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Autoantibody-targeted TAAs in pancreatic cancer: A comprehensive analysis

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ABSTRACT

Background: Pancreatic cancer is one of the leading causes of cancer mortality and lacks efficient biomarkers for early diagnosis. In the early stages of pancreatic cancer, humoral immunity can respond to a certain amount of tumor-associated antigens (TAAs) with the production of corresponding autoantibodies. Such autoantibody-targeted TAAs (autoTAAs) are highly likely to indicate early events during pancreatic carcinogenesis. Herein, we performed a comprehensive analysis of these autoTAAs to explore their physiological function and their involvement and prognostic value in pancreatic cancer.

Methods: We first searched the literature to identify the autoTAAs. A PPI network of these autoTAAs was constructed, and core network modules were extracted by Cytoscape software. GO annotation and KEGG pathway analysis were performed to analyze the main physiological functions of these autoTAAs. The prognostic value of autoTAAs in pancreatic cancer was analyzed by using RNA-seq data generated by TCGA.

Results: The PPI network including 98 autoTAAs was constructed, and 2 subgroups were extracted as core modules. GO and KEGG analysis revealed that key functions and pathways of these autoTAAs were significantly enriched in nucleotide repair, protein synthesis, and cancer-associated events. MSH2, EZR, PGK1, VCL and ANXA2 have prognostic value in pancreatic cancer, and high mRNA expression of these 5 proteins is associated with unfavorable prognosis in pancreatic cancer.

Conclusions: AutoTAAs may be associated with early events in the carcinogenesis of pancreatic cancer. MSH2, EZR, PGK1, VCL and ANXA2 predict poor prognosis in pancreatic cancer. Some autoTAAs also have prognostic value in other cancers.

Introduction

Pancreatic cancer is among the most common causes of cancer-related death worldwide, with a mortality to incidence ratio of 0.98 [1]. One of the reasons is the lack of effective markers to facilitate early diagnosis of pancreatic cancer in clinical practice, and clinical symptoms of pancreatic cancer are also easily confused with benign pancreatic diseases [2]. Studies have shown that humoral immunity responds to a certain proportion of tumor-associated antigens (TAAs) in early cancer stages, along with the production of autoantibodies [3,4]. TAAs, which are involved in the early stages of tumor development, do not always exhibit changes in their expression level, which are barely detectable by proteomics methods. In this circumstance, autoantibodies can function as sensors to report these cancer-associated abnormal proteins [5] because serum levels of autoantibodies are much higher compared to autoantibody-targeted TAAs (autoTAAs), and there are more sensitive methods to detect antibodies [6]. Based on many previous studies examining autoantibodies in cancer research, some evidence supports that these autoTAAs, which cause humoral immune responses, are highly likely to be involved in the development and early progression of pancreatic cancer [7,8]. Additionally, as these autoTAAs can induce natural autoantibodies in the human body, they are also ideal candidates for therapy [3]. It had been reported in various cancers that autoantibodies in the peripheral blood are ideal potential markers for early tumor diagnosis [9–14], but few studies have explored their corresponding autoTAAs. For this purpose, we collected autoantibodies reported in pancreatic cancer and performed a comprehensive analysis of their corresponding autoTAAs to explore their physiological functions and further analyze their prognostic value in pancreatic cancer.

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Materials and methods

Literature search of autoantibodies in pancreatic cancer

We used Medical Subject Headings (MeSH) term [("Pancreatic Neoplasms"[Mesh]) AND "Autoantibodies"[Mesh]] as keywords to perform the literature search in PubMed and set the publication period from 1963 to March 2018. An additional 31 references from the literature search results of a review article published in 2016 [14] were used as a supplement to the literature search. The inclusion/exclusion criteria were as follows: removal of duplicate literature, removal of reviews, removal of literature that was not relevant to pancreatic cancer research, removal of case reports, removal of negative results, and removal of articles that did not report the exact antigen.

Protein-protein interaction network generation and visualization

Protein-protein interaction (PPI) network analysis was performed by online tools and software, including the Retrieval of Interacting Genes/Proteins (STRING v10.0), Cytoscape (v3.5.0) and the Molecular Complex Detection (MCODE) plug-in. The STRING tool was used to generate primary PPI data. Cytoscape was then used for further PPI data editing and visualization. PPI network parameters for 98 autoTAAs were generated in the STRING online tool and then loaded into Cytoscape software (v3.5.0). Different parameter settings in Cytoscape were edited and modified before the network layout, in which node size was proportional to the number of edge-counts.

Core modules extracted by MCODE

The MOCDE plug-in in Cytoscape was used to extract core modules in the PPI network. Parameters in MCODE for subgroup identification were set as follows: network scoring not including loops and with a threshold degree cutoff value of 2, while cluster identification settings were haircut triggered and fluff inactivated; the values of node score cutoff, K-core, and maximum depth were 0.2, 2 and 100, respectively.

GO annotation and KEGG pathway analysis

Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of 98 autoTAAs and proteins in the 2 subgroups were performed by the DAVID (Database for Annotation, Visualization and Integrated Discovery, v6.8) online tool. We performed analysis in the section of Functional Annotation Chart of DAVID. For GO annotation, EASE score (p-value) <0.05, Benjamini<0.05, and Enrichment score (fold enrichment) > 1.5 were considered significant. For KEGG analysis, the cutoff threshold was set the same as for GO annotation. In addition, to obtain more information on the overall 98 autoTAAs analysis, we relaxed Benjamini's cutoff to <0.2 in the KEGG pathway analysis.

