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Comprehensive analysis of the expression and prognostic value of ARMCs in pancreatic adenocarcinoma

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Abstract

Background Pancreatic adenocarcinoma (PAAD) has a very poor prognosis, and there are few treatments for PAAD. Therefore, it is important to find some biomarkers for the diagnosis and treatment of PAAD. Although some members of Armadillo repeat containing proteins (ARMCs) have been implicated in the development of certain cancers, their relationship with PAAD remains unknown. In this study, we aimed to explore the expression and prognostic value of ARMCs in PAAD.

Methods We used the The Cancer Genome Atlas (TCGA) database for survival analysis. Then, Gene Expression Profiling Interactive Analysis (GEPIA), the cBioPortal database, the Human Protein Atlas (HPA), Kaplan-Meier Plotter, LinkedOmics Database, Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG), Cytoscape and Timer were used to analyze the relationship between ARMCs and PAAD. In addition, we established a prognostic model of ARMCs for PAAD. Immunohistochemistry (IHC) was also performed. Then Image-J was used to analyze all images obtained from the experiment, and GraphPad-Prism (9.5.1) was used for statistical analysis to verify the expression of ARMCs in PAAD.

Results In the TCGA database, the expressions of ARMC1, 2, 3, 5, 6, 7, 8, 9 and 10 in PAAD tissues were significantly higher than those in normal tissues. And higher expressions of ARMC1 and 10 were associated with lower survival rate of PAAD patients. In addition, ARMC2, 5, 6, and 10 were positively associated with advanced stages of PAAD. ARMCs mutations occur in 11% of PAAD patients, and missense mutations and amplification of ARMCs account for most of them. In addition, ARMC5 and ARMC10 were independent prognostic factors in univariate and multivariate Cox regression analyses. Finally, through our confirmation experiment, it was found that the expression of ARMC1 and 10 in PAAD tissues was significantly increased compared with those in paracancer tissue.

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Conclusion This study suggests that ARMCs may be able to play important roles in PAAD, and they can act as biomarkers, providing valuable clues for the treatment and diagnosis of PAAD.

Keywords Pancreatic adenocarcinoma, Tumor marker, ARMCs, Immune infiltration, Prognostic mode

Introduction

Pancreatic adenocarcinoma (PAAD) is a highly deadly and aggressive malignancy with a poorest prognosis of all malignancy [1]. Over 90% of deaths from PAAD are due to late diagnosis, often with metastasis, and the five-year survival rate is only seven% [2]. The only curative treatment for PAAD is surgical radical resection, but only 20% of patients can be treated [3]. For patients diagnosed at an advanced stage, the results of the operation are poor. Although a lot of research has been done on PAAD, treatments for PAAD are still limited [4]. Therefore, there is urgently needed in identifying some molecular biomarkers for the diagnosis and prognosis of PAAD, which may contribute to the development of targeted diagnosis and treatment strategies 5.

Armadillo repeat-containing proteins (ARMCs) are ubiquitous in eukaryotes. Armc1-10, ARMC12, and ARMCX1-6 constitute ARMCs. The tandem repeats of these proteins are approximately as long as 42 amino acids. Many people have studied these gene in depth and found that they play important roles not only in cell adhesion, intracellular signal transduction, but also tumorigenesis and so on [6]. Studies have shown that AMRC1 is related to mitochondria and can regulate the fission and distribution of mitochondria [7]. Other researches show that the occurrence of glioma and mitochondrial dysfunction. Therefore, ARMC1 may promote the development of glioma tumorigenesis [8]. ARMC2 is a strong risk factor for prostate cancer [9]. It has been reported that ARMC4 can inhibit the Wnt/β-catenin pathway [10]. The Wnt/β-catenin pathway is associated with colorectal cancer. Therefore, ARMC4 can inhibit the occurrence of colorectal cancer. Udolph et al. reported that ARMC6 can promote the occurrence of neuroblastoma through the NOTCH signaling pathway [11]. ARMC8 can promote the progression of lung cancer by activating the Wnt/β-catenin pathway [12]. In addition, the occurrence and development of breast cancer, ovarian cancer, hepatocellular carcinoma and bladder cancer are related to the dysregulated of ARMC8[13, 14]. ARMC10 is found in mitochondria, and its overexpression can lead to mitochondrial fission, which leads to the occurrence of liver cancer [15]. However, there are few studies on the diagnosis and prognosis of ARMCs in PAAD. Therefore, our aim is to investigate the expression and clinical significance of ARMCs in PAAD, so as to provide new ideas for the diagnosis and treatment of PAAD.

Materials and methods

Gene expression profiling interactive analysis (GEPIA)

GEPIA (http://gepia.cancer-pku.cn/) is an analysis tool based on The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression project (GTEx). And this is made up of multiple tumor and normal tissue samples. We used GEPIA to compare the differential gene expression of ARMCs in PAAD patients and normal subjects.

Human protein atlas

The Human Protein Atlas (HPA) (https://www.proteinatlas.org) contains a variety of common cancer immunohistochemical data. This resource contains Cancer information based on mRNA and protein expression data from 31 different forms of human cancer. The Cell line resource contains information on genome-wide RNA expression profiles of human protein-coding genes in 1206 human cell lines, including 1132 cancer cell lines. Confocal images of cell localization were collected using HPA. We can use these images to study the expression and localization of ARMCs in cells.

LinkedOmics Database

The LinkedOmics database is a public platform for the analysis of multiomics data and mass spectrograph-based proteomics data for all 32 TCGA cancer types. We used the LinkedOmics database to select the RNA-seq data types of PAAD patients from the TCGA database and used the statistical method of Pearson Correlation test to obtain other genes related to the target genes. We screened the top 50 genes that are obviously related to ARMCs and show them in heat maps.

cBioPortal

cBioPortal (www.cbioportal.org) is an online site that has been used to study genetic variations in cancer. These variations include mutation, amplification, and copy number variation. We used this website to study the genetic changes of ARMCs family genes in PAAD.

Protein-protein Interaction(PPI) Network Construction

The String database (http://string-db.org) contains much publicly available information about protein-protein interactions. The String database is used to build the PPI network of ARMCs. We used Cytoscape (https://metascape.org/) to visualize the genes involved in ARMCs and construct the PPI network. And the hub genes were identified by PPI network.

Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG)

GO and KEGG enrichment analyses were formed in the DAVID database (https://david.ncifcrf.gov/). GO enrich ment analysis included biological processes(BP), cellular components (CC), and molecular functions (MF). KEGG analysis could identify the neighbor genes and pathways associated with ARMCs mutations.

Timer

Timer (https://cistrome.shinyapps.io/timer/) is a online site for the systematic evaluation of immune cell correlation with cancer. We used this website to visualize the relationship between ARMCs and immune cells in PAAD and to generate several scatter plots.

Kaplan-Meier Plotter

Kaplan-Meier plotter (http://kmplot.com) was used to analyze the prognostic effects of different ARMCs expression in PAAD patients. Overall survival (OS) is the time from initial diagnosis to death of PAAD patients. Disease Special Survival is considered to be the time from the time a PAAD patient is initially diagnosed to the time of death due to PAAD. When the P value<0.05, it is considered to be statistically significant.

Prognostic modeling

We used LASSO regression to identify five genes in ARMCs that are associated with PAAD prognosis. These genes were screened for univariate and multivariate Cox proportional regression analysis.

Immunohistochemistry (IHC) analysis

25 tumor and paracancer tissues of PAAD patients were received from Fujian Provincial Hospital. The study was approved by the Ethics Committee of Fujian Provincial Hospital. IHC assays were performed using formalin-fixed PAAD tissue samples which were embedded in paraffin sections. These paraffin sections were deparaffined. And then the antigen was repaired using EDTA antigen repair solution. The paraffin sections were incubated with endogenous peroxidase blockers for 20 min, and then the sections were added with 5% BSA blocking solution and incubated for 30 min at room temperature. Antibodies against ARMC1 (Sino Biological, China) and ARMC10 (Proteintech, China) were added to the sections. The slices were kept in the refrigerator at 4℃ overnight (reheated for 45 min after taking them out of the refrigerator), and the secondary antibody was added and incubated at room temperature for 30 min. Finally, DAB chromogenic reagent was used to make the specimen chromogenic.

Image-J

All images obtained in experiments were analyzed in Image-J(9.5.1). And then we used Image-J to measure the integrated optical density (IOD) of images. Finally, the average optical density (AOD) (AOD=IOD/AREA) was determined.

GraphPad-Prism

Statistical analysis was performed using GraphPad-Prism (9.5.1). We compared the expression levels of ARMC1 and ARMC10 in pancreatic and paracancer tissues by AOD. In addition, the expression of these two genes in stage I and II PAAD tissues was compared with those in stage III and IV PAAD tissues by the same method. PAAD patients were grouped by sex, age, and presence of lymph node metastasis, and the data were analyzed using the same method. The comparison of sample means between the two groups was performed by two-tailed Student's t-test. $P<0.05$ was considered statistically significant.

Results

Gene expression of ARMCs in PAAD

R package "ggplot2" was used to analyze the differential expression of ARMCs in PAAD and normal tissues from TCGA and GTEx databases. There were 179 PAAD tissues and 171 normal and paracancerous tissues. The expression of ARMC1, 2, 3, 5, 6, 7, 8, 9 and 10 was significantly increased ($p<0.05$) when compared with normal and paracancerous human tissues (Fig. 1a).

GEPIA database was used to analyze the correlation between ARMCs and tumor stage. A total of 175 patients were enrolled, including 21 patients in Stage I, 146 in Stage II, 3 in Stage III, and 5 in Stage IV. The results showed that the expression of ARMC2, 5, 6 and 10 in different stages was significantly different ($p<0.05$), but more studies are needed to confirm it due to the small number of patients in stage III and stage IV. Other ARMCs were not significantly associated with different stages of PAAD (Fig. 1b).

