 and ROR1 as promising targets for drug repurposing in PC treatment (Table 2).

DISCUSSION

Identifying additional biomarkers for diagnosis and therapeutic targets remains essential for managing the heterogeneous nature of PC. Circulating proteomics, with its minimally invasive and comprehensive scope, presents a promising method for discovering functional biomarkers. We have established a comprehensive pipeline to explore plasma biomarkers and potential therapeutic targets in PC, based on a PWAS-MR-colocalization-clinical cohort validation approach. This pipeline boasts a notable strength in its integration of vast population genetics information. The use of PWAS and MR enables the identification of shared genetic links between circulating proteins and PC, delivering strong evidence at both the high-throughput and genetic-causal levels. Furthermore, two independent clinical cohorts were employed to validate the causal relationship between protein levels and PC risk, offering significant insights into their potential application as biomarkers for PC monitoring.

By utilizing GWAS-meta analysis and ARIC study datasets, 78 circulating proteins were identified as associated with PC risk, implicating multiple secretory pathways, including the collagen-containing extracellular matrix, extracellular matrix binding, collagen binding, and vesicle lumen, suggesting these circulating proteins may be secreted by tumor or other solid tissues. Of the 78 proteins, four (ROR1, FN1, ABO, and APOA5) showed the strongest causal associations with PC, as determined through PWAS, MR, Bonferroni correction, and colocalization analyses. We carefully considered the statistical methodology to ensure robust and reliable results while maintaining appropriate control over false positive findings. The choice of Bonferroni correction as our primary statistical method was based on its well-established role in controlling the family-wise error rate (FWER) in large-scale studies including MR.[20–24] While this method is known for its conservative nature, our comprehensive analysis demonstrated its appropriateness for our study design. To validate our methodological choice and address potential concerns about the stringency of Bonferroni correction, we conducted a complementary analysis using the false discovery rate (FDR) method. This analysis identified 24 additional proteins that met the less