Subcellular localization of subgroup proteins

We searched two human protein databases (Human Protein Atlas, https://www.ProteinAtlas.org/and HPRD, http://www.hprd.org/) to determine subcellular localization of proteins. In the Human Protein Atlas (HPA) database, subcellular localization of proteins was determined by staining cell lines with corresponding antibodies, while subcellular localization of proteins given by HPRD was derived from published literature.

Analysis of autoTAA prognostic values in pancreatic cancer and various other cancers

The prognostic capability of autoTAAs in subgroups A and B in various cancers was evaluated using the HPA website. Data analyzed by this website are derived from mRNA sequencing data from the TCGA database. The pathology atlas section in the HPA website can be used to check whether protein expression levels affect the survival of cancer patients. Prognostic analysis is based on the best separation algorithm, giving 5-year survival rate data for cancer patients (melanoma and glioma are 3-year survival rates). AutoTAAs may show either a "favorable" or "unfavorable" prognostic value. Proteins with a favorable prognosis have high mRNA expression that positively correlates with survival time, while those with an unfavorable prognosis yield the opposite effect.

Differential expression of autoTAAs in pancreatic cancer and in healthy donors

We obtained mRNA expression data from the HPA database to analyze the differential expression of autoTAAs between pancreatic cancer and in healthy donors. mRNA expression data of these proteins in tissue from pancreatic cancer was derived from RNA-seq data of 176 pancreatic cancers from the TCGA database, and mRNA expression data in pancreatic tissue from healthy donors was derived from RNA-seq data of 171 healthy donors from the Genotype-Tissue Expression (GTEx) project. GraphPad Prism software (PRISM version 7.0; GraphPad Software, San Diego, CA, USA) was used to perform statistical analysis. Comparison between the two groups was accomplished by the nonparametric Mann-Whitney test because data were not normally distributed.

Results

PPI network construction

We initially retrieved a total of 165 studies from the literature. After applying inclusion and exclusion criteria, 35 papers were ultimately included in our research (Supplemental Fig. 1), and we obtained 98 total autoTAAs in this study (Table 1). PPI parameters of autoTAAs were generated by the STRING online analytical tool, and the original PPI network layout of the 98 autoTAAs according to STRING is displayed in Supplemental Fig. 2. Then, PPI data were loaded into Cytoscape software for the extraction of core modules. Sixty-six proteins were interconnected with each other in the PPI network (Fig. 1). For the concision of the figure, the noninteractions nodes not showed.

GO annotation and KEGG analysis of the PPI network

Analysis of GO annotations and KEGG pathways of the 98 autoTAAs was performed by the DAVID online tool (Supplemental Table 1). GO annotation identified proteins enriched in biological process, cellular component, and molecular function. Biological process of these autoTAAs was significantly enriched in response to X-ray, protein sumoylation, and DNA damage response. Molecular function of the autoTAAs was enriched in poly(A) RNA binding, identical protein binding, cadherin binding involved in cell-cell adhesion, enzyme binding, and chaperone binding. Cellular components indicated that autoTAAs are located in extracellular exosomes, extracellular space, membrane, and nuclear chromosomes with telomeric region, suggesting that they belong to categories of extracellular secreted proteins or proteins involved in molecular signaling related to cancer genetics. Of note, KEGG pathway analysis suggested that a portion of autoTAAs participate in the

Table 1
The 98 autoTAAs included in this study.

GENE SYMBOL	Protein description
STK33 [15]	serine/threonine kinase 33
DTYMK [15]	deoxythymidylate kinase
SART3 [16]	squamous cell carcinoma antigen recognized by T-cells 3
RNF213 [17]	ring finger protein 213
VCL [18]	vinculin
REG3A [16]	regenerating family member 3 alpha
CDKN2A [19]	cyclin-dependent kinase inhibitor 2A
MED27 [17]	mediator complex subunit 27
CTDSP1 [20]	CTD small phosphatase 1
SMOX [17]	spermine oxidase
PMS1 [21]	PMS1 homolog 1, mismatch repair system component
CIB1 [15]	calcium and integrin binding 1
EGFR [16]	epidermal growth factor receptor
HNRNPA2B1 [22]	heterogeneous nuclear ribonucleoprotein A2/B1
KRT10 [23]	keratin 10
TMSB10 [17]	thymosin beta 10
HCFC1R1 [17]	host cell factor C1 regulator 1
AFG3L1 [17]	AFG3 like matrix AAA peptidase subunit 1, pseudogene
RRP8 [15]	ribosomal RNA processing 8, methyltransferase, homolog
HES1 [21]	hes family bHLH transcription factor 1
TAGLN [23]	transgelin
ROR2 [17]	receptor tyrosine kinase-like orphan receptor 2
MAPK9 [17,20]	mitogen-activated protein kinase 9
PSCA [24]	prostate stem cell antigen
PDCD6IP [18]	programmed cell death 6 interacting protein
MDH1 [22]	malate dehydrogenase 1
ELAC1 [17]	elaC ribonuclease Z 1
HAX1 [21]	HCLS1 associated protein X-1
IFITM3 [25]	interferon induced transmembrane protein 3
IGF2BP1 [19]	insulin-like growth factor 2 mRNA binding protein 1
ULK4 [17]	unc-51 like kinase 4
IGF2BP3 [19]	insulin-like growth factor 2 mRNA binding protein 3
CALR [26,27]	calreticulin
HNRNPL [18]	heterogeneous nuclear ribonucleoprotein L
TPI1 [23]	triosephosphate isomerase 1
EIF3G [15]	eukaryotic translation initiation factor 3 subunit G
ZNF695 [17]	zinc finger protein 695
INS [28]	insulin
MSLN [29]	mesothelin
IDH1 [23]	isocitrate dehydrogenase (NADP(+)) 1, cytosolic
C8orf34 [17]	chromosome 8 open reading frame 34
RUNX2 [21]	runt related transcription factor 2
MUC1 [30,31]	mucin 1, cell surface associated
GABARAPL2 [15]	GABA type A receptor-associated protein-like 2
HERPUD1 [17]	homocysteine inducible ER protein with ubiquitin-like domain 1
ARFIP2 [22]	ADP ribosylation factor interacting protein 2
BRCA2 [32]	BRCA2, DNA repair associated
GAS2 [17]	growth arrest-specific 2
BRCA1 [32]	BRCA1, DNA repair associated
NUP62 [19]	nucleoporin 62
PCNA [15]	proliferating cell nuclear antigen
CD79B [17]	CD79b molecule
DNAJB1 [17]	DnaJ heat shock protein family (Hsp40) member B1
RIT2 [15]	Ras-like without CAAX 2
PARP1 [32]	poly(ADP-ribose) polymerase 1
TMOD1 [17]	tropomodulin 1
ALG1 [21]	ALG1, chitobiosyldiphosphodolichol beta-mannosyltransferase
AP1AR [17]	adaptor-related protein complex 1 associated regulatory protein
PPARG [17]	peroxisome proliferator-activated receptor gamma
SHOC2 [17]	SHOC2, leucine rich repeat scaffold protein
HIST2H4A [33]	histone cluster 2 H4 family member a
ZNF207 [21]	zinc finger protein 207
BAG3 [34]	BCL2 associated athanogene 3
CEACAM5 [16,35]	carcinoembryonic antigen related cell adhesion molecule 5
CRYBB2 [17]	crystallin beta B2
SOX13 [21]	SRY-box 13
TP53 [19,21,36–41]	tumor protein p53
KTN1 [21]	kinectin 1

