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High expression of Annexin A2 is associated with DNA repair, metabolic alteration, and worse survival in pancreatic ductal adenocarcinoma

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ABSTRACT

Background: Annexin A2 (ANXA2) is a known driver of cancer progression. We investigated what mechanism associates with ANXA2 high expression and its survival impact using a bioinformatic approach in pancreatic ductal adenocarcinoma.
Methods: Primary pancreatic tumor (n = 185) cohort in The Cancer Genome Atlas and Gene set enrichment analysis were used.
Results: There were no significant associations between ANXA2 expression and clinicopathologic features of the patients investigated. The ANXA2 high tumors enriched some of the known downstream signaling, such as NF-κB ($P = .028$) and tumor necrosis factor ($P = .044$) pathways, whereas others, such as angiogenesis or epithelial-mesenchymal transition, were not associated. ANXA2 high expression tumors enriched DNA repair–related gene sets (DNA repair; $P = .011$, p53 pathway; $P = .036$) and cell proliferation–related gene sets (MYC targets; $P = .041$). In addition, new association with metabolism related gene sets, such as glycolysis ($P = .016$), nucleic acid metabolism ($P = .001$), and pyrimidine metabolism ($P = .004$) were identified in the ANXA2 high group. Patients with high ANXA2 expression demonstrated significantly worse disease-free survival ($P = .001$) and overall survival ($P = .014$), with high ANXA2 being an independent risk factor.
Conclusion: High ANXA2 expression was associated with NF-κB and tumor necrosis factor signaling, DNA repair, cell proliferation, and metabolic alteration and worse prognosis in pancreatic ductal adenocarcinoma.

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related death in the United States.[1] The overall 5-year survival is ≈8% despite the advancement in multidisciplinary cancer treatment in the past decade.[2] This poor prognosis is not only due to advanced disease stage at the time of clinical presentation, but also due to resistance to current chemotherapy and radiation therapy.[2,3] One of the unique features of PDAC is a dense fibrotic and hypovascular stroma, resulting in severe tissue hypoxia and limited nutrient availability in tumor microenvironment.[2,4] Given the harsh environment, PDAC acquires metabolic alterations for cancer cell survival, including enhanced glucose uptake,

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increased glycolysis, diversion of glucose to biosynthetic pathways, and increased macropinocytosis, scavenging of serum lipids and proteins by endocytic process.[2,5]

Annexin A2 (ANXA2) is a member of the annexin family, which is a calcium-dependent phospholipid binding protein, playing major roles in regulation of cellular growth and signaling pathways.[6] Although a small amount of ANXA2 monomer exists, ANXA2 mainly presents as a heterotetramer with S100A10 on the cell membrane and in the cytoplasm, which takes a major part in fibrinolysis by facilitating plasmin production, exocytosis, endocytosis, membrane trafficking, and cellular cytoskeleton upon phosphorylation.[7,8] Previous studies revealed that ANXA2 plays a crucial role in cancer cell proliferation, migration, invasion, and adhesion, as well as angiogenesis.[7–9] With plasmin generation from plasminogen, ANXA2 facilitates extracellular matrix degradation, promoting cell migration and tumor invasion.[8,10,11] Furthermore, intracellular ANXA2 is suggested to take part in chemotherapy resistance through NF-κB signaling.[12] Several studies demonstrated that cell-surface localization of ANXA2 was associated with cancer invasion and metastasis through enhanced epithelial-mesenchymal transition (EMT).[13–15] Furthermore, its elevated expression is associated with worse prognosis in various malignancies, including nonsmall cell lung cancer, cervical cancer, breast cancer, colorectal cancer, prostate cancer, and renal cell cancer.[7,16–18] Chaudhary et al[19] published that ANXA2 activated epidermal growth factor receptor, resulting in worse prognosis in triple-negative breast cancer.

In PDAC, ANXA2 has been demonstrated to play a role in cancer cell invasion and migration, in enhancement of metastatic activity through EMT activation, and in chemotherapy resistance.[20–23] High expression of ANXA2 has been shown to associate with worse survival, but only in small sample sizes.[20,22] Given this background, we hypothesized that high ANXA2 expression associates with DNA repair, EMT activation, and worse survival in PDAC using full genomic and clinical information from The Cancer Genome Atlas (TCGA).