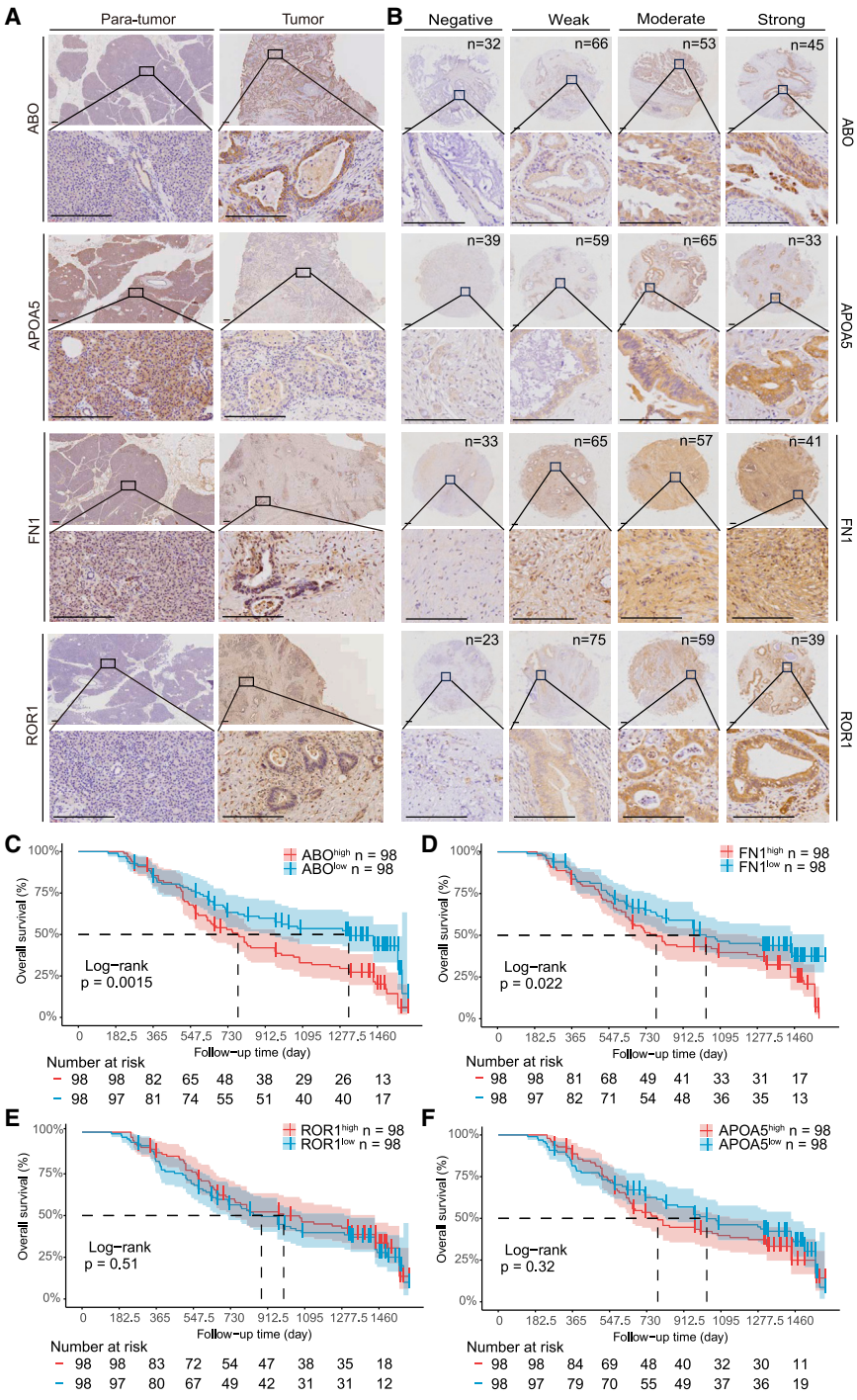

Figure 6. Tumor expression levels of four causally associated proteins and their correlations with survival of the patients

(A) Immunohistochemical staining of ABO, FN1, APOA5, and ROR1 in paired PC tumors and adjacent non-tumor tissues (n = 196) is displayed with a scale bar of 150 μm.

(B) PC tumors (n = 196) are categorized into four groups according to protein expression levels, with the scale bar set at 150 μm. The "n" in the upper right corner indicates the sample size at each expression level.

(C–F) Overall survival of PC patients stratified by median tumor expression levels of the four proteins. The number at risk reflects the count of patients still surviving at each time point. The shaded areas around the curves represent 95% confidence intervals.

stringent FDR threshold. However, crucially, none of these additional proteins passed our subsequent colocalization analysis (PPH4 < 0.8) (Figure S5). This finding provides strong support for the robustness of our Bonferroni-based approach, as it suggests that the more stringent threshold did not exclude any proteins that would have met our full set of criteria for causal association. The consistency between results from these two different statistical approaches—the more conservative Bonferroni correction and the more lenient FDR method—reinforces the reliability of our findings. This dual analytical approach not only validates our methodological choices but also enhances the confidence in our identified protein-pancreatic cancer associations.

Among the four proteins, FN1 emerged as the most clinically relevant target for PC. FN1 was significantly differentially expressed in both tumor tissues and peripheral blood samples from our real-world clinical cohorts of PC patients. Although FN1 protein overexpression in PC tumors has previously been linked to PC,[25–29] its strong correlation with both PC risk and patient survival through elevated levels in blood and tumors was not well understood. FN1 (fibronectin), a widely expressed high-molecular-weight glycoprotein in the ECM,[30] plays a significant role in promoting matrix metalloproteinase (MMP) production and interacting with ECM proteins. The observed elevation of FN1 in both blood and tumor tissues, along with its association with poor survival outcomes, further supports the hypothesis that MMP-cleaved collagens contribute to the metabolic activity and progression of PC tumors.[31–35] Notably, collagen-III and MMP1 have already been employed for early detection of hepatocellular carcinoma,[36] reinforcing the likelihood that FN1 could serve as a diagnostic and prognostic marker for PC.

While FN1 is an established therapeutic target in various cancers,[25,37] with approved drugs demonstrating favorable safety profiles in clinical settings, its role in PC is particularly notable due to the unique tumor microenvironment characteristic of PC.[25] Unlike many other solid tumors, PC is characterized by

Table 2. The actionable repurposing drugs of the causally associated targets

Proteins	Drug	Clinical Status	Molecule Type	Mechanism	Target Disease & Clinical Trials
FN1	OCRIPLASMIN	Approved	Enzyme	Fibronectin proteolytic enzyme	Uveitis (NCT01194674)
					Macular Degeneration (NCT00996684)
					Retinal Vein Occlusion (NCT02747030)
					Retinal Perforations (NCT01429441)
					Stroke (NCT00123305, NCT00123266)
					Venous Thrombosis (NCT00428129)
	L19TNFA	Phase 2 Clinical Trial	Antibody	Fibronectin binding agent	Basal Cell Carcinoma (NCT05329792)
					Malignant Melanoma (NCT02076633, NCT02938299)
	L19IL2	Phase 3 Clinical Trial			Melanoma (NCT02938299, NCT02076633, NCT01253096)
					Pancreatic Cancer (NCT01198522)
	L19SIP 131I	Phase 2 Clinical Trial		Fibronectin other	Neoplasms (NCT01242943)
	AS-1409	Phase 1 Clinical Trial		Fibronectin binding agent	Malignant Melanoma, Renal cell carcinoma (NCT00625768)
ROR1	CIRMTUZUMAB	Phase 2 Clinical Trial	Antibody	Inhibitor	Gastric Cancer, Non-Small Cell Lung Carcinoma, Breast Cancer (NCT04504916)
					Diffuse Large B-Cell Lymphoma (NCT05406401)
					Chronic Lymphocytic Leukemia (NCT04501939)
					Prostate Cancer (NCT05156905)

