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Atherosclerosis-related biomarker PABPC1 predicts pan-cancer events

Miao Lin,[1,2] Liubing Hu,[1,2,3] Si Shen,[1,4] Jiyue Liu,[1,2] Yanyan Liu,[3] Yixian Xu,[1,2] Honglin Chen,[1,4] Kazuo Sugimoto,[5,6] Jianshuang Li,[3] Ikuo Kamitsukasa,[7,8] Takaki Hiwasa,[5,9] Hao Wang ,[1,2,5] Anding Xu [1]

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ML, LH and SS contributed equally.

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For numbered affiliations see end of article.

Correspondence to
Professor Hao Wang;
haowang@jnu.edu.cn

Professor Anding Xu;
tlil@jnu.edu.cn

ABSTRACT

Background Atherosclerosis (AS) and tumours are the leading causes of death worldwide and share common risk factors, detection methods and molecular markers. Therefore, searching for serum markers shared by AS and tumours is beneficial to the early diagnosis of patients.

Methods The sera of 23 patients with AS-related transient ischaemic attack were screened by serological identification of antigens through recombinant cDNA expression cloning (SEREX), and cDNA clones were identified. Pathway function enrichment analysis was performed on cDNA clones to identify their biological pathways and determine whether they were related to AS or tumours. Subsequently, gene–gene and protein–protein interactions were performed and AS-associated markers would be discovered. The expression of AS biomarkers in human normal organs and pan-cancer tumour tissues were explored. Then, immune infiltration level and tumour mutation burden of various immune cells were evaluated. Survival curves analysis could show the expression of AS markers in pan-cancer.

Results AS-related sera were screened by SEREX, and 83 cDNA clones with high homology were obtained. Through functional enrichment analysis, it was found that their functions were closely related to AS and tumour functions. After multiple biological information interaction screening and the external cohort validating, poly(A) binding protein cytoplasmic 1 (PABPC1) was found to be a potential AS biomarker. To assess whether PABPC1 was related to pan-cancer, its expression in different tumour pathological stages and ages was screened. Since AS-associated proteins were closely related to cancer immune infiltration, we investigated and found that PABPC1 had the same role in pan-cancer. Finally, analysis of Kaplan-Meier survival curves revealed that high PABPC1 expression in pan-cancer was associated with high risk of death.

Conclusions Through the findings of SEREX and bioinformatics pan-cancer analysis, we concluded that PABPC1 might serve as a potential biomarker for the prediction and diagnosis of AS and pan-cancer.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Both atherosclerosis (AS) and tumours are the major causes of death. These two diseases share many factors, and the same strategies used to predict AS can also detect certain cancers. We have used serological identification of antigens through recombinant cDNA expression cloning (SEREX) technology to find a variety of biomarkers common to cancer in AS serum, such as Aldolase A, BRCA1-associated ATM activator 1, etc. However, due to the limited number of markers and the need for verification of biological data, we need to conduct this study.

WHAT THIS STUDY ADDS

⇒ Used SEREX to screen 23 AS-related sera and obtained 83 cDNA clones with high homology to known genes. Functional enrichment analysis of these 83 genes found that they were consistent with AS and tumour-related biological pathways. Gene–gene and protein–protein interactions were performed and poly(A) binding protein cytoplasmic 1 (PABPC1) was identified as a potential AS biomarker. Further explored the expression of PABPC1 in normal organs and pan-cancer tumour tissues in the human body, and found significant differences in the expression of PABPC1 in different pathological stages or ages, and found that PABPC1 was related to immune infiltration level and tumour mutation burden. Analysis of survival curves showed that high expression of PABPC1 in pan-cancer was significantly more correlated with risk of death than low expression. Through the results of SEREX and bioinformatics pan-cancer analysis, we concluded that PABPC1 might serve as a potential cobiomarker for AS and pan-cancer.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study of PABPC1 can develop new serum markers, provide new indicators for further clinical liquid biopsy and may promote the prevention and treatment of AS and tumour diseases to a certain extent, thereby improving the survival rate and quality of life of patients.