Material and Methods

Data acquisition from the TCGA

Genomic and clinicopathologic data were obtained from TCGA pancreatic cancer cohort (https://cancergenome.nih.gov/) through cBioportal,[24,25] as described previously.[26–29] Among 185 primary tumors, 154 patients were registered as pancreas-adenocarcinoma ductal type in histologic diagnosis section. Of those, 147 patients were identified to have both gene expression from RNA-sequence and overall survival (OS) data. The median observation period was 15 months (inter-quartile range [IQR]: 8–21 months). Because TCGA is a deidentified, publicly accessible database, institutional review board approval was waived. The pathologic assessments of the PDAC cohort of TCGA, such as perineural invasion (PNI) and lymphovascular invasion (LVI) were manually obtained from TIES system that include pathological reports of part of TCGA cohort (http://ties.dbmi.pitt.edu/#) through Roswell Park Comprehensive Cancer Center. A validation cohort (GSE85916) that contained genomic information and survival information of 79 patients was identified through Gene Expression Omnibus datasets. No validation cohorts were available for disease-free survival (DFS).

Gene set enrichment analysis

Gene set enrichment analysis was performed comparing the ANXA2 low and high expression tumors using the Hallmark gene sets[30] and GO Biological Process gene sets[31,32] with the software provided by the Broad Institute (https://software.broadinstitute.org/gsea/index.jsp), as described before.[33,34] Significantly enriched GO Biological Process gene sets were categorized using GO classification system (http://geneontology.org/).[31,32]

Survival analysis

OS was defined as the time from date of diagnosis to the date of death by any cause, and DFS was defined as the time from date of diagnosis to the date of recurrence. Univariate and multivariate analyses for OS and DFS were conducted to estimate hazard ratios and 95% confidence intervals (CIs) with several valuables using age, sex, tumor size, American Joint Committee on Cancer (AJCC) Staging T, N, and M categories, histologic grade, pathologic AJCC stage, residual tumor status, ANXA2 status, PNI, and LVI. Some of the parameters were dichotomized as follows: age <65 and age ≥65, tumor size <3.5 cm and ≥3.5 cm, AJCC T classification T1+T2 and T3+T4, histologic grade G3 (poorly differentiated) and G1+G2 (well-differentiated + moderately differentiated), pathologic AJCC stage I+II and III+IV, and residual tumor status R1+R2 and R0. Parameters with *P* value <.20 on univariate analyses were included in the subsequent multivariate analyses.

Statistical analysis

Statistical comparisons of the clinicopathologic parameters were performed by Fisher exact test. Continues values were compared by ANOVA or Student's *t* test. All statistical analyses were performed using R software (http://www.r-project.org/) together with Bioconductor (http://bioconductor.org/) and JMP 14.0 (SAS, Cary, NC). In gene sets enrichment analysis, false discovery rates (FDRs), cut of 0.25, is commonly considered a reasonable testing adjustment in the setting of exploratory discovery study, such as ours, where the interest is in finding candidate hypothesis to be further validated.[35]

Results

Patient demographics were not significantly different between high and low expression of ANXA2 in TCGA PDAC cohort

The patients were divided into 2 groups based on ANXA2 mRNA expression level using a higher tertile. This is based on the previous reports that defined the cutoffs of ANXA2 expression between 50 to 80 percentile of their cohorts.[16,36,37] First, we investigated whether there was any association between the patient clinicopathologic features and ANXA2 expression levels to rule out the possibility of confounding factors. There was no statistically significant difference between these 2 groups in any of the features analyzed, which were age, sex, diabetes, chronic pancreatitis, race, tumor location, AJCC categories (T, N, M), tumor size, and residual tumor, shown in Table I.

ANXA2 level is not associated with known pathologic characteristics in PDAC

Several pathologic characteristics have been reported to associate with the prognosis of PDAC. To rule out the possible confounding factors, association between known prognostic pathologic features of PDAC and ANXA2 expression level was analyzed. Neither PNI nor LVI was associated with ANXA2 expression levels (Fig 1, *A* and *B*). Also, histologic grades that indicate aggressiveness of the PDAC and AJCC pathologic stages that predict patient survival did not associate with high ANXA2 levels (Fig 1, *C* and *D*).