Table 1 (*continued*)

GENE SYMBOL	Protein description
TIMM44 [21]	translocase of inner mitochondrial membrane 44
RAD51 [42]	RAD51 recombinase
EIF4A3 [43]	eukaryotic translation initiation factor 4A3
G6PD [23]	glucose-6-phosphate dehydrogenase
CFL1 [23]	cofilin 1
HAUS8 [17]	HAUS augmin-like complex subunit 8
TG [44]	thyroglobulin
MIA [25]	melanoma inhibitory activity
TUFM [23]	Tu translation elongation factor, mitochondrial
HIST2H2BE [45]	histone cluster 2 H2B family member e
C6orf141 [17]	chromosome 6 open reading frame 141
FAM13A [17]	family with sequence similarity 13 member A
ALDH1A1 [23]	aldehyde dehydrogenase 1 family member A1
MRPL12 [21]	mitochondrial ribosomal protein L12
EZR [18,46]	ezrin
TGM1 [27]	transglutaminase 1
ENO1 [47,48]	enolase 1
SPATA32 [17]	spermatogenesis associated 32
MSH2 [21]	mutS homolog 2
PTPRA [17]	protein tyrosine phosphatase, receptor type A
ANXA1 [18]	annexin A1
TNP1 [15]	transition protein 1
BIRC5 [19]	baculoviral IAP repeat containing 5
COTL1 [49]	coactosin like F-actin binding protein 1
LRRC49 [17]	leucine rich repeat containing 49
ANXA2 [18,22]	annexin A2
PGK1 [22,33]	phosphoglycerate kinase 1
ARAP2 [21]	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2
NR2E3 [17,20]	nuclear receptor subfamily 2 group E member 3
PNLIPRP2 [25]	pancreatic lipase related protein 2 (gene/pseudogene)

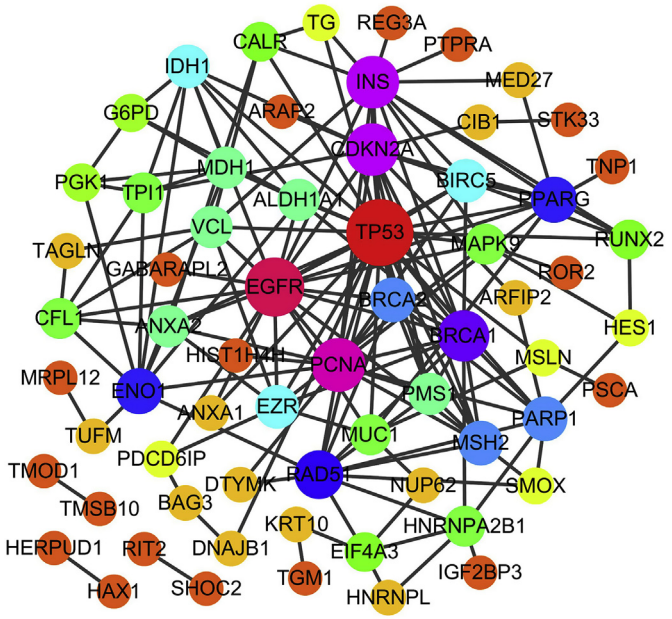

Fig. 1. Protein-protein interaction (PPI) network of 98 autoTAAs. The PPI network was generated from Cytoscape software (ver. 3.5.0). Node size is based on edge counts, and larger nodes in the core show priority importance in this network.

processes of various cancers. Nine of 98 proteins were associated with pathways in cancer, including EGFR, CDKN2A, MSH2, PPARG, TP53, BRCA2, MAPK9, BIRC5, and RAD51, 6 of 98 proteins were associated with pathways of pancreatic cancer, including EGFR, CDKN2A, TP53, BRCA2, MAPK9, and RAD51, 4 of 98 proteins were associated with Fanconi anemia pathway, including HES1, BRCA2, BRCA1, and RAD51, and 4 of 98 proteins were associated with

colorectal cancer, including MSH2, TP53, MAPK9, and BIRC5.