INTRODUCTION

Cardiovascular diseases, including atherosclerosis (AS) and severe hypertension,[1] are among the leading causes of death worldwide.[2] AS is an important component of cardiovascular disease and is the main cause of coronary artery diseases, such as acute myocardial infarction, as well as ischaemic vascular diseases, such as transient ischaemic attack (TIA) and cerebral infarction,[3 4] which often leads to insufficient oxygen supply to tissues, causing sudden death or permanent

disability.[5 6] Cancer, which belongs to the chronic inflammatory diseases along with AS, has one of the highest mortality rates in the world.[7 8] Cancer incidence is increasing, prompting many investigators to accelerate research of early signs, symptoms and testing for cancer.[9 10] Pan-cancer research, as a general study of cancer treatment, aims to examine the similarities and differences between genomic and cellular changes found in various tumour types.[11–13]

Numerous studies have demonstrated an association between cancer and cardiovascular diseases.[14] For example, age,[15] lack of exercise,[16] tobacco use,[17] alcohol consumption[18] and obesity[19] are traditional common risk factors. More recently, recognised entities have appeared, such as clonal haematopoiesis,[20] chronic inflammation[21] and oxidative stress.[22 23] Certain associations were also observed in the serum markers common to both groups of diseases. As a case, plasminogen activator inhibitor-1 was a potential factor that increased cancer-associated thrombosis.[24] In addition, our research team successfully applied serological identification of antigens through recombinant cDNA expression cloning (SEREX) to AS-related diseases in previous studies, and identified multiple tumour-associated comarkers in AS-related TIA sera. Among them, low-density lipoprotein receptor-related protein-associated protein 1 (LRPAP1) played an important role in AS and digestive organ cancers,[25] and BRCA1-associated ATM activator 1 (BRAT1) was significant for gastrointestinal cancers and AS.[26] These common factors suggested that markers associated with AS might also be important in predicting the occurrence of cancer, but the discovery of common markers between them remained insufficient. Meanwhile, biomarkers found in our previous studies were based only on digestive system cancers, and studies using AS serum screening to identify pan-cancer markers were still limited.

Currently, the commonly used methods for molecular marker screening of AS and tumours include radioimmunoassays, chemiluminescence immunoassays, liquid biopsies, etc. Among these, liquid biopsy only uses blood, urine or other body fluid to detect disease-related markers, which are rapid, less invasive and predictive.[27 28] For instance, SEREX, one of representative technologies of liquid biopsy, can effectively screen and identify relevant target antigen genes on genome scale and provide molecular targets for AS and tumour treatment.[29] Simultaneously, recent popular bioinformatics technology has had a significant effect on understanding the molecular mechanism of AS and pan-cancer by using interaction between genes and the related pathways involved in biological processes (BPs). In recent years, pan-cancer analysis significantly affects the understanding to molecular mechanism of cancer by using interaction between genes and pathways involved in BPs.[30 31] Pan-cancer analyses can leverage human genome sequence data and a large compendium of relevant molecular and phenotypic features, as well as functional genomic data of genome-scale expression and epigenomics to understand the impact of variation, and elucidate impacts of dysregulated genes on organism pathway in specific disease and tissue settings.[32 33] Combining SEREX liquid biopsy and bioinformatic pan-cancer analysis will greatly facilitate the discovery of common markers of AS and cancer.[34]

In this study, based on SEREX and pan-cancer analysis bioinformatics technology, poly(A) binding protein cytoplasmic 1 (PABPC1) was discovered as a potential common molecular marker of AS and pan-cancer. The study of PABPC1 may promote the prevention and treatment of these two diseases to an extent.

MATERIALS AND METHODS

Serum samples

Nineteen serum samples were obtained from 19 patients with AS-confirmed TIA who were treated at the hospital of Japan. The remaining four AS-related TIA serum samples were obtained from hospital of China. All patients were diagnosed with TIA according to the latest TIA diagnostic criteria of the American Stroke Association.[35 36] All of them understood the purpose and steps of the experiments, and agreed to participate in the study before sera collection. Written informed consent was obtained from all participants. Thereafter, peripheral blood (5 mL) was collected from each patient and centrifuged at 3000×g for 10 min after being kept at room temperature for 2 hours. The supernatant was regarded as a serum sample and stored in a −80°C refrigerator until further use. Repeated freezing and thawing was avoided.

Immunoscreening of cDNA expression clones

Sera from 23 patients with AS-related TIA were subjected to 3 rounds of SEREX immunological screening of a human aortic endothelial cell cDNA expression library (Stratagene, USA; concentration: 2.0×10^9 pfu/mL). XL1-Blue strain (2 µL) was extracted and added to 10 mL of Lysogeny Broth (LB) medium containing 12.5 g/L tetracycline. It was placed in an incubator at 30°C and a rotation speed of 210 rpm for overnight incubation. A concentration of $2–5\times10^4$ pfu containing the human aortic endothelial cell cDNA library was mixed with 600 µL *Escherichia coli*(*E-coli*) XL1-Blue bacterial solution (A600=0.5). The plate was incubated until plaques formed, and preheated upside down at 37°C for 1 hour. The nitrocellulose (NC) membranes were pretreated with 10 mmol/L isopropyl β-D-thiogalactoside (IPTG) solution for 30 min and then the surface of the plate was covered. The NC membranes were pretreated with 10 mmol/L IPTG solution for 30 min, covered on the surface of the plate and incubated upright at 37°C for 2.5 hours to let the plaques be transferred to NC membranes. The NC membranes were gently peeled off and washed three times with Tris Buffered Saline with Tween 2o (TBS-T) buffer. After the NC membrane was blocked with 10 mg/g bovine serum albumin (Nacalai Tesque, Japan), it was transferred to TIA patient serum (primary antibody), which had been diluted to 1:2000, and shaken slowly for 1 hour. The NC