Table I
Patient demographics in TCGA cohort

	ANXA2 high (49)	ANXA2 low (98)	*P* value
Age (median IQR)	65 (56–75)	66 (58–72)	.856
Sex (M/F)	28/21	51/47	.558
Diabetes (yes/no)	10/34	23/55	.420
Chronic pancreatitis (yes/no)	6/37	5/69	.198
Race (Asian/Black/White)	3/0/46	6/6/82	.193
Tumor location (head/body and tail)	41/8	83/13	.652
AJCC T (T1/T2/T3/T4)	2/5/41/1	2/11/82/2	.919
AJCC N (N0/N1)	10/39	27/70	.324
AJCC M (M0/M1)	22/0	48/3	.137
Tumor size (cm; median IQR)	4 (3–4.5)	3.2 (2.8–4.5)	.982
Residual tumor (R0/R1/R2)	29/17/1	55/30/4	.753

IQR, interquartile range.

High ANXA2 expression is associated with reported roles, NF-κB and tumor necrosis factor signaling, in PDAC

ANXA2 is known to be involved in the number of malignant processes in cancer cells including cell cycle regulation, cell proliferation, endocytosis, exocytosis, and downstream signaling pathways. A gene sets enrichment analysis using 50 Hallmark gene sets[30] and GO Biological Process gene sets[31,32] were conducted to identify the possible roles of ANXA2 in PDAC. All Hallmark gene sets enriched in the ANXA2 high tumors were shown in Supplementary Table I. Gene sets that correspond to the previously characterized mechanisms of ANXA2, such as NF-κB (Normalized Enrichment Score [NES] = 1.83, *P* = .028, FDR = 0.243) and tumor necrosis factor (TNF; NES = 1.68, *P* = .044, FDR = 0.342) pathways were significantly enriched in the ANXA2 high PDACs, whereas angiogenesis (NES = −1.21; *P* = .267, FDR = 0.536) or EMT (NES = −0.94; *P* = .539, FDR = 0.629) were not (Fig 2, *A* to *D*).

High ANXA2 expression is associated with DNA repair, cell proliferation, and metabolic alteration in PDAC

Gene sets of DNA repair (NES = 1.78; *P* = .011, FDR = 0.182) and p53 pathway (NES = 1.47; *P* = .036, FDR = 0.228) were also significantly enriched in the ANXA2 high tumors, suggesting