Protein expression and Cellular localization of ARMCs in PAAD

The Human Protein Atlas (HPA) were used to explore the expression of ARMCs in human PAAD. The localization of gene expression in cells often determines different important functions. We used confocal images from the Human Protein Atlas (HPA) database to study the expression localization of ARMCs in human PAAD cell lines (Fig. 2). The results showed that ARMC1 was detected in mitochondria, ARMC2 was detected in nucleoplasm, ARMC5 was detected in nucleoplasm and cytoplasm, ARMC6 was detected in cytoplasm, ARMC7 was detected in cytoplasm, ARMC8 was detected in

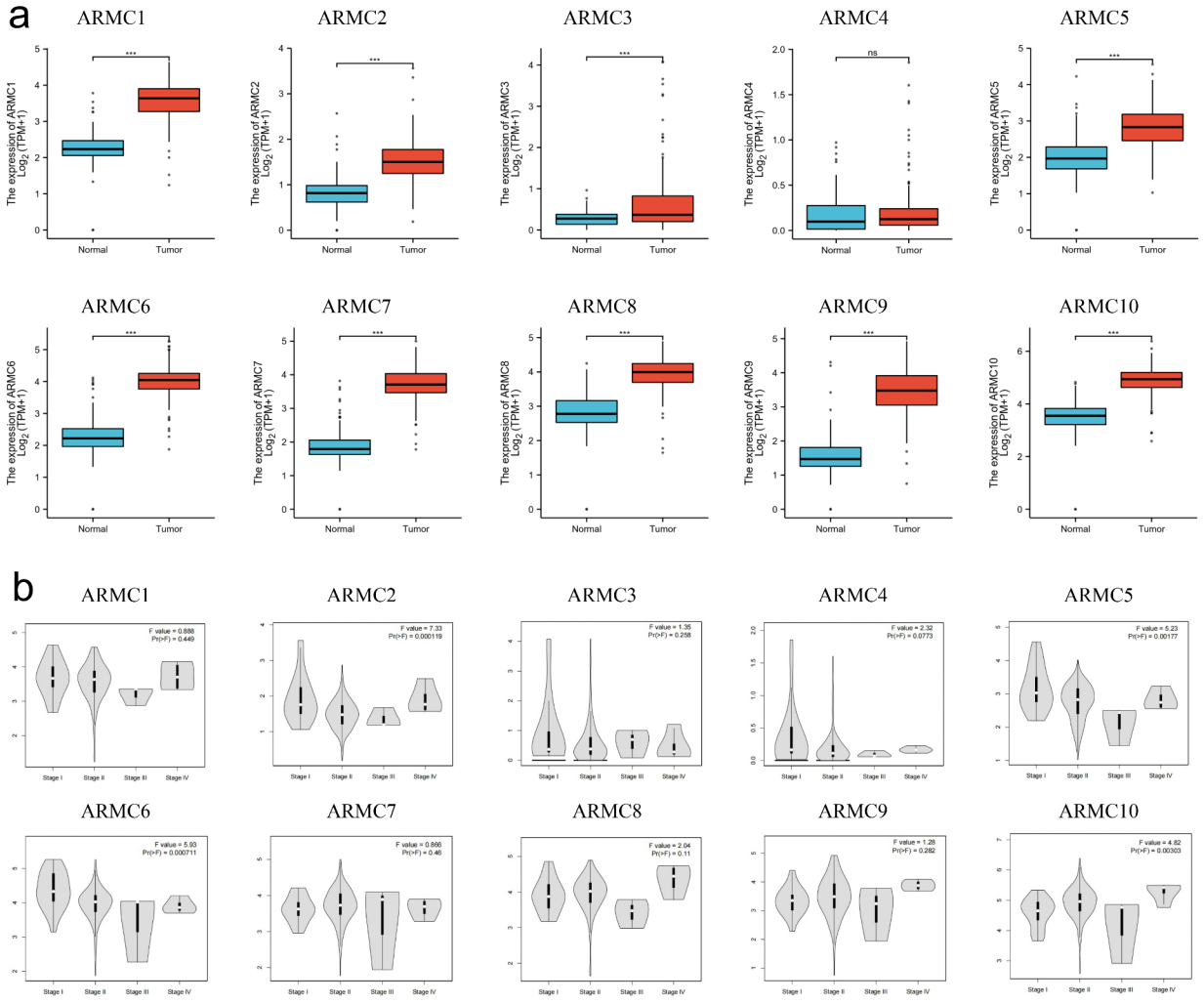

Fig. 1 Correlation between ARMCs expression and the clinicopathological characteristics of PAAD patients. **a** The expression of ARMCs gene in PAAD. **b** The expression levels of ARMCs in different pathological stages using GEPIA. *$P<0.05$, **$P<0.005$, ***$P<0.001$. "ns" was regarded as no statistically significant

nucleoplasm and vesicles, and ARMC10 was detected in mitochondria. In this image, each expression was from the same cell line. Moreover, the results showed that the proteins in these different human PAAD cell lines were distributed in the same subcell. The colocation results of ARMC3, ARMC4, and ARMC9 are not found in the HPA database.

Diagnostic value of ARMCs for PAAD patients

In order to assess ARMCs value to the diagnosis of patients with PAAD, we used the UCSC XENA database (https://xenabrowser.net/datapages/) to analyze TCGA database data. And the receiver operating characteristic (ROC) curve analysis was made (Fig. 3). The results showed that most of these genes have high diagnostic value for distinguishing PAAD patients from healthy people, including ARMC1 (0.963), ARMC2 (0.918), ARMC5 (0.857), ARMC6 (0.973), ARMC7 (0.984), ARMC8 (0.934), ARMC9 (0.970), ARMC10 (0.957). ARMC3 (0.639) and ARMC4 (0.545) had moderate value.

Correlated genes with ARMCs in PAAD

TCGA database was used to explore the genes associated with ARMCs. Using the top 25 related genes, we created volcano maps and heat maps (Fig. 4a, b). It can be seen intuitively in the volcano maps that the differential genes were concentrated in the upper left and upper right corners of the figure. It can be seen that the most positive genes related to ARMC1 include RB1CC1, VCPIP1 and NBN, while the most negative genes related to ARMC1 include C1orf24 and TIAF1. Among the 241 differentially expressed genes, there are 35 up-regulated and 206 down-regulated genes. The most positive genes associated with ARMC2 included LOC338758 and GNG2. The