Core modules extracted by MCODE

Two subgroups were extracted by MCODE as core modules of the PPI network (Fig. 2). Subgroup A included BIRC5, RAD51, PPARG, PCNA, INS, CDKN2A, MSH2, TP53, EGFR, PMS1, BRCA1, and BRCA2. Subgroup B included ALDH1A1, VCL, EZR, ENO1, TPI1, PGK1, G6PD, CALR, and ANXA2. Then, we analyzed the physiological functions and involved pathways of the proteins in these two subgroups through GO annotation and KEGG analysis (Table 2 and Table 3).

Biological process and molecular function of the autoTAAs in subgroup A were significantly enriched in DNA-associated bioprocesses, including DNA damage response, strand displacement/repair, negative/positive regulation of transcription/telomere maintenance, enzyme binding, protein binding, and DNA binding. The corresponding cellular component analysis showed that 10 of 12 proteins were located in the nucleus (Table 2). For KEGG pathway analysis, autoTAAs in subgroup A were clustered in pancreatic cancer (5 of 12: EGFR, CDKN2A, TP53, BRCA2, and RAD51) and a wide variety of cancers (bladder cancer, Fanconi anemia, non-small cell lung cancer, colorectal cancer, glioma, and melanoma) (Table 3). Proteins in subgroup B were significantly enriched in gluconeogenesis/glycolysis, and subcellular localization of the 9 proteins in this subgroup included both that cytosol and extracellular exosomes (Table 2). Four of 9 proteins in subgroup B were the composed of cell-cell adherens junctions, and some are anchored on membranes (6 of 9). Pathway analysis revealed that autoTAAs in subgroup B play roles in carbon metabolism, biosynthesis of antibiotics or amino acids, and glycolysis/gluconeogenesis (Table 3).

GO annotation of plasma membrane proteins

Twenty-three autoTAAs were validated in the HPA database as plasma membrane proteins via experimentally verified (immunofluorescence assay) and bioinformatics predictions (Supplemental Table 2). These autoTAAs are initially localized or ectopically expressed on the cell membrane. Biological function analysis of these membrane proteins was significantly enriched in negative regulation of apoptotic process, regulation of cell shape, positive regulation of cell migration, and cadherin binding involved in cell-cell adhesion.

Prognostic values of autoTAAs in pancreatic cancer and various other cancers

We further explored the prognostic capacity of 21 proteins in subgroups A and B for pancreatic cancer and other cancers. The prognostic value of these autoTAAs was obtained in the pathology section of the HPA database. The HPA pathology section provides analysis for the correlation between mRNA expression levels of proteins and patient survival. For pancreatic cancer analysis, the HPA database analyzed RNA-seq data from 176 cancer patients generated by TCGA database. We found that mRNA expression of ANXA2, EZR, MSH2, PGK1, and VCL was inversely correlated with patient survival in pancreatic cancer (Fig. 3). As shown in Fig. 3, patients were divided based on the mRNA expression level of proteins into two groups: the 5-year survival low (under cut off) and 5-year survival high (over cut off) groups. Survival analysis suggested that the 5 proteins with high expression were unfavorable in pancreatic cancer. For the ANXA2 protein, the 5-year survival rate of the 60 patients with mRNA expression lower than the cutoff reached 35%, while the 5-year survival rate of 116 patients with mRNA expression higher than the cutoff was only 16% ($p = 0.0000085$). Unfavorable prognosis was also observed in EZR, MSH2, PGK1, and VCL proteins.

Most of the autoTAAs possessed unfavorable prognostic capabilities when their mRNA expression level was higher than the expression cutoff, while only a few autoTAAs were associated with favorable prognosis. The prognostic values of 21 autoTAAs in various cancers are listed in Supplemental Table 3. Furthermore, we explored the subcellular localization of proteins in subgroups A and B, and information was obtained from HPA and HPRD databases (Supplemental Table 3). Most of these proteins were expressed in subcellular compartments, and EZR, ENO1, and EGFR were also expressed on the cell membrane.

Differential expression of autoTAAs between pancreatic cancer tissues and healthy donors

Because ANXA2, EZR, MSH2, PGK1, and VCL have prognostic value for pancreatic cancer, we further validated the differential expression of these autoTAAs at the mRNA level in pancreatic cancer tissues and healthy donors. mRNA expression data is reported as RPKM (reads per kilobase per million mapped reads). The median RPKM of ANXA2, EZR, MSH2, PGK1, and VCL in pancreatic cancer and healthy donors were 177.1 versus 31.5, 182.3 versus 31.3, 4.3 versus 2.0, 67.2 versus 14.5, and 24.3 versus 10.9, respectively (Supplemental Fig. 3). Results showed that expression of ANXA2, EZR, MSH2, PGK1, and VCL in pancreatic cancer tissues was significantly higher than that in normal pancreatic tissue.