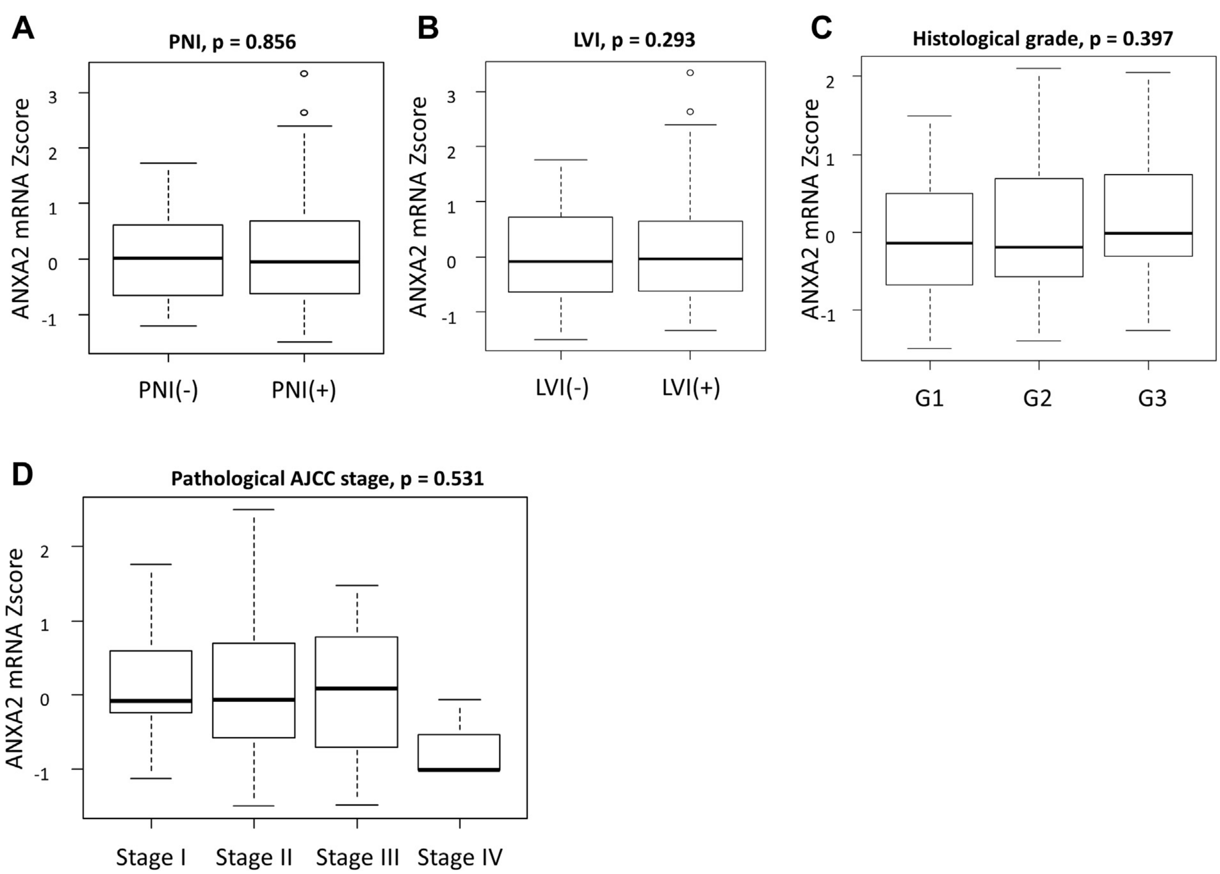

Fig 1. ANXA2 mRNA expression levels of pathologic features known to be related with PDAC aggressiveness. (*A*) Perineural invasion (PNI); PNI (−) (*n* = 14) versus PNI (+) (*n* = 110; *P* = .856). (*B*) Lymphovascular invasion (LVI); LVI (−) (*n* = 45) versus LVI (+) (*n* = 78; *P* = .293). (*C*) Histologic grade; G1 (*n* = 21) versus G2 (*n* = 84) versus G3 (*n* = 42; *P* = .397). (*D*) Pathologic AJCC stage; stage I (*n* = 12) versus stage II (*n* = 128) versus stage III (*n* = 3) versus stage IV (*n* = 3; *P* = .531).