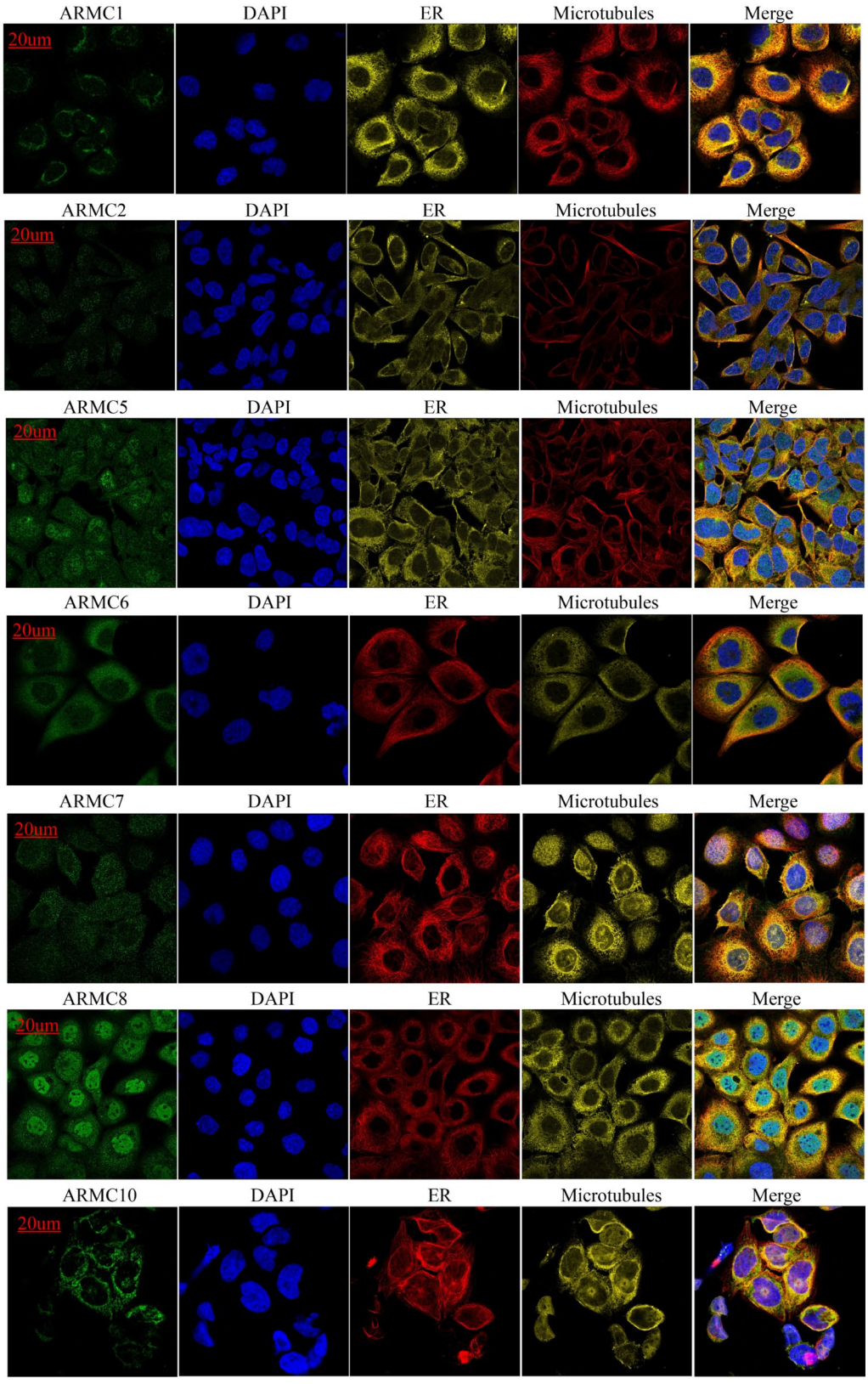

Fig. 2 Cell colocalization image of ARMCs

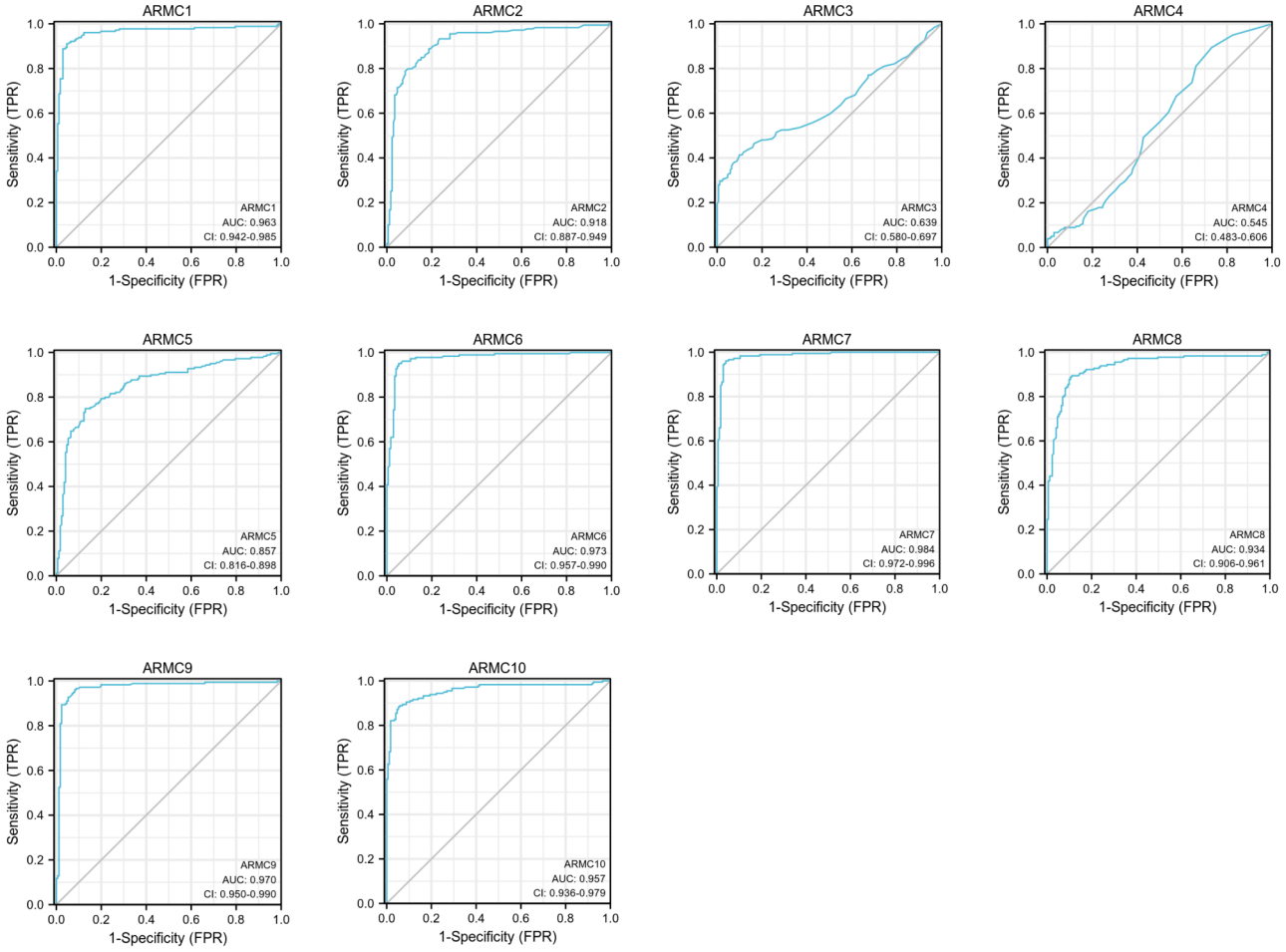

Fig. 3 ROC analysis of individual ARMCs in TCGA database

most negative genes associated with ARMC2 included LRRC8E and MST1R. The most active genes associated with ARMC3 included C7orf57 and C1orf192. The most related negative genes of ARMC3 include MCOLN2 and C3. Among the 274 differentially expressed genes, there are 85 up-regulated and 189 down-regulated genes. The most active genes associated with ARMC4 included C1orf194 and KNCN. The most associated negative genes of ARMC4 included RHOD and C19orf33. Among the 465 differentially expressed genes, there are 351 up-regulated and 114 down-regulated genes. The most active genes associated with ARMC5 include MBD3 and THAP7. The most associated negative genes of ARMC5 included WDFY1 and ZFP91. Among the 363 differentially expressed genes, there are 266 up-regulated and 97 down-regulated genes. The most active genes associated with ARMC6 included ASPSCR1 and COPE. The most associated negative genes of ARMC6 included PDLIM5 and ATP11B. Among the 583 differentially expressed genes, there are 448 up-regulated and 135 down-regulated genes. The most positive genes associated with ARMC7 included CCDC137 and DUS1L. The most negative genes associated with ARMC7 included PCNP and PJA2. Among the 135 differentially expressed genes, there are 29 up-regulated and 106 down-regulated genes. The most positive genes associated with ARMC8 included PIK3CA and FYTTD1. The most associated negative genes of ARMC8 included DDRGK1 and TECR. Among the 355 differentially expressed genes, there are 58 up-regulated and 297 down-regulated genes. The most active genes associated with ARMC9 included COL1A2 and COL3A1. The most negative genes associated with ARMC9 included SNHG8. Among the 331 differentially expressed genes, there are 47up-regulated and 284 down-regulated genes. The most active genes associated with ARMC10 include MET and LRRC8E. The most related negative genes of ARMC10 include PDZD4 and SLC26A11. Among the 669 differentially expressed genes, there are 91 up-regulated and 578 down-regulated genes.

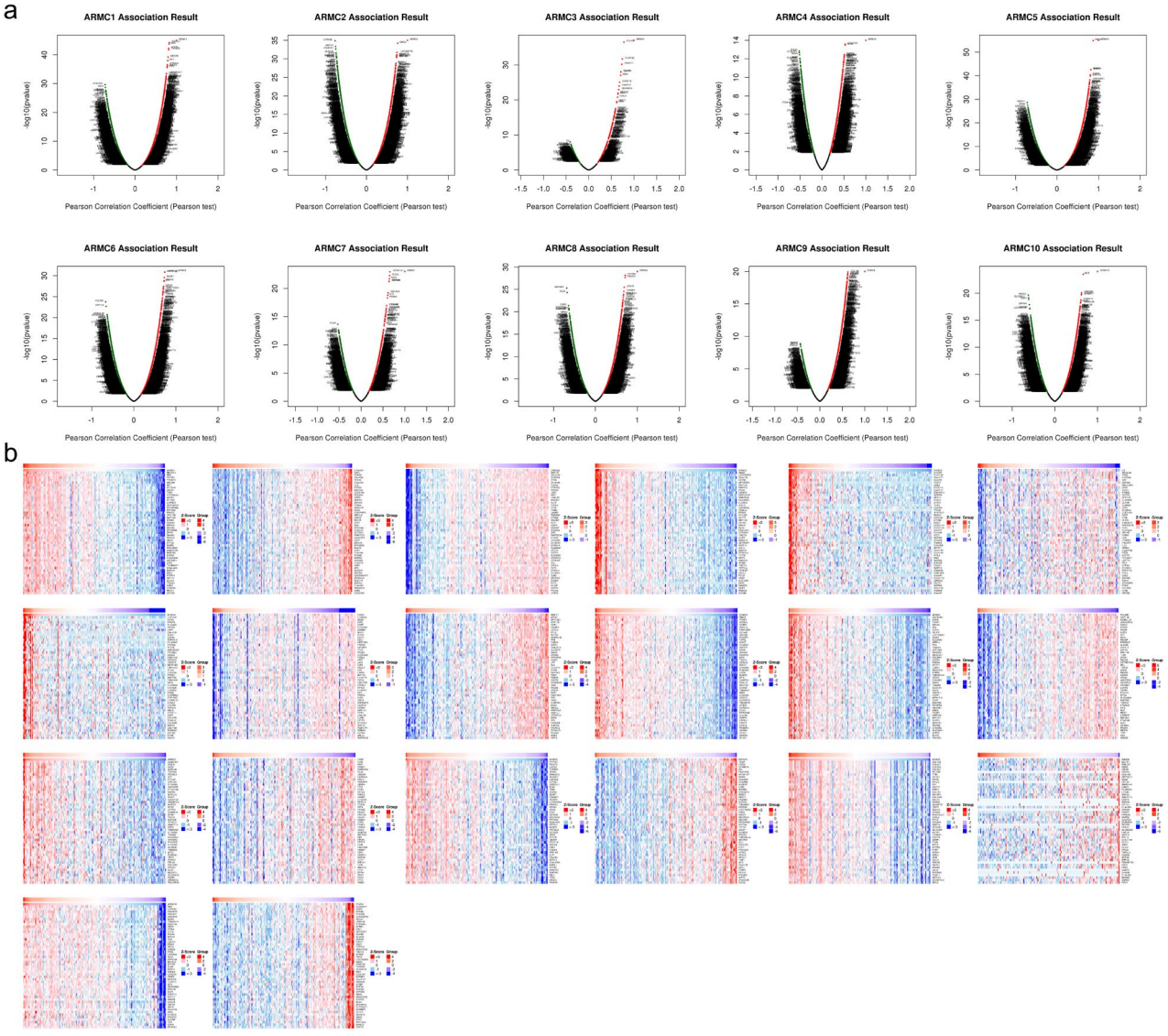

Fig. 4 Correlated genes with ARMCs in PAAD. **a** Volcano plot of the 25 genes most associated with ARMCs. **b** Heatmap plot of the 50 genes most associated with ARMCs

Genetic alterations, and correlation analyses of ARMCs in PAAD

We used the cBioPortal platform (http://www.cbioportal.org) to study gene alternations of ARMCs (Fig. 5). ARMC4 is not shown in the results. Three alterations were found in different subtypes of ARMCs, and the amplification was most common mutation in PAAD samples (Fig. 5a). 20 samples of ARMCs of 175 patients were found to have genetic mutations, accounting for 11% (Fig. 5b). ARMC1, 7 and 9 included missense mutations and amplification. ARMC2 included deep deletion and missense mutations. ARMC3 and 6 included missense mutations. ARMC5 was not mutated. ARMC8 included amplification and splice mutations. ARMC10 included amplification. ARMC1, ARMC2, ARMC3, ARMC5, ARMC6, ARMC7, ARMC8, ARMC9 and ARMC10 were altered in 5, 1.1, 1.7, 0, 0.6, 2.3, 1.1, 1.7, 1.7% of the PAAD samples.

In addition, we used the Pearson correlation analysis to examine the correlation among the ARMC members (Fig. 5c). The results showed, clearly positive correlations were observed between ARMC1 and 2, 4, 8, 9; ARMC2 and 4, 5, 6, 8, 9; ARMC3 and 10; ARMC5 and 6, 7; ARMC6 and 7; ARMC8 and 9, 10; ARMC9 and 10. Clearly negative correlation was observed between ARMC5 and 10.