an extensive desmoplastic reaction, resulting in a dense stromal compartment that can comprise up to 90% of the tumor volume.[38] This distinctive feature of PC significantly influences the behavior of FN1 and its interactions within the tumor microenvironment. In PC, FN1 closely interacts with SPARC (secreted protein acidic and rich in cysteine), a matricellular protein that regulates cell-ECM interactions and tumor progression.[39] This interaction is particularly important in PC compared to other cancers due to the heightened expression of SPARC in the pancreatic tumor stroma.[40] SPARC enhances fibronectin matrix deposition and modulates FN1's effects on cancer cell survival and proliferation, likely through integrin signaling pathways.[41] This FN1-SPARC interaction contributes to the unique stromal architecture of PC, which is not as prominent in many other cancer types.[42] Furthermore, the extensive desmoplasia in PC creates a hypoxic environment, which can upregulate FN1 expression through hypoxia-inducible factor (HIF)-1α.[43] This hypoxia-induced FN1 overexpression is more pronounced in PC compared to less desmoplastic tumors, contributing to increased chemoresistance and poor drug delivery—challenges that are particularly severe in PC treatment.[44] The role of FN1 in promoting pancreatic stellate cell (PSC) activation is another aspect that distinguishes its function in PC from other cancers.[45,46] PSCs, when activated, are the primary source of ECM proteins in PC, including FN1.[46,47] This creates a feedforward loop where FN1 promotes PSC activation, leading to more FN1 production, which is a cycle more prominent in PC than in many other cancer types.[48] Additionally, FN1's interaction with integrins in PC leads to the activation of focal adhesion kinase (FAK) and subsequent stimulation of the AKT/CREB pathway.[49] This pathway is particularly important in PC as it promotes cell survival and inhibits chemotherapy-induced apoptosis, allowing PC cells to generate an ECM that supports survival and increases overall tumor resistance to treatment.[50] While this mechanism exists in other cancers, the extensive stromal component of PC amplifies its effects.

For instance, ocriplasmin, an FDA-approved drug for diabetic retinopathy treatment,[51] presents a potential new strategy for PC therapy due to its role as an alpha-2 antiplasmin reducer and its truncated form of plasmin, which targets FN1. Ocriplasmin's capacity to degrade the protein matrix responsible for mechanical compression, lower interstitial fluid pressure, and enhance drug uptake suggests its broader applicability in slowing the progression of other desmoplastic cancers. Additionally, FN1 splice variant ED-B (ED-B), consisting of 91 amino acids, is able to insert into the FN1 molecule during the active tissue remodeling associated with angiogenesis which is critical to tumorigenesis,[52] and has been identified in the vascular regions of PC.[53,54] L19-IL2, an anti-ED-B antibody, significantly suppressed PC tumor growth and metastasis in preclinical mouse models, with its safety validated in early clinical trials involving patients with metastatic melanoma[55] and advanced renal cell carcinoma.[56] Furthermore, a multicenter European phase 1 trial (NCT01198522), employing an open-label, non-randomized, single-group assignment design, corroborated the safety and efficacy of L19-IL2 in combination with gemcitabine for advanced PC, although the trial was terminated due to lack of recruitment. These data indicate that FN1 represents a promising candidate for drug repurposing in PC, offering a rapid transition to clinical trials and increasing the likelihood of achieving impactful therapeutic outcomes.