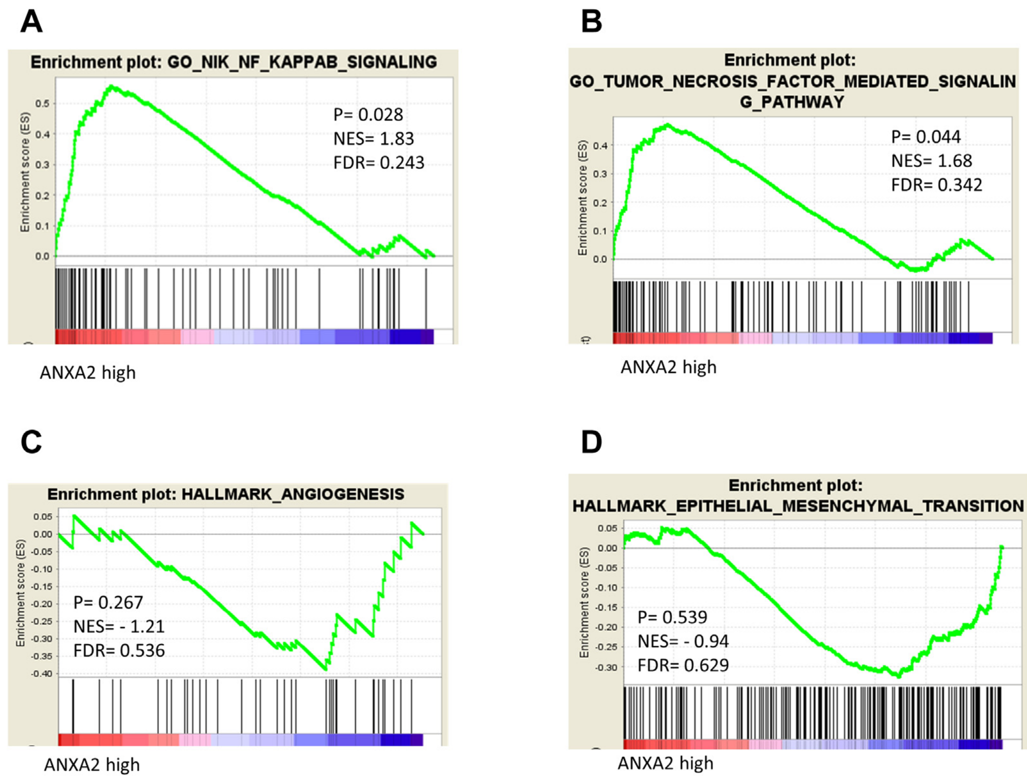

Fig 2. The association of ANXA2 and its known role by gene sets enrichment analysis comparing ANXA2 high and low PDACs. (*A*) Enrichment plot with NF-κB NES = 1.83; *P* = .028, FDR = 0.243). (*B*) Enrichment plot with TNF (NES = 1.68; *P* = .044, FDR = 0.342). (*C*) Enrichment plot with angiogenesis (NES = −1.21; *P* = .267, FDR = 0.536). (*D*) Enrichment plot with EMT (NES = −0.94; *P* = .539, FDR = 0.629).

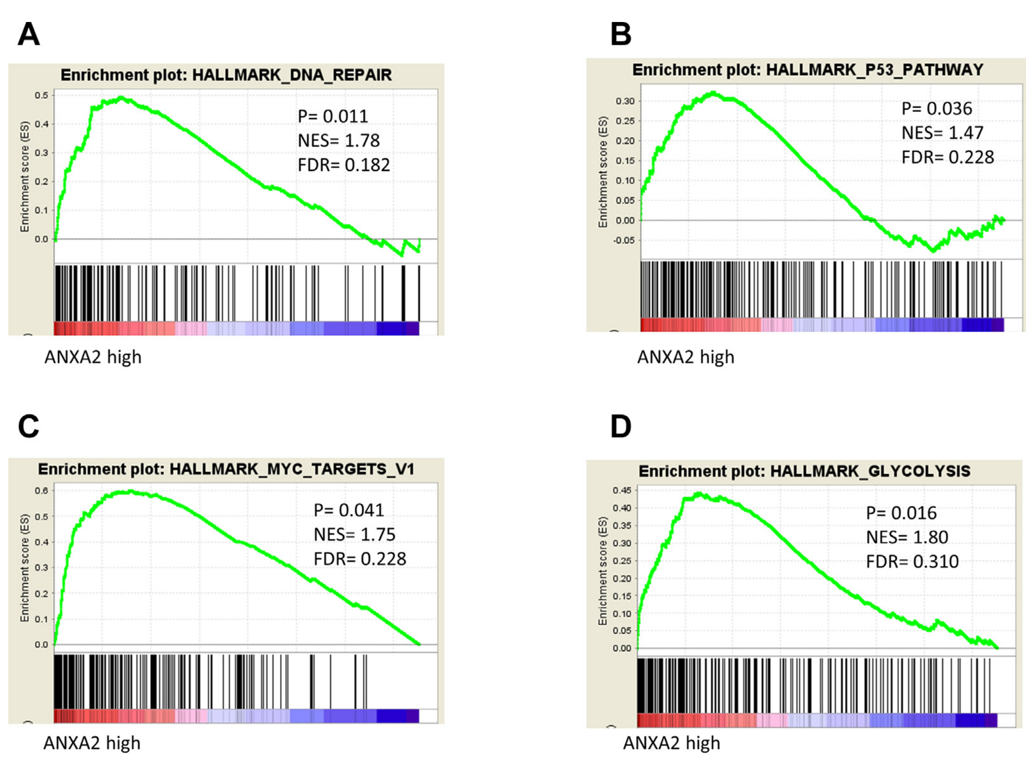

Fig 3. The association of ANXA2 and unreported signaling of ANXA2 by gene sets enrichment analysis. (*A*) Enrichment plot with DNA repair (NES = 1.78; *P* = .011, FDR = 0.182). (*B*) Enrichment plot with p53 pathway (NES = 1.47; *P* = .036, FDR = 0.228). (*C*) Enrichment plot with MYC targets v1 (NES = 1.75; *P* = .041, FDR = 0.228). (*D*) Enrichment plot with glycolysis (NES = 1.80; *P* = .016, FDR = 0.310).