Functions and pathways of ARMCs in PAAD patients

We analyzed 200 genes that are highly associated with ARMCs mutations and used the cBioPortal database

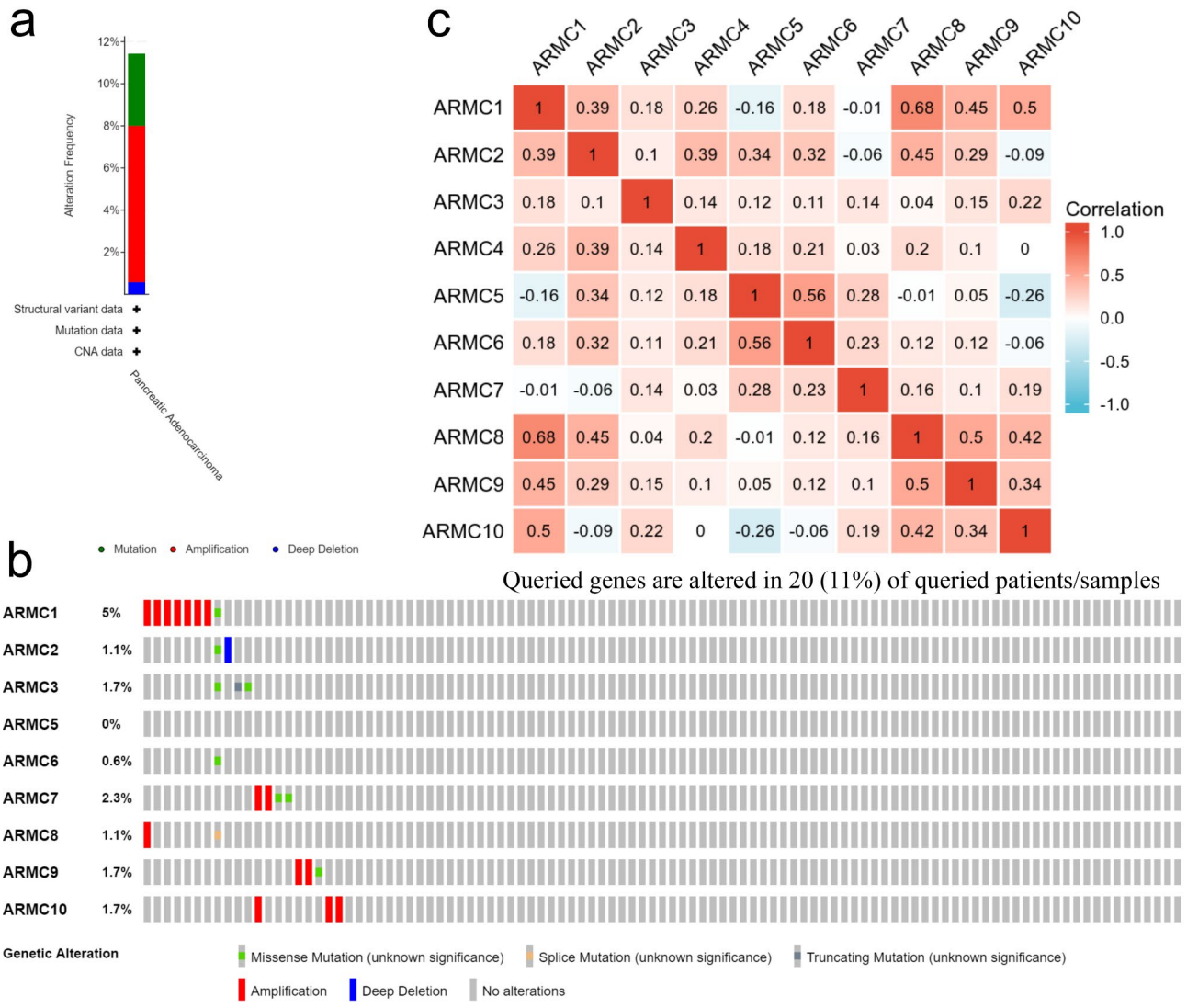

Fig. 5 Correlation and Mutation analysis of ARMCs in PAAD. **a** Mutation frequency of ARMCs. **b** correlation between ARMCs. **c** the visual summary Oncoprint based on a query of the ARMC family genes (not including ARMC4)

(www.cbioportal.com) and Cytoscape to build a protein-protein interaction (PPI) network (Fig. 6a). In addition, we used GO and KEGG in DAVID database to analyze the functions of ARMCs (Fig. 6b, c, d, e). And we used the Bioinformatics database for mapping. PPI network shows, including XPO1 RPTOR, ELAVL1, HNRNPA2B1, FAF2 and POLR2C mutations in multiple genes and ARMCs significant correlation. In GO term analysis and KEGG pathway enrichment analysis, it can be seen that ARMCs and their neighbor genes are mainly involved in Cilium assembly, Ubiqutin-dependent protein catabolic process, nucleotides, Cytosol indicates protein binding, and they are mainly involved in Nucleocytoplasmic transport.

Immune cell infiltration of ARMCs in PAAD patients

Immune cells plays an important role in tumorigenesis. Using the TIMER database, we investigated the relationship between immune cell infiltration and ARMCs expression (Fig. 7). The expression of ARMC1 was positively correlated with B cells, CD8+T cells, macrophages, neutrophils and dendritic cells. The expression of ARMC2 was positively correlated with B cells, CD8+T cells, CD4+T cells, macrophages, neutrophils and dendritic cells. The expression of ARMC3 was positively correlated with CD8+T cells. The expression of ARMC4 was positively correlated with B cells, CD8+T cells, macrophages and dendritic cells. The expression of ARMC5 was positively correlated with CD4+T cells. The expression of ARMC6 was positively correlated with CD4+T cells and macrophages. The expression of ARMC7 was

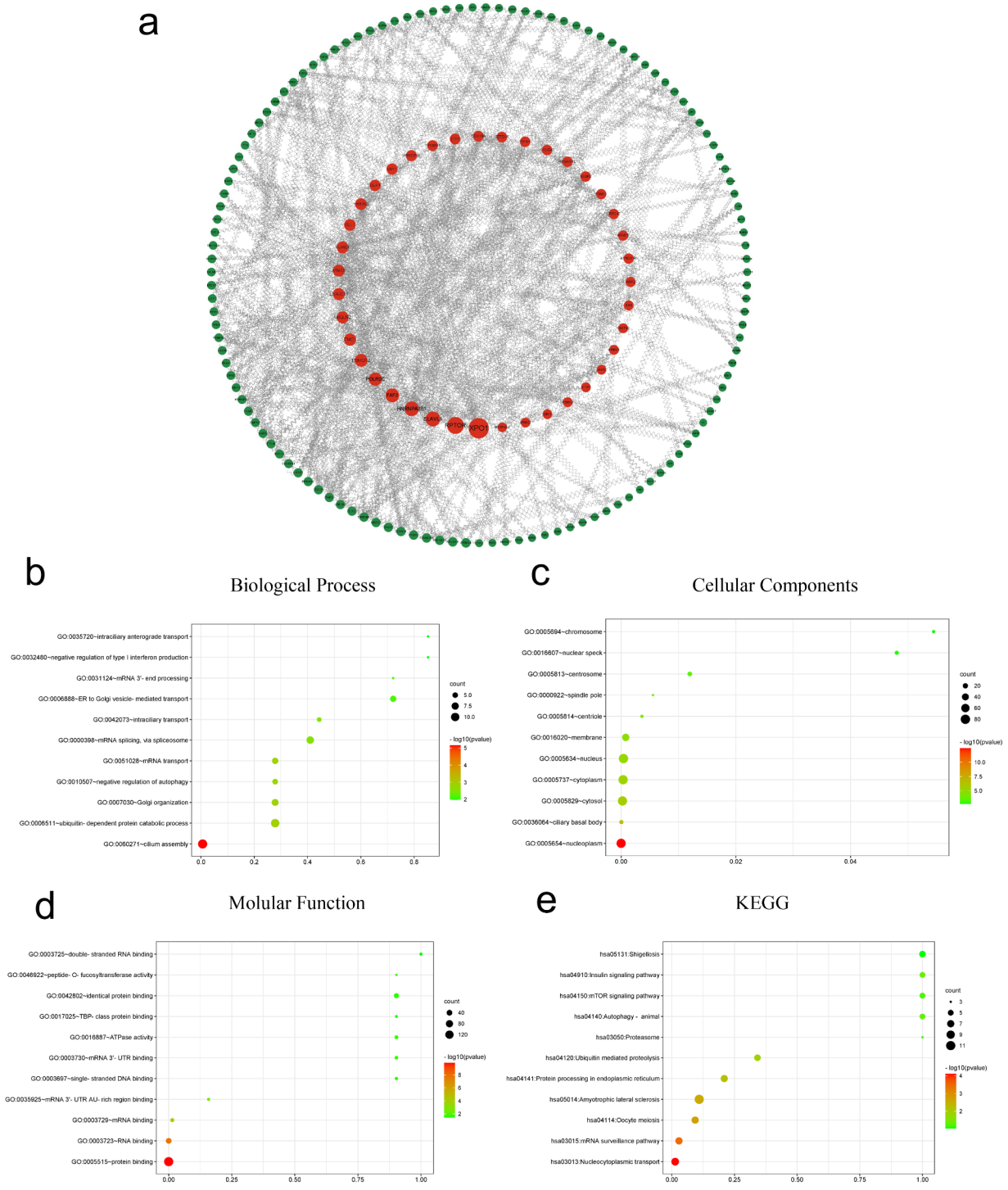

Fig. 6 Functional enrichment analysis and correlation of ARMCs. **a** Protein-protein interaction network analysis. **b** Biological process. **c** Cellular components. **d** Molecular function. **e** KEGG