Aside from FN1, ABO also exhibited significant differential expression in both pancreatic tumor tissues and peripheral blood. The ABO gene encodes the glycosyltransferase enzyme responsible for determining blood types through major alleles (A and B).[57] Our findings suggest that tumor-secreted ABO may contribute significantly to PC progression, highlighting the need to identify novel glycosylated substrates of ABO. Although variants in the ABO locus have been linked to an elevated risk of PC in individuals with non-O blood types,[58,59] and the relationship between ABO at both genetic and transcriptional levels and PC susceptibility is well-established,[57,60–62] a direct causal link between ABO protein expression and PC risk has not been previously demonstrated. Given that proteins are the direct mediators of cellular functions, our results firmly establish the connection between ABO and PC risk. However, unlike FN1, no monoclonal antibodies or antagonists targeting ABO have yet progressed to clinical trials via ChEMBL, Drugbank, and OpenTargets datasets. From both clinical and economic standpoints, developing ABO as a therapeutic target for PC may be a longer-term endeavor, as it requires extensive drug development and validation.

Through comprehensive analysis of peripheral blood plasma proteomics using MR and PWAS with stringent Bonferroni correction, we identified a genetic-level causal relationship between ROR1 and PC. Our analysis revealed an inverse correlation between ROR1-associated genetic and PC-related variants,[63] suggesting ROR1 as a potential protective factor against PC in peripheral blood. This finding presents an intriguing contrast to previous tissue-based studies of ROR1, highlighting a possible context-dependent function of this protein. ROR1, a receptor tyrosine kinase (RTK) family member, has been extensively studied as an oncogenic factor[64] due to its reported specific expression in tumor cells, including PC, and minimal expression in normal tissues.[65] This expression profile has led to multiple clinical trials investigating ROR1-targeted therapies for various cancers, including PC.[66,67] Traditional histological and functional analyses of PC tissue have linked ROR1 to malignancy-related mechanisms, including enhanced cell proliferation and epithelial-mesenchymal transition (EMT).[68] However, these studies primarily focused on isolated tumor tissues or cell cultures, potentially overlooking systemic effects.

The apparent dichotomy in ROR1's function between blood and tissue compartments aligns with established paradigms in cancer biology. Similar tissue-specific effects have been observed with other key proteins, such as IL-6,[69] which demonstrates both pro- and anti-inflammatory properties, and TGF-β,[70] which acts as a tumor suppressor in early stages but promotes tumor progression in advanced cancer. Recent clinical data appears to support this complexity. Despite significant investment in ROR1-targeted therapies, interim results have been mixed, with limited efficacy in solid tumors except for specific lymphomas.[71] Furthermore, recent pan-tissue analyses have challenged the assumed tumor-specific expression of ROR1, revealing significant expression in various normal tissues.[72] While direct evidence for ROR1's protective mechanism in circulation remains to be established, our findings suggest that its function may be highly context-dependent. Circulating blood, which interfaces with multiple organs and tumor sites, may provide a more comprehensive view of systemic disease states compared to isolated tumor samples. The source and precise role of circulating ROR1 require further investigation, particularly given its known expression patterns in normal lymphocytes and hematologic malignancies.[73] These findings underscore the need for a more nuanced understanding of ROR1's biological functions and its potential utility as part of multi-marker panels for disease monitoring.

It is important to note that our study's identification of both risk factors (ABO, FN1) and protective factors (ROR1, APOA5) through genetic colocalization analysis reflects the complex nature of PC biology. While our survival analyses emphasized FN1 and ABO due to their significant associations with patient outcomes, the identification of protective factors, particularly ROR1, provides crucial insights into PC pathophysiology. The observed tissue-specific dichotomy of ROR1 function—protective in plasma but potentially harmful in tumor tissue—adds another layer of complexity to therapeutic development. This finding suggests that careful consideration is needed when developing targeted therapies, as systemic inhibition might have opposing effects in different compartments. Similarly, while APOA5's protective association with PC did not translate into significant survival outcomes in our analysis, its identification contributes to our understanding of potential protective mechanisms in PC development. These findings underscore the importance of comprehensive protein analysis in cancer research, where both risk and protective factors can provide valuable insights for therapeutic development, particularly in the context of PC's complex tumor microenvironment and systemic effects.

In summary, this study identifies causative circulating proteins associated with PC risk and highlights FN1 as a promising therapeutic target. Given that FN1 is already addressed by existing drugs, it offers substantial translational potential for PC treatment. Through an integrated multi-omics approach and analysis of two single-center retrospective clinical cohorts, the research provides robust evidence supporting circulating proteins as viable therapeutic targets, along with corresponding biomarkers. Additionally, potential molecular mechanisms involving these proteins in PC pathogenesis are explored. This work contributes new insights into both the therapeutic landscape and the molecular understanding of PC.