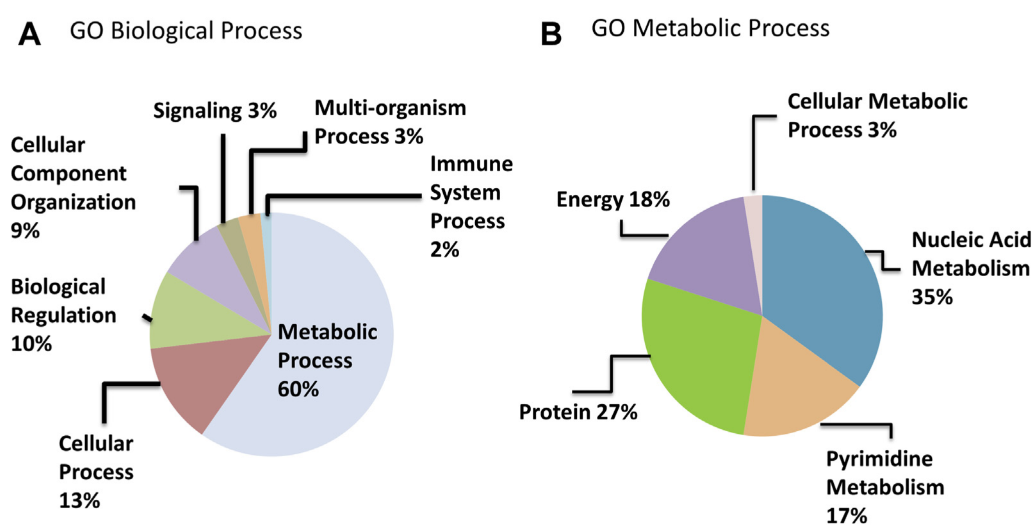

Fig 4. Gene sets enrichment analysis with GO Biological Process gene sets. (*A*) Categories in GO biological process enriched in the high ANXA2 expression group. Numbers within the pie indicate percentage of the enriched categories. (*B*) Subcategories of metabolic process in the high ANXA2 expression group. Numbers within the pie indicate percentage of the enriched categories.

ANXA2 may be associated with DNA repair mechanism in PDAC (Fig 3, *A* and *B*). MYC targets v1 gene set (NES = 1.75; P = .041, FDR = 0.228) was also enriched in the ANXA2 high group, indicating that higher ANXA2 tumors may have enhanced proliferative capability (Fig 3, *C*). Additionally, glycolysis gene set was enriched (NES = 1.80; P = .016, FDR = 0.31), which may illustrate a potential association of ANXA2 with glucose metabolism in PDAC (Fig 3, *D*). Among GO Biological Process 886 gene sets, 67 gene sets were significantly enriched in the ANXA2 high tumors. Of those, 40 gene sets were categorized as metabolic process in GO classification, which further implies that ANXA2 may modulate metabolic alterations in PDAC either directly or indirectly (Fig 4, *A*). Metabolic process gene sets were further subcategorized, which revealed that more than half of the genes were involved in nucleic acid or pyrimidine metabolism (Fig 4, *B*, Supplementary Table II-V). Taken together, these findings indicate that ANXA2 high PDACs may have inherent metabolic propensity to synthesize increased amounts of nucleotides to sustain rapid cellular proliferation and DNA repair.

High ANXA2 expression is associated with worse prognoses in PDAC

We found that the ANXA2 high tumors were associated with aggressive features of cancer, such as DNA repair, higher cell proliferation, and metabolic alteration. These findings led us to further hypothesize that patients with high ANXA2 tumors may have worse survival. As we expected, patients with ANXA2 high expression tumor demonstrated significantly worse DFS than the low expression group (median DFS time: 8.5 months vs 17.3 months, P = .001; Fig 5, *A*), which suggests that those tumors are more likely to recur. Furthermore, the ANXA2 high expression group demonstrated significantly worse OS compared with the low expression group (median OS time: 12.5 months vs 20.6 months, P = .004; Fig 5, *B*). This association between ANXA2 high expression and poor OS was validated in another cohort (GSE85916; median OS time: 14.6 months vs 21.1 months, P = .041; Fig 5, *C*). To investigate if ANXA2 is an independent prognostic factor, univariate and multivariate analyses were performed. High ANXA2 expression (hazard ratios = 2.85, P = .001) remained an independent risk factor for DFS in the patients with PDAC (Table II). Similarly, ANXA2 high expression was the only significant independent risk factor for OS (HR=1.90, P = .014; Table III).