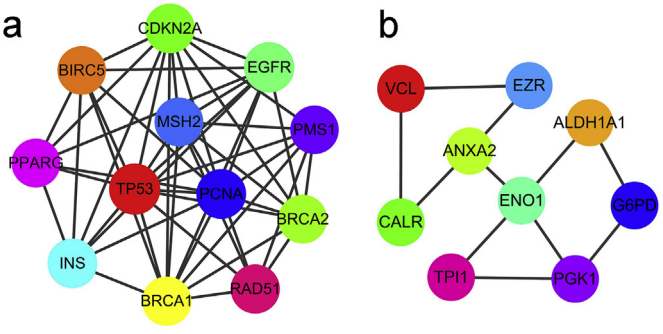

Fig. 2. Two subgroups were extracted as core modules of the PPI network. a) Subgroup A contained 12 proteins: BIRC5, RAD51, PPARG, PCNA, INS, CDKN2A, MSH2, TP53, EGFR, PMS1, BRCA1, and BRCA2. b) Subgroup B contained 9 proteins: ALDH1A1, VCL, EZR, ENO1, TPI1, PGK1, G6PD, CALR, and ANXA2.

Discussion

Ninety-eight autoTAAs were included in this study. We first constructed a PPI network of these 98 proteins and then extracted 2 core modules as subgroups. The physiological processes and involved pathways of these autoTAAs were analyzed by GO annotation and KEGG pathway analysis. Next, we explored the prognostic value of these autoTAAs in pancreatic cancer and in various other cancers. Survival analysis was performed using mRNA sequence data derived from The Cancer Genome Atlas (TCGA) database. Results showed that when mRNA expression levels of autoTAAs increased, patient survival significantly decreased, indicating that these autoTAAs have prognostic value.

Overall, GO term annotation indicated the main function of 98 autoTAAs at the genetic level responds to DNA damage and repair, while at the protein level, autoTAAs participate in binding enzymes, chaperone proteins, and RNA, suggesting that the human body may be challenged by external environmental disturbances or malignancies that are accompanied by enhanced related protein function. Most of the proteins in subgroup A were located in the nucleus

Table 2
GO annotation of subgroup A and B.

	Category	Term	Count	Genes	P Value	Fold Enrichment	Benjamini
subgroup A: (12 proteins): BIRC5, RAD51, PPARG, PCNA, INS, CDKN2A, MSH2, TP53, EGFR, PMS1, BRCA1, BRCA2	GOTERM_BP_DIRECT	GO:0010165~response to X-ray	4	MSH2, TP53, BRCA2, RAD51	0.000000320	254.4242424	0.000140094
	GOTERM_BP_DIRECT	GO:0016925~protein sumoylation	5	CDKN2A, PCNA, TP53, BIRC5, BRCA1	0.000000711	59.8005698	0.000155780
	GOTERM_BP_DIRECT	GO:0006978~DNA damage response, signal transduction by p53 class mediator resulting in transcription of p21 class mediator	3	TP53, BRCA2, BRCA1	0.000046582	262.375	0.006778112
	GOTERM_BP_DIRECT	GO:0071158~positive regulation of cell cycle arrest	3	CDKN2A, TP53, BRCA1	0.000116082	167.92	0.012631261
	GOTERM_BP_DIRECT	GO:0000732~strand displacement	3	BRCA2, BRCA1, RAD51	0.000125711	161.4615385	0.010952514
	GOTERM_BP_DIRECT	GO:0045739~positive regulation of DNA repair	3	EGFR, PCNA, BRCA1	0.000168018	139.9333333	0.012191443
	GOTERM_BP_DIRECT	GO:0042771~intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	3	MSH2, TP53, BRCA2	0.000179542	135.4193548	0.011172305
	GOTERM_BP_DIRECT	GO:0000722~telomere maintenance via recombination	3	PCNA, BRCA2, RAD51	0.000191443	131.1875	0.010427737
	GOTERM_BP_DIRECT	GO:0045892~negative regulation of transcription, DNA-templated	5	CDKN2A, PPARG, TP53, BIRC5, BRCA1	0.000215313	14.02137609	0.010424995
	GOTERM_BP_DIRECT	GO:0006298~mismatch repair	3	MSH2, PCNA, PMS1	0.000229408	119.9428571	0.009998891
	GOTERM_BP_DIRECT	GO:0000731~DNA synthesis involved in DNA repair	3	BRCA2, BRCA1, RAD51	0.000229408	119.9428571	0.009998891
	GOTERM_BP_DIRECT	GO:0045893~positive regulation of transcription, DNA-templated	5	CDKN2A, PPARG, TP53, BRCA2, BRCA1	0.000243053	13.58576052	0.009632410
	GOTERM_BP_DIRECT	GO:0045740~positive regulation of DNA replication	3	EGFR, INS, PCNA	0.000331137	99.95238095	0.012015734
	GOTERM_BP_DIRECT	GO:0006302~double-strand break repair	3	MSH2, BRCA2, BRCA1	0.000817912	63.60606061	0.027192076
	GOTERM_BP_DIRECT	GO:0000724~double-strand break repair via homologous recombination	3	BRCA2, BRCA1, RAD51	0.001026980	56.72972973	0.031635124
	GOTERM_MF_DIRECT	GO:0019899~enzyme binding	7	EGFR, MSH2, PPARG, PCNA, TP53, BIRC5, BRCA1	0.000000024	29.57132132	0.000002634
	GOTERM_MF_DIRECT	GO:0042802~identical protein binding	7	EGFR, INS, PPARG, PCNA, TP53, BIRC5, RAD51	0.000002854	13.14719626	0.000156940
	GOTERM_MF_DIRECT	GO:0003684~damaged DNA binding	4	MSH2, PCNA, TP53, BRCA1	0.000008001	89.31746032	0.000293337
	GOTERM_MF_DIRECT	GO:0003690~double-stranded DNA binding	4	EGFR, MSH2, TP53, RAD51	0.000017081	69.4691358	0.000469621
	GOTERM_MF_DIRECT	GO:0003677~DNA binding	8	CDKN2A, MSH2, PPARG, PCNA, TP53, BRCA1, PMS1, RAD51	0.000021449	6.722819594	0.000471770
	GOTERM_MF_DIRECT	GO:0003697~single-stranded DNA binding	4	MSH2, BRCA2, PMS1, RAD51	0.000025868	60.50537634	0.000474145
	GOTERM_MF_DIRECT	GO:0003682~chromatin binding	5	EGFR, PPARG, PCNA, TP53, RAD51	0.000082212	17.98913043	0.001291128
	GOTERM_MF_DIRECT	GO:0019903~protein phosphatase binding	3	EGFR, PPARG, TP53	0.000737744	66.98809524	0.010096406
	GOTERM_MF_DIRECT	GO:0019901~protein kinase binding	4	EGFR, CDKN2A, MSH2, TP53	0.001583472	14.96542553	0.019182511
	GOTERM_MF_DIRECT	GO:0002020~protease binding	3	INS, TP53, BRCA2	0.001882034	41.78465347	0.020508654
	GOTERM_CC_DIRECT	GO:0000800~lateral element	3	BRCA2, BRCA1, RAD51	0.000030023	325.4285714	0.002638577
	GOTERM_CC_DIRECT	GO:0005654~nucleoplasm	9	CDKN2A, MSH2, PPARG, PCNA, TP53, BRCA2, BIRC5, BRCA1, RAD51	0.000031386	4.909482759	0.001380036
	GOTERM_CC_DIRECT	GO:0000784~nuclear chromosome, telomeric region	4	MSH2, PCNA, BRCA2, RAD51	0.000056128	46.72820513	0.001645104
	GOTERM_CC_DIRECT	GO:0005634~nucleus	10	EGFR, CDKN2A, PPARG, PCNA, TP53, BRCA2, BIRC5, BRCA1, PMS1, RAD51	0.000531591	2.804555248	0.011629955
	GOTERM_CC_DIRECT	GO:0043234~protein complex	4	CDKN2A, TP53, BRCA2, BRCA1	0.001653442	14.74433657	0.028704636
subgroup B: (9 proteins): ALDH1A1, VCL, EZR, ENO1, TPI1, PGK1, G6PD, CALR, ANXA2	GOTERM_BP_DIRECT	GO:0061621~canonical glycolysis	3	TPI1, PGK1, ENO1	0.000064181	215.2821	0.010280314
	GOTERM_BP_DIRECT	GO:0006096~glycolytic process	3	TPI1, PGK1, ENO1	0.000110576	164.6275	0.008862335
	GOTERM_BP_DIRECT	GO:0006094~gluconeogenesis	3	TPI1, PGK1, ENO1	0.000186017	127.2121	0.009934172
	GOTERM_MF_DIRECT	GO:0098641~cadherin binding involved in cell-cell adhesion	4	EZR, ANXA2, ENO1, VCL	0.000263593	25.87126	0.015433678