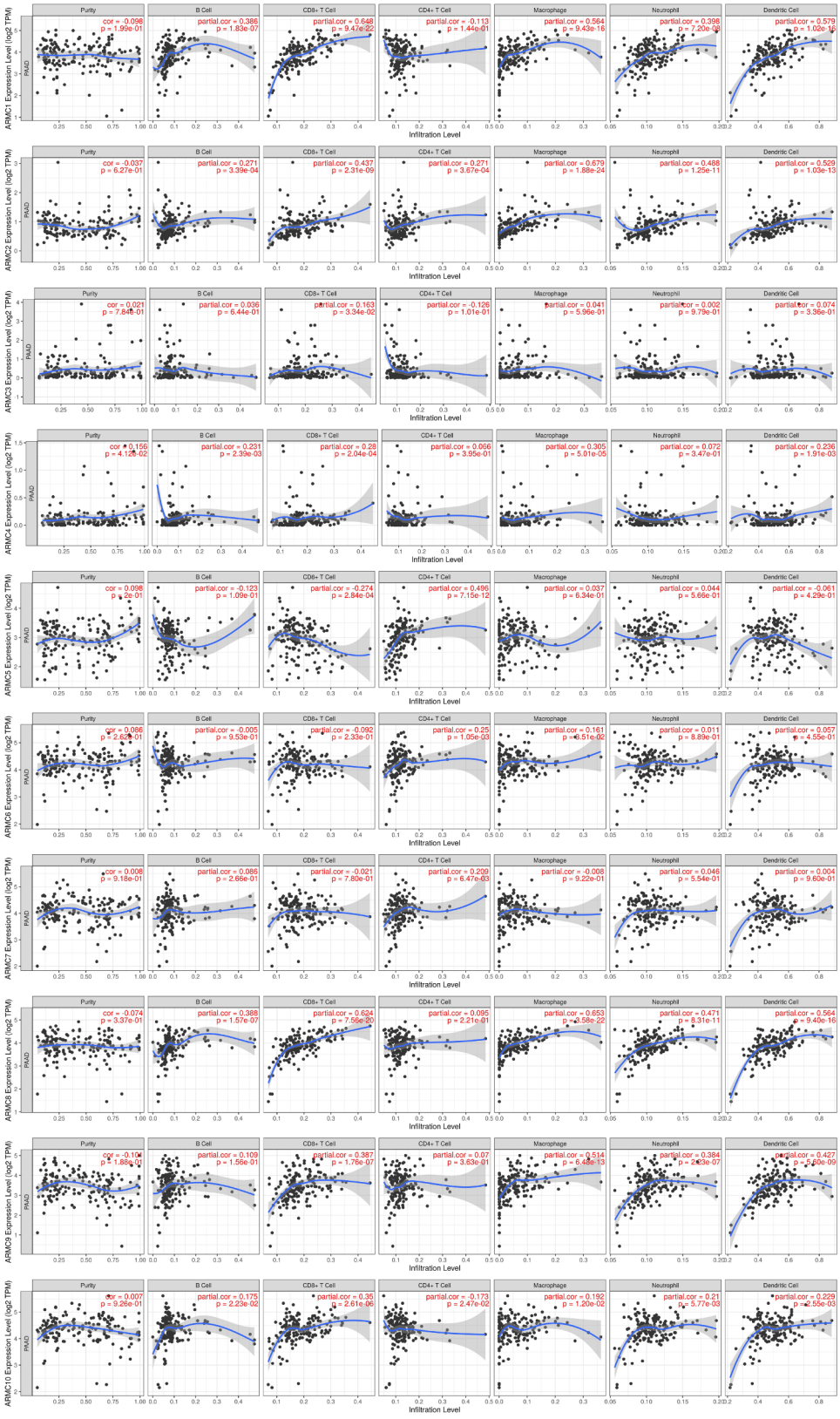

Fig. 7 Correlation between 7 kinds of immune cells and ARMCs

positively correlated with CD4+T cells. The expression of ARMC8 was positively correlated with B cells, CD8+T cells, macrophages, neutrophils and dendritic cells. The expression of ARMC9 was positively correlated with CD8+T cells, macrophages, neutrophils and dendritic cells. The expression of ARMC10 was positively correlated with B cells, CD8+T cells, macrophages, neutrophils and dendritic cells.

Prognosis of ARMCs in PAAD

In addition, the Kaplan–Meier Plotter was used to demonstrate whether ARMCs had an impact on the prognosis of PAAD (Fig. 8). Patients with low expression of ARMC1 were significantly correlated with better OS and DSS ($P<0.05$). In contrast to ARMC1, high expression of ARMC2, ARMC4 and ARMC5 were correlated with better OS and DSS ($P<0.05$). The high expression of ARMC3 was more associated with better OS, however, the results showed that the expression of ARMC3 had no significant relationship with DSS. The result for ARMC10 was the same as for ARMC1, with a more significant correlation between patients in the low-expression group and better OS and DSS ($P<0.001$). The expression of ARMC6, 7, 8 and 9 was not significantly associated with the OS and DSS of PAAD.

Prognostic modeling

A LASSO logistic regression analysis was performed on the gene expression matrix of 182 PAAD patients in TCGA database to determine the risk scores of the 6 most relevant gene (ARMC1, 2, 4, 5, 7 and 10) constructs based on whether they are associated with the development of PAAD (Fig. 9a, b).

Then, we used TCGA database to establish the KM survival curve distribution of the risk model, and the results showed that the survival status of patients in the high-risk group was significantly lower than that of patients in the low-risk group (HR=2.78, $p<0.001$). In addition, we also established the ROC curve of this model, and the AUC values were 0.681,0.714 and 0.855, for the ROC curves of this model at 2, 3 and 5 years, respectively (Fig. 9c).

In addition, univariate and multivariate Cox regression analyses were performed (Table 1). Results showed that cancer stage and radiation therapy were prognostic predictors in TCGA-PAAD in univariate Cox regression analysis, but not age, gender, histological grade, smoking, alcohol and family history. Histological grade and radiotherapy were prognostic factors in multivariate Cox regression analysis.

Univariate and multivariate cox regression analysis of the above 6 genes showed that ARMC2, 4, 5 and 10 were prognostic factors in univariate Cox regression analysis, while ARMC4, 5 and 10 were independent prognostic factors in multivariate Cox regression analysis (Table 2).

Immunohistochemical in PAAD

Immunohistochemical staining was performed from human PAAD (Table 3). The results showed that ARMC1 and ARMC10 are mainly distributed in the cytoplasm of PAAD cells. The positive expression of PAAD cells stained by chromogenic reagent shows obvious brown-yellow color, while the positive expression of PAAD cells in the nuclei and adjacent cells is relatively rare. The cell nucleus stained by hematoxylin shows blue color under the naked eye. The staining intensity of ARMC1 and ARMC10 in PAAD tissues was significantly higher than that in paracancer tissues ($P<0.0001$) (Fig. 10a). In addition, we compared the specimens from stage I and II with those from stage III and IV. The results showed that the staining intensity of ARMC1 in PAAD tissues at III and IV stage was significantly higher than that at stage I and II ($P<0.0001$). The staining intensity of ARMC10 in PAAD tissues at stage III and IV was also higher than that at stage I and II ($P<0.002$) (Fig. 10b). The positive expression rate of ARMC1 in patients with lymph node metastasis was not significantly different from those without lymph node metastasis ($P>0.05$). The positive expression rate of ARMC10 in patients with lymph node metastasis was significantly different from that in patients without lymph node metastasis ($P<0.05$) (Fig. 10c). In addition, the images of ARMC1 and ARMC10 from IHC were presented (Fig. 10d). The figure included cancer tissue and paracancer tissue. The paracancer tissue we selected was the area 1.0–2.0 cm away from the tumor margin. In PAAD tissues, the brown-yellow region is the region where DAB chromogenic agent was positively expressed, and the nuclei were stained blue by hematoxylin, which can be distinguished from the paracancerous tissue. In the figure of cancer tissue, the nuclei of PAAD cells were enlarged and obvious, and the nuclear division was easy to see.

Discussion

PAAD has a poor prognosis and is highly metastatic [16]. PAAD has become the seventh leading cause of cancer death in the world [17]. PAAD is usually diagnosed at an advanced stage [18]. Therefore, it is important to find molecular markers for early diagnosis of PAAD.

ARMCs were first discovered in drosophila segment polarity protein [19]. They are made up of 42 repeating amino acids [20]. ARMCs are related to a variety of signaling pathways, so they are closely related to intracellular signaling and tumorigenesis. Studies have shown that ARMCs are associated with the development of a variety of cancers [21].

ARMC1

ARMC1 exists in mitochondria and is a component of the MICOS/MIB complex, which is involved in the