Limitations of the study

Our study is subject to several limitations. First, although 1,345 instrumental variants from the ARIC study were utilized, they did not capture the full spectrum of actionable and druggable circulating proteins. Expanding the protein range could enhance the identification of more robust therapeutic targets and blood biomarkers. Secondly, we incorporated multiple data sources for a comprehensive meta-analysis and used diverse protein validation cohorts, including the ARIC study, deCODE, Interval, and KORA F4, all of which have been widely utilized in research aimed at identifying drug targets for cancer and inflammatory diseases, including breast cancer[74] and COVID-19.[75] However,

we acknowledge that our study primarily focuses on individuals of European descent, which may introduce selection bias. This limitation could affect the generalizability of our findings to populations from Asia, Africa, or the Middle East. Therefore, we emphasize the critical need for more inclusive, multiethnic research initiatives. Such expanded studies are essential for identifying population-specific therapeutic targets and ensuring the broader applicability of our findings across diverse ethnic groups. Thirdly, our study specifically targeted cis instrumental variables linked to pQTLs, which unfortunately did not account for *trans*-acting regions or the intricate dynamics of gene isoforms, potentially impacting the accuracy of the causal estimates. Besides, our initial protein screening using the ARIC cohort (ages 45–64) had potential age-related limitations for PC research. We addressed this through a comprehensive pipeline integrating PWAS, MR, colocalization analysis, and clinical validation across diverse cohorts. This approach effectively mitigated the initial limitations and enhanced the generalizability of our findings, as evidenced by the consistent identification of key proteins like ABO and FN1 across different age groups. Furthermore, while two cohorts were used to evaluate the feasibility of identified targets as therapeutic candidates with corresponding biomarkers, the limited sample size, drawn from a single center in Shanghai, should be acknowledged. To rigorously confirm the efficacy of these targets, larger-scale drug sensitivity tests, extensive animal studies, and prospective research are required.

RESOURCE AVAILABILITY

Lead contact

For additional details or specific inquiries, please reach out to the lead contact, Lei Zhang (zhang.lei@zs-hospital.sh.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- GWAS summary statistics from the UK Biobank were sourced from Neale Lab (http://www.nealelab.is/uk-biobank), while those from the FinnGen consortium (R9 release) were accessed via https://finngen.fi/en. Human blood pQTL data used in PWAS were retrieved from http://nilanjanchatterjeelab.org/pwas/. *Cis*-pQTLs from deCODE, employed in mendelian randomization and colocalization analyses, were acquired from https://www.decode.com/. All raw data including immunostaining and ELISA were uploaded to Mendeley Data (https://data.mendeley.com/datasets/jyyvrrsz44/1).
- This paper does not report the original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

H.F., H.S., L.Z., X.H., and L.L. supervised and designed the study; H.F., Z.C., F.Y., F.M., and J.F. conducted the statistical analysis and experiments; H.S., H.F., and Z.C. drafted the manuscript; H.S., L.Z., X.H., and L.L. provided guidance and made revisions to the manuscript; H.Y., Y.G., H.X., Y.L., R.L., and W.L. collected experimental samples and clinical data. All authors contributed to and critically reviewed the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
ABO Antibody	Affinity	Cat#DF6481; RRID: AB_2838443
Fibronectin Antibody	Affinity	Cat#AF5335; RRID: AB_2837820
ROR1 Monoclonal antibody	Proteintech	Cat#66923-1-1g; RRID: AB_2882250
Apolipoprotein AV (APOA5) Mouse Monoclonal Antibody	Origene	Cat#TA507140; RRID: AB_2624105
Biological samples		
Human peripheral blood	Cohort from Zhongshan Hospital	Healthy donors and pancreatic cancer patients
Human pancreatic cancer tumor tissue	Cohort from Zhongshan Hospital	Pancreatic cancer patients
Deposited data		
pQTLs Weights for PWAS	Zhang, Chaterjee et al.[12]	nilanjanchatterjeelab.org/pwas/
pQTLs for MR	Ferkingstad et al.[76]	https://www.decode.com/
GWAS of Pancreatic Cancer	FinnGen R9.[77]	https://r9.finngen.fi/
	UK Biobank from Neale Lab.	https://www.nealelab.is/uk-biobank
pQTLs of ABO for MR replication cohort	Sun et al.[78]	https://www.ebi.ac.uk/gwas/downloads/summary-statistics
pQTLs of FN1 for MR replication cohort	Suhre et al.[79]	https://metabolomips.org/pgwas/
pQTLs of ROR1 for MR replication cohort	Sun et al.[78]	https://www.ebi.ac.uk/gwas/downloads/summary-statistics
pQTLs of APOA5 for MR replication cohort	Sun et al.[78]	https://www.ebi.ac.uk/gwas/downloads/summary-statistics
Software and algorithms		
TwoSampleMR R package	Hemani et al.[80]	https://github.com/MRCIEU/TwoSampleMR
Coloc R package	Wallace et al.	https://CRAN.R-project.org/package=coloc
METAL	Abecasis et al.[81]	https://csg.sph.umich.edu/abecasis/metal/
LiftOver	Hyun et al.	https://genome.sph.umich.edu/wiki/LiftOver
FUSION	Gusev et al.[16]	http://gusevlab.org/projects/fusion/
PLINK	Sham et al.[82]	https://www.cog-genomics.org/plink/1.9/
enrichplot R package	Yu et al.	https://bioconductor.org/packages/release/bioc/html/enrichplot.html
ggplot2 R package	Wickham et al.[83]	https://cran.r-project.org/web/packages/ggplot2/index.html
MatchIt R package	Ho et al.[84]	https://cran.r-project.org/web/packages/MatchIt/vignettes/MatchIt.html#ref-ho2007
survival R package	Therneau et al.	https://cran.r-project.org/web/packages/survival/index.html
survminer R package	Kassambara et al.	https://cran.r-project.org/web/packages/survminer/index.html
pROC R package	Robin et al.	https://cran.r-project.org/web/packages/pROC/index.html
caret R package	Kuhn et al.	https://cran.r-project.org/web/packages/caret/index.html
Code deposited in Zenodo	Feng et al.	https://doi.org/10.5281/zenodo.14517486