Table 2 (*continued*)

	Category	Term	Count	Genes	P Value	Fold Enrichment	Benjamini
	GOTERM_CC_DIRECT	GO:0070062~extracellular exosome	9	ALDH1A1, TPI1, EZR, G6PD, CALR, PGK1, ANXA2, ENO1, VCL	0.000000318	6.483102	0.000029232
	GOTERM_CC_DIRECT	GO:0005829~cytosol	9	ALDH1A1, TPI1, EZR, G6PD, CALR, PGK1, ANXA2, ENO1, VCL	0.000001190	5.497436	0.000054760
	GOTERM_CC_DIRECT	GO:0005913~cell-cell adherens junction	4	EZR, ANXA2, ENO1, VCL	0.000289167	25.07602	0.008829866
	GOTERM_CC_DIRECT	GO:0016020~membrane	6	EZR, G6PD, CALR, PGK1, ANXA2, ENO1	0.001042212	5.522424	0.023698058
	GOTERM_CC_DIRECT	GO:0005615~extracellular space	5	TPI1, EZR, CALR, ANXA2, ENO1	0.001633204	7.516291	0.029627748

Abbreviations: BP = biological process, MF = molecular function, CC = cellular component.
EASE score (p value) < 0.05, Enrichment score (fold enrichment) > 1.5, and benjamini<0.05 was considered significant.

Table 3
KEGG Pathway analysis of subgroup A and B.