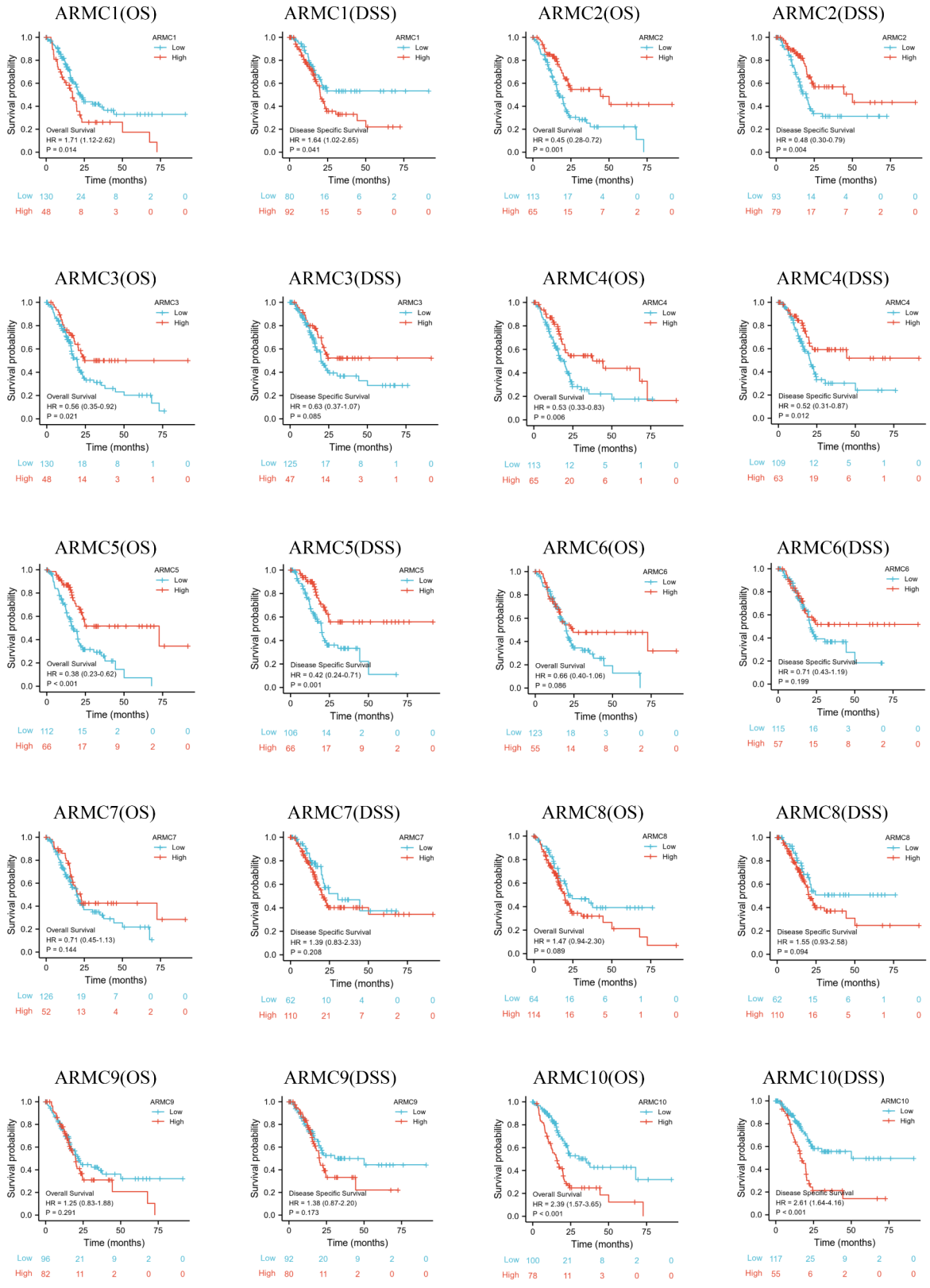

Fig. 8 Correlation between the expression of ARMCs with OS and DSS in PAAD

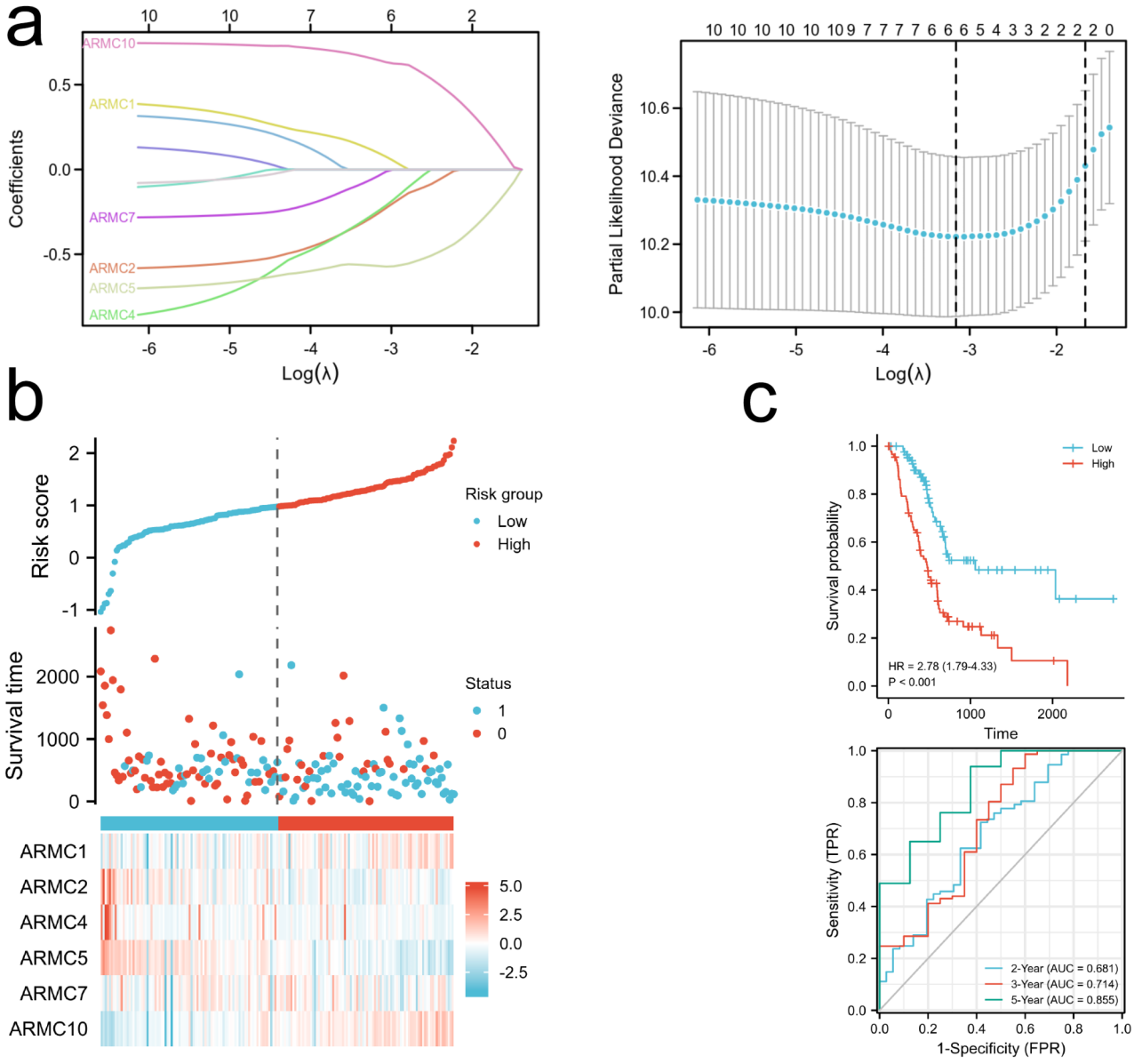

Fig. 9 Construction of the prognostic risk model. **a** LASSO analysis of related genes. **b** Risk scores, survival time, and survival status in the TCGA dataset. Top: scatterplot of risk scores from low to high; middle: scatterplot distribution of survival time and survival status corresponding to risk scores of different samples; bottom: heat map of gene expression in the prognostic model. **c** Top: K-M curves for high-risk patients and low risk patients; bottom: ROC curves for two, three, and five years for this risk model

transduction of Wnt signals [22]. Moreover, ARMC1 interacts with mitochondrial fission regulator 1, which leads to mitochondrial fission [23]. Through the TCGA database, we found that the expression level of ARMC1 in PAAD patients was significantly higher than that in normal controls. To verify the accuracy of the results, some human PAAD specimens were collected and analyzed by immunohistochemistry. The results showed that the expression level of ARMC1 protein in PAAD patients was significantly higher than that in paracancer tissues. And the expression in stage I and II PAAD tissues was significantly lower than that in stage III and IV PAAD tissues. The positive rate of ARMC1 expression was not associated with PAAD in patients with or without lymph node metastasis. It can be found from ROC curve that ARMC1 has high diagnostic value. The Kaplan-Meier analysis indicated that the upregulation of ARMC1 is significantly associated with lower survival in PAAD patients. In 175 samples from PAAD patients, the ARMC1 mutation rate was 5%, included missense mutations and amplification. And ARMC1 was correlated with B cells, CD8+T cells, macrophages, neutrophils and dendritic cells.

Table 1 Univariate/multivariate Cox regression analysis of clinicopathological features of PAAD associated with OS

Characteristics		Patient(*N*)	Univariate analysis		Multivariate analysis	
			Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age	<=65	93	Reference			
	> 65	85	1.290 (0.854–1.948)	0.227		
Gender	Female	80	Reference			
	Male	98	0.809 (0.537–1.219)	0.311		
Histologic grade	G1&G2	126	Reference			
	G3&G4	50	1.538 (0.996–2.376)	0.052	1.764 (1.122–2.773)	**0.014**
Pathologic stage	Stage I	21	Reference			
	Stage II	146	2.333 (1.069–5.089)	**0.033**		
	Stage III	3	1.255 (0.153–10.275)	0.832		
	Stage IV	5	1.566 (0.321–7.637)	0.579		
Radiation therapy	No	118	Reference			
	Yes	45	0.508 (0.298–0.866)	**0.013**	0.495 (0.289–0.848)	**0.010**
Smoker	No	65	Reference			
	Yes	79	1.086 (0.687–1.719)	0.724		
Alcohol history	No	65	Reference			
	Yes	101	1.147 (0.738–1.783)	0.542		
Family history	No	47	Reference			
	Yes	63	1.117 (0.650–1.920)	0.689		

The meaning of the bold values was regarded as statistically significant

Table 2 Univariate/multivariate Cox regression analysis of ARMCs of PAAD associated with OS

Characteristics		Patient *N*(178)	Univariate analysis		Multivariate analysis	
			Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
ARMC1	Low	89	Reference			
	High	89	1.422 (0.941–2.149)	0.095	1.140 (0.718–1.812)	0.578
ARMC2	Low	89	Reference			
	High	89	0.529 (0.347–0.807)	**0.003**	0.742 (0.470–1.169)	0.198
ARMC4	Low	89	Reference			
	High	89	0.584 (0.385–0.886)	**0.011**	0.508 (0.327–0.789)	**0.003**
ARMC5	Low	89	Reference			
	High	89	0.493 (0.322–0.754)	**0.001**	0.616 (0.399–0.953)	**0.029**
ARMC7	Low	89	Reference			
	High	89	0.761 (0.503–1.149)	0.194		
ARMC10	Low	89	Reference			
	High	89	2.541 (1.638–3.940)	**< 0.001**	2.443 (1.488–4.012)	**< 0.001**

The meaning of the bold values was regarded as statistically significan

Table 3 Clinicopathological characteristics of 12 PAAD patients stained with ARMC1 antibody and 13 PAAD patients stained with ARMC10

Characteristics	PAAD patients with ARMC1(*N* = 12)	PAAD patients with ARMC10(*N* = 13)
Gender (Female/male)	3/9	5/8
Age (years, mean ± SD)	63 ± 9.53	60.46 ± 10.18
Histology Grade (I, II/III, IV)	6/6	6/7
Lymph node metastasis(with/without)	7/5	4/9

ARMC2

A follow-up study showed a strong association between ARMC2 and chronic obstructive pulmonary disease(COPD) [24]. Studies have shown that ARMC2 is a strong risk factor for prostate cancer [9]. In the TCGA database, the expression level of ARMC2 was significantly increased in PAAD patients. ARMC2 also has high diagnostic value according to ROC curve.