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Other		
Human ABO quickly Elisa Kit	Weiaobi	Cat#EH11248
Human FN quickly Elisa Kit	Weiaobi	Cat#EH10725
Human ROR1 quickly Elisa Kit	Weiaobi	Cat#EH11260
Human APOA-V quickly Elisa Kit	Weiaobi	Cat#EH11252

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

This meta-analysis incorporated GWAS data of PC obtained from the UK Biobank (accessed via Neale Lab) and the FinnGen consortium. The included UK Biobank cohort was composed of 403 European individuals with PC and 360,791 controls, with ages spanning from 40 to 70 years during the period from 2006 to 2010. The ethical clearance for this cohort was granted by the National Information Governance Board for Health and Social Care and the NHS North West Multicenter Research Ethics Committee (REC; approval number: 11/NW/0382). The included FinnGen consortium cohort consisted of 1,416 European patients and 287,137 controls with mean age 53 when participating. Participants within the FinnGen consortium furnished their informed consent in line with the stipulations of the Finnish Biobank Act. The research protocol of the FinnGen study received endorsement from the Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS), with the approval number being HUS/990/2017. In addition, two independent clinical cohorts from Zhongshan Hospital were incorporated. One cohort comprised 160 PC patients and 200 healthy Chinese controls, with a median age ranging from 53 to 66 years, and was enrolled in 2022. The other cohort consisted of 196 surgically resected PC participants, with a median age of 65 years, and was recruited between 2014 and 2017. These cohorts adhered to the ethical standards set by the Declaration of Helsinki and were approved by the Ethics Committees of Zhongshan Hospital, Fudan University (approval number: B2023 - 342). The aforementioned participants diagnosed with PC were assigned to the relevant experimental groups. The diagnosis of PC was conducted in accordance with the International Classification of Diseases, 10th Revision (ICD-10).

METHOD DETAILS

Human blood pQTL for PWAS

A total of 1,345 plasma protein data points were acquired from the ARIC study, which included 7,213 European participants (http://nilanjanchatterjeelab.org/pwas/).[12] Comprehensive details can be accessed in the original publication.

Meta-GWAS for Pancreatic Cancer

A meta-analysis was conducted utilizing data from the UK Biobank (http://www.nealelab.is/uk-biobank) and the FinnGen consortium database (version R9, https://finngen.fi/en),[77] integrating GWAS on PC. The study included 403 European PC patients and 360,791 controls from the UK Biobank, alongside 1,416 European PC patients and 287,137 controls from the FinnGen database. Meta-analysis was performed using inverse-variance weighting and fixed effects via METAL software (version 2011-03-25, https://csg.sph.umich.edu/abecasis/Metal).[81] After assessing genomic inflation, a quantile-quantile (QQ) plot was generated to assess the alignment between observed and expected P-value distributions under the null hypothesis (Figure S1A), thereby mitigating false positive risks. A Manhattan plot visualizing the genome-wide distribution of P-values for genetic variants was also produced (R package qqman, https://github.com/stephenturner/qqman, Figure S1B).[85,86]