	Term	Count	Genes	P Value	Fold Enrichment	Benjamini
Subgroup A (12 proteins): BIRC5, RAD51, PPARG, PCNA, INS, CDKN2A, MSH2, TP53, EGFR, PMS1, BRCA1, BRCA2	hsa05200:Pathways in cancer	8	EGFR, CDKN2A, MSH2, PPARG, TP53, BRCA2, BIRC5, RAD51	0.000000189	12.78742	0.000015113
	hsa05212:Pancreatic cancer	5	EGFR, CDKN2A, TP53, BRCA2, RAD51	0.000001436	48.32168	0.000057423
	hsa05219:Bladder cancer	3	EGFR, CDKN2A, TP53	0.001499956	45.96452	0.039238292
	hsa03460:Fanconi anemia pathway	3	BRCA2, BRCA1, RAD51	0.002497393	35.55746	0.048780402
	hsa05223:Non-small cell lung cancer	3	EGFR, CDKN2A, TP53	0.002784527	33.6526	0.043633990
	hsa05210:Colorectal cancer	3	MSH2, TP53, BIRC5	0.003403365	30.39589	0.044437966
	hsa05214:Glioma	3	EGFR, CDKN2A, TP53	0.003734852	28.99301	0.041862449
	hsa05218:Melanoma	3	EGFR, CDKN2A, TP53	0.004441426	26.54289	0.043537015
Subgroup B (9 proteins): ALDH1A1, VCL, EZR, ENO1, TPI1, PGK1, G6PD, CALR, ANXA2	hsa01200:Carbon metabolism	4	TPI1, G6PD, PGK1, ENO1	0.000142098	30.57522	0.004112667
	hsa01130:Biosynthesis of antibiotics	4	TPI1, G6PD, PGK1, ENO1	0.000909638	16.29717	0.013109076
	hsa00010:Glycolysis/Gluconeogenesis	3	TPI1, PGK1, ENO1	0.001884947	38.67537	0.018073036
	hsa01230:Biosynthesis of amino acids	3	TPI1, PGK1, ENO1	0.002294895	35.01689	0.016519150

EASE score (p value) < 0.05, Enrichment score (fold enrichment) > 1.5, and benjamini<0.05 was considered significant.

and were functionally associated with DNA regulation, including DNA damage response, strand displacement/repair, and negative/positive regulation of transcription. Proteins in subgroup B displayed roles in carbon metabolism, biosynthesis of antibiotics or amino acids, and glycolysis/gluconeogenesis. Pathway analysis suggested that these autoTAAs do indeed play roles in several cancers, including pancreatic cancer, indicating that their bioprocessing functions are highly related to cancer development. Moreover, some of the proteins in subgroup A were also involved in carbon metabolism, and glycolysis/gluconeogenesis may be responsible for providing ATP to excessively active proteins, thus contributing to the proliferation of malignant cells. AutoTAAs located at the plasma membrane, as predicted by bioinformatics algorithms or experimentally verified data, should also draw the attention. In our study, 23 autoTAAs were experimentally confirmed or predicted by bioinformatics to be plasma membrane proteins in the HPA database, and 26 autoTAAs were predicted by bioinformatics to be plasma membrane proteins by GO annotation in the DAVID website.

Benign pancreatic diseases mimic malignancy in their clinical symptoms. For instance, autoimmune pancreatitis (AIP) is considered susceptible to being confused with pancreatic cancer, as they are similar in clinical symptoms and serum markers. In addition, traditional clinical markers in pancreatic cancer, such as CA19-9 and CEA, are insufficient to differentiate the two diseases. Researchers are committed to identifying new markers for differential diagnosis of AIP from pancreatic cancer. Carbonic anhydrase 2 (CA2) [50], carbonic anhydrase 4 (CA4) [51], lactoferrin (LF) [52], pancreatic secretory trypsin inhibitor (PSTI) [53], and amylase-2α (AMY2A) [54] are the most promising autoantibody markers reported in AIP that possess high specificity and sensitivity. Compared to bioinformatics analysis in this article, autoantibody markers in pancreatic cancer and AIP show little overlap, indicating they do have different mechanisms. Accordingly, bioinformatics tools can efficiency help to develop new panels for differentiating pancreatic cancer from benign pancreatic disease.

In this study, we further explored the prognostic value of 21 autoTAAs (from subgroup A and subgroup B) in pancreatic cancer and other cancers. Among these autoTAAs, 5 proteins exhibited prognostic significance for pancreatic cancer, and some of these autoTAAs also have prognostic capability the pan-cancer level, such as in lung cancer, liver cancer, renal cancer, breast cancer, ovarian cancer, and glioma, most of which convey unfavorable prognosis with a few conveying a favorable prognosis. ANXA2, EZR, MSH2, PGK1 and VCL have prognostic significance in patients with pancreatic cancer, and these five proteins all showed an unfavorable prognosis when their mRNA is highly expressed. We further analyzed the differential expression of these five proteins in pancreatic cancer tissue and normal human pancreatic tissue. The results indicated that the expression levels of these proteins were significantly higher in patients with pancreatic cancer than in normal pancreas tissues, suggesting that these five proteins exhibit abnormal expression patterns in patients with pancreatic cancer. Perhaps they are involved in the occurrence and development of pancreatic cancer, or they may represent products in the development of pancreatic cancer. These results indicate that autoTAAs have valuable prognostic capability and can be used as markers to observe progression in pancreatic cancer.

Identification and evaluation of novel protein biomarkers via proteomics relies on fold change in protein expression levels compared to healthy individuals or noncancerous controls. However, in the early stages of tumorigenesis, most tumor-associated antigens have not yet undergone changes that are detectable by proteomics methods [10,11]. In tumor cells, abnormal