ARMC3

The C terminal of ARMC3 can hydrolyze PKAI-mRNA-cAMP, which deactivates its inhibition of the activation of **protein kinase A** (PKA), and subsequently can actively promote capacitive and acrosome reactions [25, 26]. When exon 11 of ARMC3 is deleted, translation stops prematurely, which affects sperm activity, resulting in sperm quality defects, and ultimately male infertility [27]. When ARMC3 binds to CRACR2A, it can inhibit the expression of ORAI1 and STIM1, thus inhibiting the

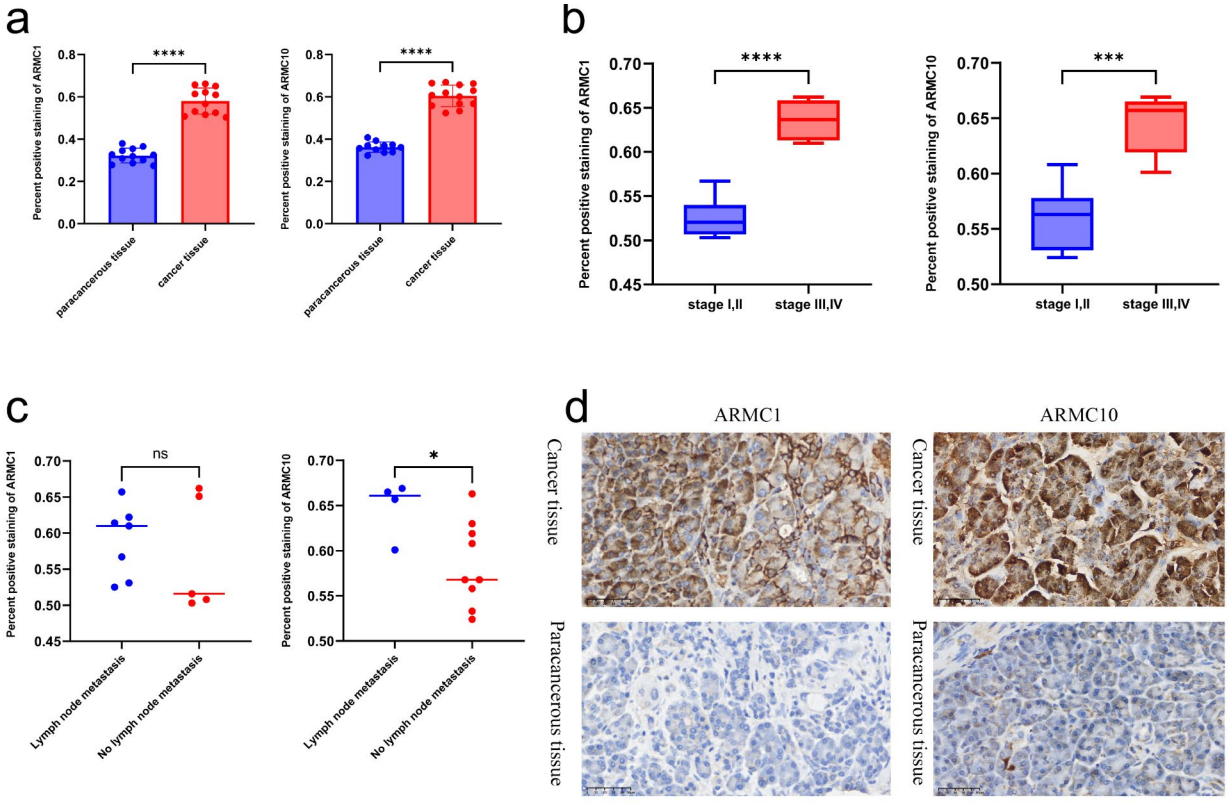

Fig. 10 Expression of ARMC1 and ARMC10 in PAAD tissue and paracancer tissues by immunohistochemistry. **a** Expression difference of ARMC1 and ARMC10 in pancreatic and paracancer tissues. **b** Expression levels of ARMC1 and ARMC10 in stage I, II and III, IV PAAD tissues. **c** Expression levels of ARMC1 and ARMC10 in PAAD patients with or without lymph node metastasis. **d** The protein expression of ARMC1 and ARMC10 in PAAD and paracancer tissues using immunohistochemistry (IHC)

occurrence of hypertension [10]. Our results showed that the expression of ARMC3 in PAAD patients was significantly higher than that in normal tissues, and ARMC3 had moderate diagnostic value for PAAD. The mutation rate of ARMC3 was 1.7%, which mainly included truncated mutation and missense mutation.

ARMC4

ARMC4 is a tumor suppressor gene. The Wnt/β-catenin pathway can negatively regulate the embryonic mitotic cell cycle, which can actively promote the development of tumors [28]. However, ARMC4 can interact with GSK3B gene to help control the unstable phosphorylation of β-catenin, which further leads to the inhibition of Wnt/β-catenin pathway [10]. This will promote the development of embryos and inhibit the development of tumors. Therefore, the mutation of ARMC4 causes the cell to proliferate continuously, which leads to the occurrence and development of cancer. Our study found that there was no significant difference in the expression level of ARMC4 in pancreatic and normal tissues in the TCGA database. Pancreatic cancer patients with high ARMC4 expression are associated with higher survival rates. The above prognostic models indicated that ARMC4 was an independent prognostic factor in univariate and multivariate Cox regression analyses.

ARMC5

Human ARMC5 consists of 935 amino acids. ARMC5 can inhibit the Wnt/β-catenin pathway and thus inhibit the cell cycle [29]. In addition, when ARMC5 and CUL3 bind to each other, ARMC5 is degraded to promote the cell cycle [30]. Thus, when ARMC5 is mutated, cell cycle progression is deregulated in patients with prostatic cortical tumors and primary aldosteronism [31, 32]. It was found that the expression of ARMC5 in PAAD patients was higher than that in normal people. According to ROC curve, ARMC5 has good diagnostic value for PAAD. The above prognostic models showed that ARMC5 was an independent prognostic factor in univariate and multivariate Cox regression analyses.

ARMC6

ARMC6 is involved in cytoskeleton composition, nervous system development, and cell division, and is important for protein localization [33, 34]. ARMC6 disrupts genes by the duplications. Studies have shown that ARMC6 is involved in the development of neuroblastoma through the MYCN&MAX/ARMC6/NOTCH/NICD/CSL pathway [11]. In addition, ARMC6 is also involved in PAAD formation [35]. The expression of ARMC6 in PAAD patients was significantly different from that in normal controls. ROC curve showed that ARMC6 had a high accuracy in the diagnosis of PAAD.

ARMC7

ARMC7 may be linked to Alzheimer's disease(AD) [36]. ARMC7 can involve in the development of AD through ARMC7/ APP/ clusterin/ p53/ Dkk1/ Wnt/ PCP/ JNK pathway. ARMC7 was significantly upregulated in PAAD tissue. Moreover, ARMC7 had high diagnostic value for PAAD.

ARMC8

ARMC8 is an important component of the C-terminal of lissencephaly type-1-like homology motif complex [10]. The complex is involved in the regulation of cell adhesion, migration, proliferation and programmed cell death [37]. In addition, ARMC8 interacts with α-catenin to participate in cell adhesion [38]. Therefore, ARMC8 has been implicated in the development and progression of various cancers, such as hepatocellular carcinoma, breast cancer, ovarian cancer, bladder cancer, osteosarcoma, and lung cancer [14, 39, 40]. The expression level of ARMC8 was significantly increased compared with the paracancer tissues. According to ROC curve, ARMC8 also had a high diagnostic basis for PAAD.

ARMC9

ARMC9 interacts with phosphatase SWI4 and DCR2 to regulate the cell cycle [41]. In addition, ARMC9 also affects cell division [42]. Therefore, when ARMC9 is mutated, it can stall the cell cycle and lead to cancer. The mRNA expression of ARMC9 is associated with the occurrence of gastric cancer [43]. The expression level of ARMC9 was also significantly elevated in PAAD patients. ROC curve showed that ARMC9 had high diagnostic value for PAAD.

ARMC10

ARMC10 is located in mitochondria. When ARMC10 is phosphorylated by AMPK, it leads to mitochondrial dysfunction, which can lead to diabetes and Alzheimer's disease [8, 15, 44]. ARMC10 will interact with EPHA1 to promote the occurrence of tumors and the formation of blood vessels [45]. The combination of ARMC10 and temozolomide (TMZ) will lead to the occurrence of TMZ drug-resistant glioma [46]. We found that expression of ARMC10 was significantly elevated in PAAD patients. The results showed that the expression level of ARMC10 protein in PAAD patients was significantly higher than that in paracancer tissues. And the expression in stage I and II PAAD tissues was significantly lower than that in stage III and IV PAAD tissues. The positive rate of ARMC10 expression was significant associated with PAAD in patients with or without lymph node metastasis($p<0.05$). ARMC10 has high diagnostic value for PAAD. PAAD patients with low ARMC10 expression had better OS and DSS than those with high ARMC10 expression. The prognostic models showed that ARMC10 was an independent prognostic factor in univariate and multivariate Cox regression analyses.

Conclusions

In conclusion, the expression of ARMC1, 2, 3, 5, 6, 7, 8, 9 and 10 was significantly increased in PAAD patients. ARMC1, 2, 5, 6, 7, 8, 9 and 10 may be helpful in the diagnosis of PAAD. ARMC1 and ARMC10 were significantly associated with the prognosis of PAAD. Based on public databases, ARMCs is closely related to the clinicopathological features, immune infiltration and gene mutation of PAAD. Up to now, the role of ARMCs in PAAD has not been studied in the literature. Therefore, the specific role and potential mechanism of ARMCs in PAAD still need to be further investigated through many experiments. These studies suggest that ARMCs may play important roles in PAAD and act as biomarkers. They can provide valuable clues for the treatment and diagnosis of PAAD.

Abbreviations

PAAD	Pancreatic adenocarcinoma
ARMCs	Armadillo repeat containing proteins
TCGA	The Cancer Genome Atlas
GEPIA	Gene Expression Profiling Interactive Analysis
GO	Gene ontology
KEGG	Kyoto encyclopedia of genes and genomes
IHC	Immunohistochemistry
HPA	Human Protein Atlas
PPI	Protein-Protein Interaction
OS	Overall survival

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

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Author contributions
G.Z., H.C. and S.L. performed study concept and design; G.Z. and F.Y. performed development of methodology and writing, review, and revision of the paper; G.Z., Y.G., Q.Z., Z.W., J.H., M.Y., and F.Z. provided acquisition, analysis and interpretation of data, and statistical analysis; H.C. Y.C. and S.C. provided technical and material support. All authors reviewed the manuscript.