PWAS

The PC meta-GWAS was systematically integrated with human blood proteomes from the ARIC study. PWAS analysis was performed using FUSION (https://github.com/gusevlab/fusion_twas),[16] applying weights derived from cis summary statistics of plasma proteins with significant non-zero cis-heritability ($P < 0.01$), estimated via GCTA. Additionally, linkage disequilibrium (LD) reference weights tailored to individuals of EA from the 1000 Genomes Project (https://data.broadinstitute.org/alkesgroup/FUSION/LDREF.tar.bz2) were incorporated to enhance accuracy. Genetic variant locations were mapped across different versions of the human reference genome using LiftOver (http://genome.ucsc.edu/cgi-bin/hgLiftOver). The genetic influence of PC, quantified by the PC GWAS z-score, was combined with protein weights through a linear summation of z-score × weight for independent SNPs at the locus, utilizing the FUSION tool.

Instrumental variables selection

IVs for PWAS-significant proteins of PC were selected based on summary statistics from the deCODE proteome study. IVs were identified following specific selection criteria: (1) cis-pQTL variants confined to a ±1 MB region around the target protein-coding

genes; (2) exclusion of variants located in the Major Histocompatibility Complex (MHC) region (chr6 25.5–34.0 Mb); (3) a significant pQTL-serum protein association ($P \leq 5 \times 10^{-8}$); (4) independent pQTLs identified through LD clumping ($r^2 < 0.1$) using PLINK (v1.9)[82]; and (5) SNPs demonstrating a strong correlation with the exposure, with an F statistic >10. The F statistic was calculated as $F = R^2(N-K-1)/K(1-R^2)$, with R^2 derived from $R^2 = 2 \times MAF \times (1-MAF) \times Beta^2$.[87] The replication cohort for plasma protein pQTL data was sourced from the INTERVAL study by Sun et al.,[78] encompassing measurements of 2,994 plasma proteins in 3,301 European participants using the SomaLogic platform. Validation data were further supplemented by the KORA F4 study by Suhre et al.,[79] which included 1,000 European participants and utilized the SOMAscan platform.

Mendelian Randomization

In the MR analysis, cis-pQTLs from the discovery cohorts served as the exposure variable, while outcomes were derived from PC meta-GWAS results. MR analyses utilized the TwoSampleMR R package (https://mrcieu.github.io/TwoSampleMR/). The inverse-variance weighted (IVW) method was applied for instruments with multiple variants, and the Wald-ratio method was used for single-variant instruments.[88] Cochrane's Q statistics assessed heterogeneity across genetic instruments,[89] and MR-Egger was implemented to detect horizontal pleiotropy.[90] The significance threshold was set at $P < 6.7 \times 10^{-4}$ (0.05/74) after Bonferroni correction. In the replication cohort, MR was repeated for the identified proteins or genes with a significance threshold of $P < 0.05$.

Bayesian Colocalization analysis

Colocalization analysis using the R package coloc (https://github.com/chr1swallace/coloc) was performed to evaluate the causal potential of disease-associated variants across expression, protein, and phenotypic levels. Five hypotheses (H0-H4) were defined as follows: $p1 = 1 \times 10^{-4}$, representing the probability that a causal variant is exclusively associated with plasma proteins; $p2 = 1 \times 10^{-4}$, denoting the likelihood of a variant solely affecting PC; and a joint prior probability, $p12 = 5 \times 10^{-5}$, indicating the chance of a variant influencing both traits. A posterior probability for H4 (PPH4) exceeding 80% was considered indicative of a strong association. Additionally, sensitivity analysis on prior values conducted via the R package coloc[91] confirmed that $p12 = 5 \times 10^{-5}$ produced consistent results across different conditions (Figure S2).

Establishment of single-center clinical cohorts

To assess the clinical potential of the identified actionable causal targets for PC, two cohorts were established, adhering to the ethical standards set by the Declaration of Helsinki and approved by the Ethics Committees of Zhongshan Hospital, Fudan University (B2023-342). Prior to participation, all patients provided written informed consent.

The first cohort comprised 160 patients with pancreatic cancer who received either single or combined modalities of surgical treatment (pancreaticoduodenectomy or distal pancreatectomy), neoadjuvant chemotherapy, chemoradiotherapy, immunotherapy, or targeted therapy, along with 200 healthy controls recruited at Zhongshan Hospital between January and September 2022. To address potential selection bias and balance baseline characteristics between groups, we performed propensity score matching (PSM) using the 'MatchIt' package in R (version 4.2.3). The propensity score was calculated using a logistic regression model that included demographic variables such as age, gender, and presence of diabetes. A 1:1 nearest neighbor matching algorithm was applied with a caliper width of 0.2 standard deviations of the logit of the propensity score, resulting in 60 matched pairs of patients and controls. Circulating protein levels were quantified using enzyme-linked immunosorbent assay (ELISA) on fresh peripheral blood samples from all participants, focusing on four specific proteins: ABO, FN1, APOA5, and ROR1. Protein expression differences between matched patients and healthy individuals were analyzed using two-tailed Mann-Whitney tests.