Discussion

In the present study, we demonstrated that ANXA2 did not associate with the known clinical nor pathologic features that indicate cancer aggressiveness, including PNI, LVI, histological grade, and

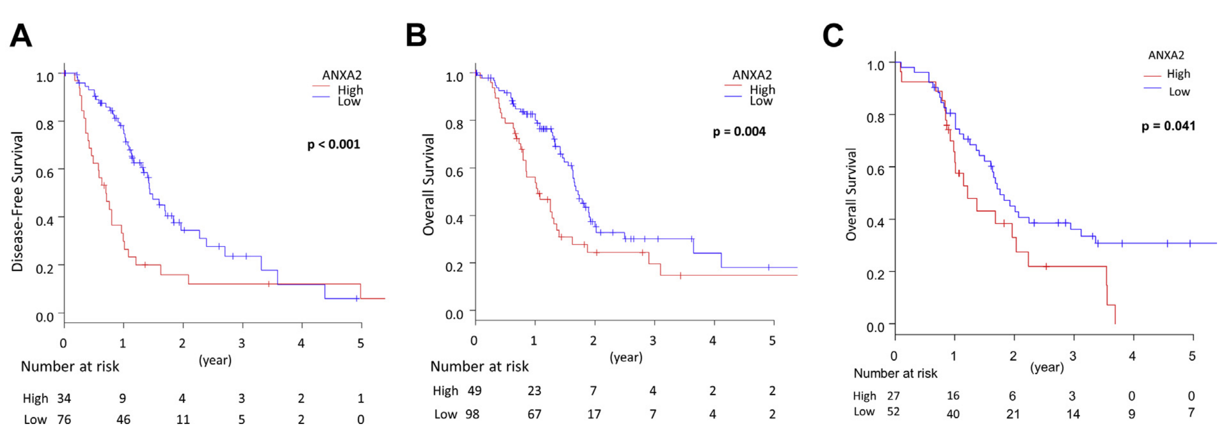

Fig 5. Kaplan-Meier curves depicting patient survivals by expression of ANXA2 in the TCGA pancreatic cancer cohort. (*A*) DFS. Median survival time: ANXA2 low 17.3 months versus ANXA2 high 8.5 months. (*B*) OS. Median survival time: ANXA2 low 20.6 months vs. ANXA2 high 12.5 months. (*C*) Overall survival (OS) with a validation cohort (GSE85916). ANXA2 low 21.1 months versus ANXA2 high 14.6 months.

Table II
Univariate/multivariable analysis (COX proportional hazards model) for DFS

	Univariate analysis			Multivariate analysis		
	P value	Hazard Ratio	95% CI	*P* value	Hazard Ratio	95% CI
Age (>65)	.839	0.953	0.601–1.515			
Sex (M)	.479	0.845	0.531–1.351			
Tumor size (≥3.5 cm)	.035	1.695	1.038–2.798	.114	1.642	0.888–3.035
AJCC T (T3+T4/T1+T2)	.960	0.983	0.523–2.048			
AJCC N (N1)	.142	1.476	0.881–2.592	.753	1.131	0.525–2.436
AJCC M (M1)	.873	0.853	0.048–3.989			
Histologic grade (G3/G1+G2)	.039	1.712	1.028–2.780	.263	1.500	0.737–3.050
Pathologic stage (III+IV/I+II)	.833	0.863	0.141–2.758			
Residual tumor status (R1+R2/R0)	.017	1.879	1.123–3.109	.076	1.856	0.937–3.679
Perineural invasion (PNI +)	.458	1.339	0.644–3.266			
Lymphovascular invasion (LVI +)	.063	1.679	0.973–3.031	.736	1.128	0.559–2.279
ANXA2 expression (high)	.002	2.246	1.360–3.649	.001	2.847	1.504–5.388

pathologic AJCC stage. On the other hand, high ANXA2 expressing PDACs were associated with DNA repair, metabolic alterations, and cell proliferation, but not angiogenesis or EMT gene sets. We further found that PDAC patients with high ANXA2 expression tumors had significantly worse prognoses. To our knowledge, this is the first study demonstrating association between ANXA2 and DNA repair, metabolic alteration and survival in PDAC.