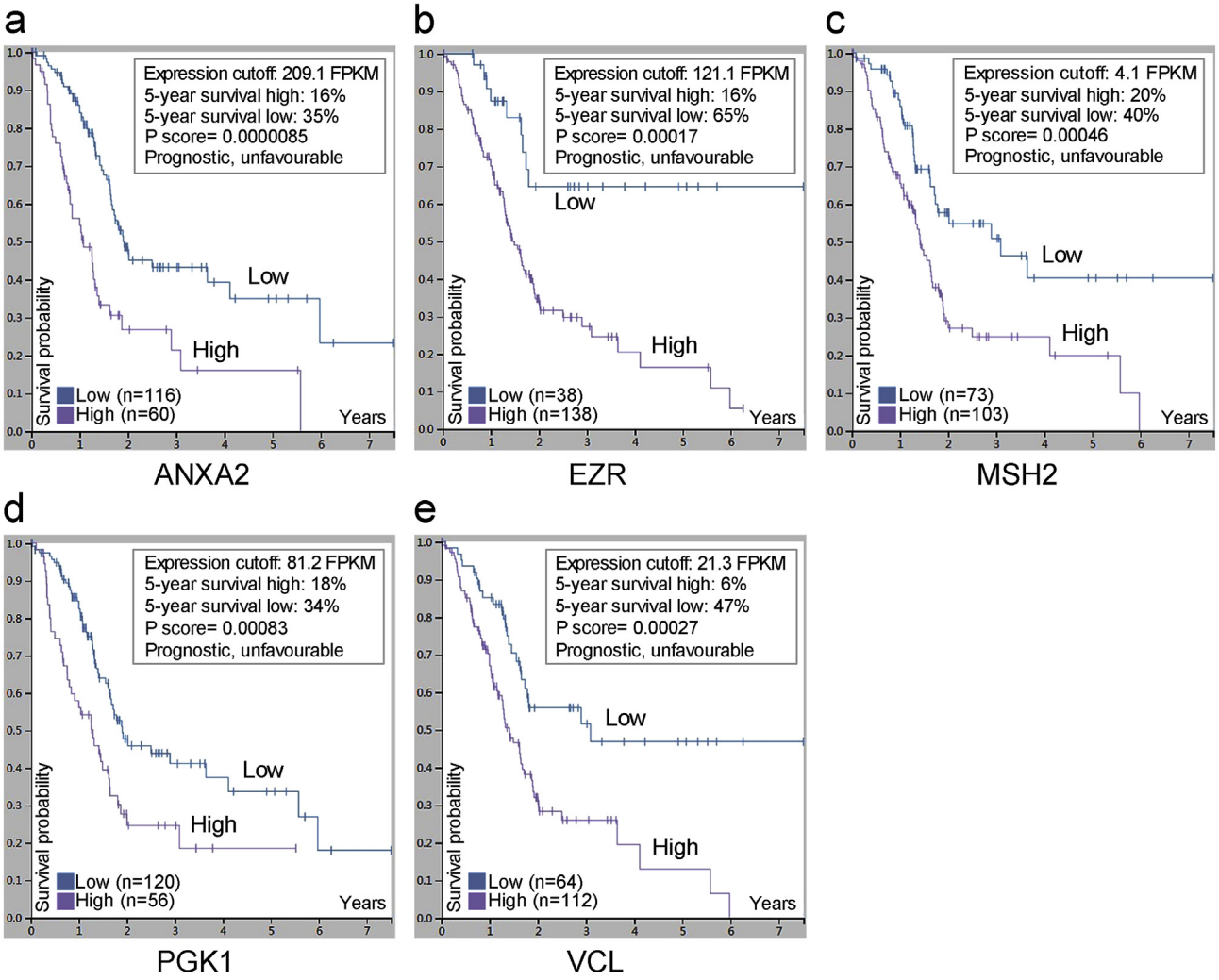

Fig. 3. Prognostic value of the autoantibodies in pancreatic cancer. a, b, c, d, and e show the 5-year survival rate assessed by mRNA levels of ANXA2, EZR, MSH2, PGK1, and VCL, respectively. The aquamarine line indicates 5-year survival for patients with higher expression than the expression cutoff. The purple line indicates 5-year survival for patients with lower expression than the expression cutoff.

posttranslational modification or gene mutations of cancer-related proteins cause alterations of protein domains and neo-epitope exposure, resulting in the production of corresponding autoantibodies [55]. In the early stages of cancer, the abundance of TAAs in the target organ or peripheral circulation will not increase or decrease sufficiently to be detected by proteomics methods. Additionally, some cancer-related proteins in the peripheral blood exhibit no changes in expression level compared to healthy individuals. Thus, proteomic methods barely detect and screen proteins of interest, which exhibit only weak variations in expression levels or even remain unchanged. This may be one reason for the markers identified via proteomic methods only being effective during the late stages of pancreatic cancer. It is difficult to identify proteins that have not significantly changed in expression level, leading to the omission of many important cancer-associated proteins that contain key information on the early stages of pancreatic cancer. Abnormal autoTAAs may participate in bioprocessing of malignancy, apoptosis, necrosis, and oxidative stress, as the immune system is very sensitive to these abnormal changes, sometimes producing autoantibodies against these abnormal proteins [56]. Some evidence indicates that autoantibodies always produced during the precancer stage may help to clear cancer-related cells or molecules [10]. Considering these findings, in cancerous cells, the immune system is very sensitive to tiny deviations from normality. Serological autoantibodies may be ideal reporters to identify and detect TAAs with no obvious change in expression levels.

Our study demonstrates that autoTAAs have significant prognostic value in pancreatic cancer, as well as in other cancers. Identification of autoantibodies is very promising work because autoantibodies can be used as diagnostic markers in the early stages of cancer. In addition, these autoantibodies can be used as reporters to identify abnormalities in corresponding autoTAAs. These autoTAAs, which can induce an immune response, have a high probability of being involved in early physiological processes of cancer. AutoTAAs are promising candidates for prognostic markers of pancreatic cancer or as candidates for tumor-targeted therapy.

At the same time, we should note that most of the results of this analysis are based on bioinformatics predictions; therefore, it is necessary to verify the prognostic value of these autoTAAs experimentally. Well-designed experiments need to be performed to explore the physiological mechanisms of autoTAAs in pancreatic cancer.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pan.2019.06.009.

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