To evaluate the consistency of our findings across different demographic characteristics, we conducted subgroup analyses stratified by potential confounding factors: age ($\geq$ 65 years vs. < 65 years), gender (male vs. female), and diabetes status (YES vs. NO). Within each subgroup, we compared the expression levels of ABO and FN1 between pancreatic cancer patients and healthy controls. Interaction tests were performed to assess whether these demographic factors significantly modified the association between protein levels and disease status. A $P > 0.05$ for interaction terms indicated that the relationship between protein expression and disease status remained consistent across subgroups.

The second retrospective analysis was conducted on 196 consecutive PC patients who underwent pancreaticoduodenectomy at Zhongshan Hospital from March 2015 to September 2017. Tumor and adjacent tissue samples during surgery were collected for immunohistochemistry (IHC) analysis, targeting the expression of four circulating markers (ABO, FN1, APOA5, and ROR1) in both tumor and para-tumor tissues. IHC scores, based on expression levels in epithelial cells or the tumor microenvironment (TME), were categorized into four groups: negative, weak, moderate, and strong. Tumors with negative or weak scores were classified as the low-expression group, while those with moderate or strong scores were assigned to the high-expression group. The primary endpoint was overall survival (OS), measured from the date of diagnosis to death or last follow-up.[34] The association between expression levels and survival outcomes was evaluated using log-rank tests.

Univariate and multivariate logistic regression analyses

Univariate and multivariate logistic regression analyses were performed to evaluate the association between protein levels and pancreatic cancer diagnosis. For univariate analysis, each potential predictor variable was individually assessed. Variables with $P < 0.1$ in univariate analysis were subsequently included in the multivariate model to adjust for potential confounding factors.

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the strength of associations. Continuous variables were standardized before entering the regression models to facilitate comparison of effect sizes. All statistical analyses were performed using R software (version 4.2.3), and two-sided P less than 0.05 were considered statistically significant.

Diagnostic performance analysis

To evaluate the diagnostic performance of plasma FN1 and ABO levels, we performed receiver operating characteristic (ROC) curve analysis using the 'pROC' package in R (version 4.2.3). ROC curves were generated by plotting sensitivity against 1-specificity at various threshold settings. The area under the ROC curve (AUC) was calculated to quantify the overall diagnostic accuracy. We constructed ROC curves for FN1 and ABO individually and in combination using a multivariate logistic regression model. The optimal cutoff values for classifying PC versus healthy controls were determined using Youden's index (maximizing the sum of sensitivity and specificity). Using these cutoff values, confusion matrices were constructed using the 'caret' package in R to provide detailed information on true positives, true negatives, false positives, and false negatives, allowing us to calculate accuracy (proportion of correct predictions), sensitivity (true positive rate), and specificity (true negative rate). For the combined model of FN1 and ABO, predictions were based on the probability outputs from the multivariate logistic regression model.

Actionable druggable targets

To determine if any approved or clinically tested medications target the identified causative proteins, the ChEMBL dataset (version 32, https://www.ebi.ac.uk/chembl/),[92] Drugbank dataset (version 5.1.10, https://go.drugbank.com/),[93] and OpenTargets platform (https://platform.opentargets.org/)[94] were analyzed. These resources provided detailed information on the disease diagnosis and treatment authorization status associated with the relevant compounds.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses and data visualization were conducted using R software (version 4.2.3) and GraphPad Prism 9.4.1. In the context of two-group comparisons, the Student's t-test was utilized when the data followed a normal distribution, while the Two-tailed Mann-Whitney test was employed for non-normal distributions. To mitigate potential selection bias and ensure the balance of baseline characteristics between groups, PSM with a 1:1 ratio and a caliper of 0.2 standard deviations was implemented. Univariate and multivariate logistic regression analyses were carried out to assess the relationship between protein levels and PC. The log-rank test was applied to determine the association between the expression levels of the targeted protein and survival outcomes. The ROC curves were used to evaluate the diagnostic performance of plasma FN1 and ABO levels. All the detailed statistical analyses can be located in the corresponding METHOD DETAILS and LEGEND. Two-sided *P* less than 0.05 were considered statistically significant.