ANXA2 expression is elevated in various solid organ malignancies and associated with recurrence, metastasis, and worse survival, which implicates that ANXA2 is a molecular signature for aggressive cancers.[7,8] It has been reported that silencing ANXA2 resulted in cell cycle arrest in nonsmall cell lung cancer and downregulated MYC, contributing loss of invasive capacity of breast cancer.[17,38] These reports support our results that ANXA2 was associated with MYC targets genes, suggesting the potential ANXA2 function in tumor proliferation. Our finding that ANXA2 was associated with TNF and NF-κB signaling pathway is in agreement with previous reports demonstrating that ANXA2 mediates upregulation of NF-κB signaling and subsequent chemotherapy resistance in PDAC.[12,39] Also, TNF is one of the major stimulation of NF-κB, and there were several reports demonstrating correlation between ANXA2 and TNF in inflammatory disease.[40,41] It was also reported that ANXA2 is accumulated in the nucleus to reduce DNA damage in response to genotoxic agents in normal tissues.[42,43] Enhanced DNA repair is known to be one of the mechanisms of chemotherapy resistance in cancer cells.[12,14] Our finding that DNA repair was associated with ANXA2 expression may explain that ANXA2 high group has enhanced chemo-resistance that resulted in poor prognosis.

Although some of the known ANXA2 functions were comparable in our results as indicated earlier, we did not find any association of ANXA2 with some of other known roles, such as angiogenesis or EMT. In contrast, we identified the association of ANXA2 and metabolic alteration, which is one of the hallmarks of cancer.[44] Accelerated glycolysis has been well documented in cancer as a Warburg effect, although it is relatively less efficient to generate ATP compared with mitochondrial oxidative phosphorylation. Increased glycolysis was found to divert glycolytic intermediates into various biosynthetic pathways such as generating nucleosides and amino acids for assembling new cells.[2,3,45] Increased metabolism, such as enhanced nucleic acid metabolism, and increased carbohydrate and protein metabolism are also well known phenomena in PDAC to compensate the increased cellular demand.[46] Glycolysis and subsequent pentose phosphate pathway are being utilized to generate the pentose sugars, which serve as primary intermediates in the nucleotides and nucleic acid synthesis in PDAC.[46] Although there are limited reports demonstrating the involvement of ANXA2 in tumor metabolic alterations, our results suggest that in PDAC ANXA2 is associated with glucose metabolism and pyrimidine metabolism, resulting in poor prognosis of PDAC.

There are limitations with this study. What we found in this study is only the association of high ANXA2 expression with worse OS and possible aggressive tumor biology features using TCGA. TCGA has a few disadvantages. Although it does offer significant benefits with a myriad of gene expression data associated with clinical information, the median follow-up of PDAC was 15 months (interquartile range 8–21 months), which is rather short. TCGA provides only gene expression of the surgically resected primary tumor that account for <20% of pancreatic cancer population; hence, ANXA2 role in metastatic tumor is unclear. Last, this study does not include any in vitro or in vivo experiments, thus all our findings are mere associations and causality is unknown. To prove the role of ANXA2 in PDAC, the experimental results will be required.

Table III
Univariate and multivariable analysis (Cox proportional hazards model) for OS

	Univariate analysis			Multivariable analysis		
	P value	Hazard Ratio	95% CI	*P* value	Hazard Ratio	95% CI
Age (>65)	.731	1.076	0.710–1.645			
Sex (M)	.875	0.975	0.709–1.344			
Tumor size (≥3.5 cm)	.529	0.898	0.641–1.256			
AJCC T (T3+T4/T1+T2)	.717	1.128	0.611–2.326			
AJCC N (N1)	.080	1.556	0.950–2.691	.686	1.139	0.607–2.138
AJCC M (M1)	.605	1.385	0.333–3.859			
Histologic grade (G3/G1+G2)	.132	1.412	0.898–2.173	.192	1.453	0.829–2.544
Pathologic stage (III+IV/I+II)	.997	1.001	0.351–2.240			
Residual tumor status (R1+R2/R0)	.024	1.703	1.076–2.667	.087	1.616	0.933–2.799
Perineural invasion (PNI +)	.282	1.472	0.747–3.342			
Lymphovascular invasion (LVI +)	.011	1.883	1.150–3.216	.376	1.303	0.725–2.343
ANXA2 expression (high)	.006	1.872	1.201–2.887	.014	1.902	1.114–3.175

In conclusion, ANXA2 high expression is significantly associated with NF-κB and TNF signaling, cell proliferation, DNA repair, metabolic process gene signatures, and worse prognosis in patients with PDAC. Given its association with survival, ANXA2 expression can be a potential candidate as a prognostic biomarker in PDAC. Additional investigation is required to elucidate the mechanism of ANXA2 role in DNA repair and altered metabolism.

Conflict of interest

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.surg.2019.04.011.

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