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Data availability
Data were download from the TCGA database- PAAD (Pancreatic adenocarcinoma (https://portal.gdc.cancer.gov). All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
All procedures involving human samples were approved by the Ethics Committee of Fujian Provincial Hospital. Informed consent was obtained from all the participants and/or their legal guardians.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. LUCHINI C, BROSENS L A A, WOOD L D, et al. Comprehensive characterisation of pancreatic ductal adenocarcinoma with microsatellite instability: histology, molecular pathology and clinical implications [J]. Gut. 2021;70(1):148–56.
2. ROSSI SEBASTIANO M, POZZATO C, SALIAKOURA M et al. ACSL3-PAI-1 signaling axis mediates tumor-stroma cross-talk promoting pancreatic cancer progression [J]. Sci Adv, 2020, 6(44).
3. LIU Y, LYU S C, WANG X Q, et al. Application of preoperative three-dimensional model design in radioactive particle implantation for advanced pancreatic cancer [J]. Onco Targets Ther. 2018;11:8685–93.
4. MA C, HUANG T, DING Y C, et al. MicroRNA-200c overexpression inhibits chemoresistance, invasion and colony formation of human pancreatic cancer stem cells [J]. Int J Clin Exp Pathol. 2015;8(6):6533–9.
5. LI X, LI Y, WAN L, et al. Mir-509-5p inhibits cellular proliferation and migration via targeting MDM2 in pancreatic cancer cells [J]. Onco Targets Ther. 2017;10:4455–64.
6. HUANG Y, JIANG Z, GAO X, et al. ARMC Subfamily: structures, functions, evolutions, interactions, and diseases [J]. Front Mol Biosci. 2021;8:791597.
7. WAGNER F, KUNZ T C, CHOWDHURY S R, et al. Armadillo repeat-containing protein 1 is a dual localization protein associated with mitochondrial intermembrane space bridging complex [J]. PLoS ONE. 2019;14(10):e0218303.
8. JIN Y, CUI D, REN J, et al. CACNA2D3 is downregulated in gliomas and functions as a tumor suppressor [J]. Mol Carcinog. 2017;56(3):945–59.
9. RAND K A, ROHLAND N, TANDON A, et al. Whole-exome sequencing of over 4100 men of African ancestry and prostate cancer risk [J]. Hum Mol Genet. 2016;25(2):371–81.
10. PIERCE B G, WIEHE K, HWANG H, et al. ZDOCK server: interactive docking prediction of protein-protein complexes and symmetric multimers [J]. Bioinformatics. 2014;30(12):1771–3.
11. UDOLPH G, RATH P. On the roles of Notch, Delta, kuzbanian, and inscuteable during the development of Drosophila embryonic neuroblast lineages [J]. Dev Biol. 2009;336(2):156–68.
12. XIE C, JIANG G, FAN C, et al. ARMC8alpha promotes proliferation and invasion of non-small cell lung cancer cells by activating the canonical wnt signaling pathway [J]. Tumour Biol. 2014;35(9):8903–11.
13. JIANG F, SHI Y, LU H, et al. Armadillo repeat-containing protein 8 (ARMC8) silencing inhibits Proliferation and Invasion in Osteosarcoma cells [J]. Oncol Res. 2016;24(5):381–9.
14. ZHAO Y, PENG S, JIA C, et al. Armc8 regulates the invasive ability of hepatocellular carcinoma through E-cadherin/catenin complex [J]. Tumour Biol. 2016;37(8):11219–24.
15. CHEN Z, LEI C, WANG C, et al. Global phosphoproteomic analysis reveals ARMC10 as an AMPK substrate that regulates mitochondrial dynamics [J]. Nat Commun. 2019;10(1):104.
16. SHAO H, IM H, CASTRO C M, et al. New technologies for analysis of Extracellular vesicles [J]. Chem Rev. 2018;118(4):1917–50.
17. ZHANG Z, TANG H, CHEN P, et al. Demystifying the manipulation of host immunity, metabolism, and extraintestinal tumors by the gut microbiome [J]. Signal Transduct Target Ther. 2019;4:41.
18. ZHAO J, WANG H, HSIAO C H, et al. Simultaneous inhibition of hedgehog signaling and tumor proliferation remodels stroma and enhances pancreatic cancer therapy [J]. Biomaterials. 2018;159:215–28.
19. PEIFER M, BERG S, REYNOLDS A B. A repeating amino acid motif shared by proteins with diverse cellular roles [J]. Cell. 1994;76(5):789–91.
20. COATES JC. Armadillo repeat proteins: beyond the animal kingdom [J]. Trends Cell Biol. 2003;13(9):463–71.
21. LIANG X, MEN Q L, LI YW, et al. Silencing of Armadillo repeat-containing protein 8 (ARMc8) inhibits TGF-beta-Induced EMT in bladder carcinoma UMUC3 cells [J]. Oncol Res. 2017;25(1):99–105.
22. XU W. Mechanistic insights from structural studies of beta-catenin and its binding partners [J]. J Cell Sci. 2007;120(Pt 19):3337–44.
23. ROLLAND T, TASAN M. A proteome-scale map of the human interactome network [J]. Cell. 2014;159(5):1212–26.
24. SOLER ARTIGAS M, LOTH D W, WAIN L V, et al. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function [J]. Nat Genet. 2011;43(11):1082–90.
25. VIJAYARAGHAVAN S, LIBERTY G A, MOHAN J, et al. Isolation and molecular characterization of AKAP110, a novel, sperm-specific protein kinase A-anchoring protein [J]. Mol Endocrinol. 1999;13(5):705–17.
26. VETTER M M, ZENN H M MENDEZE, et al. The testis-specific Calpha2 subunit of PKA is kinetically indistinguishable from the common Calpha1 subunit of PKA [J]. BMC Biochem. 2011;12:40.
27. PAUSCH H, VENHORANTA H, WURMSER C, et al. A frameshift mutation in ARMC3 is associated with a tail stump sperm defect in Swedish Red (Bos taurus) cattle [J]. BMC Genet. 2016;17:49.
28. DEMA A, SCHROTER M F PERETSE, et al. The A-Kinase anchoring protein (AKAP) glycogen synthase kinase 3beta Interaction protein (GSKIP) regulates beta-catenin through its interactions with both protein kinase A (PKA) and GSK3beta [J]. J Biol Chem. 2016;291(37):19618–30.
29. FAUCZ F R BERTHONA, ESPIARD S, et al. Age-dependent effects of Armc5 haploinsufficiency on adrenocortical function [J]. Hum Mol Genet. 2017;26(18):3495–507.

30. CAVALCANTE I P, VACZLAVIK A. Cullin 3 targets the tumor suppressor gene ARMC5 for ubiquitination and degradation [J]. Endocr Relat Cancer. 2020;27(4):221–30.
31. ZILBERMINT M, XEKOUKI P, FAUCZ F R, et al. Primary aldosteronism and ARMC5 variants [J]. J Clin Endocrinol Metab. 2015;100(6):E900–9.
32. BERTHON A. Update of genetic and molecular causes of Adrenocortical Hyperplasias causing Cushing syndrome [J]. Horm Metab Res. 2020;52(8):598–606.
33. LI P, YANG X. Inscuteable and Staufen mediate asymmetric localization and segregation of prospero RNA during Drosophila neuroblast cell divisions [J]. Cell. 1997;90(3):437–47.
34. CAI Y, YU F, LIN S, et al. Apical complex genes control mitotic spindle geometry and relative size of daughter cells in Drosophila neuroblast and pI asymmetric divisions [J]. Cell. 2003;112(1):51–62.
35. GRESS T M, MULLER-PILLASCH F, GENG M, et al. A pancreatic cancer-specific expression profile [J]. Oncogene. 1996;13(8):1819–30.
36. SOLEIMANI ZAKERI N S PASHAZADEHS, MOTIEGHADER H. Gene biomarker discovery at different stages of Alzheimer using gene co-expression network approach [J]. Sci Rep. 2020;10(1):12210.
37. HUFFMAN N, PALMIERI D. COPPOLA V. The CTLH Complex in Cancer Cell Plasticity [J]. J Oncol, 2019, 2019: 4216750.
38. SUZUKI T, UEDA A, KOBAYASHI N, et al. Proteasome-dependent degradation of alpha-catenin is regulated by interaction with ARMc8alpha [J]. Biochem J. 2008;411(3):581–91.
39. JIANG G, YANG D, WANG L, et al. A novel biomarker ARMc8 promotes the malignant progression of ovarian cancer [J]. Hum Pathol. 2015;46(10):1471–9.
40. GUL I S, HULPIAU P. SANDERS E, Armc8 is an evolutionarily conserved armadillo protein involved in cell-cell adhesion complexes through multiple molecular interactions [J]. Biosci Rep, 2019, 39(8).
41. PATHAK R, BOGOMOLNAYA L M, GUO J, et al. Gid8p (Dcr1p) and Dcr2p function in a common pathway to promote START completion in Saccharomyces cerevisiae [J]. Eukaryot Cell. 2004;3(6):1627–38.
42. KUSSEL P. Yeast Srp1, a nuclear protein related to Drosophila and mouse pendulin, is required for normal migration, division, and integrity of nuclei during mitosis [J]. Mol Gen Genet. 1995;248(3):351–63.
43. LIU D, LI L, WANG L, et al. Recognition of DNA methylation molecular features for diagnosis and prognosis in gastric Cancer [J]. Front Genet. 2021;12:758926.
44. CHOW J, RAHMAN J, ACHERMANN JC, et al. Mitochondrial disease and endocrine dysfunction [J]. Nat Rev Endocrinol. 2017;13(2):92–104.
45. YAMAZAKI T, MASUDA J, OMORI T, et al. EphA1 interacts with integrin-linked kinase and regulates cell morphology and motility [J]. J Cell Sci. 2009;122(Pt 2):243–55.
46. CAI H Q, LIU A S, ZHANG MJ, et al. Identifying Predictive Gene expression and signature related to Temozolomide Sensitivity of glioblastomas [J]. Front Oncol. 2020;10:669.

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