membrane was washed with TBS-T again and soaked in 1:5000 diluted goat anti-human immunoglobulin (Ig) G H&L (HRP) (secondary antibody) (Jackson Immunology Research Laboratory, Japan) for 1 hour. The NC membrane was immersed in nitro blue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate solution in dark for colour reaction to occur. To obtain purified positive single clones, the second and third rounds of screening were performed by diluting titre of cDNA expression library according to the first round of screening.

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Intraclonal excision and restriction enzyme digestion identification

A mixture of 100 µL XL1-Blue (A600=1.0), 125 µL positive monoclonal colony dilution and 0.5 µL ExAssist helper phage (>1.0×10^{10} pfu/mL) were prepared. The mixture was added to 3 mL LB medium and incubated at 30°C with shaking for 2.5–3.0 hours. The mixture was heated and incubated in a dry thermostatic metal bath at 65°C–70°C for 20 min, and then centrifuged at 1000×g for 15 min. The pBluescript phagemid was obtained from the supernatant, transfected with *E-coli* SOLR strain and spread on a petri dish, which was incubated upside down overnight at 37°C. The transformed positive monoclonal pBluescript plasmid was obtained using ampicillin selection. The plasmid was identified using *Eco*RI and *Xho*I double digestion to determine whether it contained target gene fragment and size of the inserted fragment.

DNA sequencing

After adding the universal forward primer T3 and reverse primer T7 to the pBluescript SK (+) plasmid containing the cDNA insert, forward and reverse sequencings of target gene were performed. The primer sequences were as follows: T3-20mer forward primer (100 µM), ATTAACCCTCACTAAAGGGA; and T7-20merreverse primer (100 µM), TAATACGACTCACTATAGGG. Homology analysis of the determined sequences to known genes was performed using the National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) search tool in the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Function enrichment analysis

Gene Ontology (GO)[37] is a powerful bioinformatics tool for annotating genes, and Kyoto Encyclopedia of Genes and Genomes (KEGG)[38] is a set of databases for systematic analysis of biological pathway information. To study the pathogenesis of AS, the Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/)[39] was used to facilitate functional annotation. Using Metascape (http://metascape.org/gp/index.html)[40] to perform functional enrichment analysis of target genes, we gained information on BPs, cytogenetics and signalling pathways. Using these two tools, a correlation between the function of screened genes and AS was found.

Gene–gene and protein–protein interactions

The BisoGenet and CytoNCA in Cytoscape were used to construct and analyse gene interactions. A bisogenet network can extract subnetwork topological features, based on nodes and connections to construct a gene screening network.[41–43]

GEO database (http://www.ncbi.nlm.nih.gov/geo) contains extensive data on disease-related gene expression. GEO database was used to determine whether identified genes were AS related. We searched GEO database and identified five series related to AS (GSE16561, GSE66724, GSE58294 GSE22255 and GSE230214). Peripheral blood samples of GSE16561 were from 39 patients with MRI-diagnosed ischaemic stroke and 25 controls who were non-stroke neurologically healthy.[44] GSE66724 included eight stroke patients and eight controls, and microarray analysis of peripheral blood cells identified gene expression patterns associated with stroke.[45] Blood samples from 69 patients with stroke and 23 controls were collected in GSE58294 and whole genome U133 Affymetrix arrays were performed.[46] GSE22255 reported data on gene expression levels observed in peripheral blood mononuclear cells from 20 patients and 20 controls.[47] The GSE230214 dataset reflected the genetic differences between patients with AS and healthy controls. After comparing gene expression levels of AS-related stroke patients and controls, the differentially expressed genes observed were used and significance level was set at 0.05.

The STRING V.11.0 Database (https://string-db.org/)[48] was used to predict protein–protein associations. The minimum required interaction score was set to 0.4. The nodes and lines in the network graph represented target proteins and their interactions. The stronger interaction between two proteins, the thicker their connection.

Protein interaction function of Metascape was also used to synchronise protein–protein associations. The resulting network contains a subset of proteins that physically interact with at least one other member of the list.

The ConsensusPathDB database (http://consensuspathdb.org)[49] integrates protein interactions, genetic interactions, metabolomes and drug-target interaction information. Interaction and function analyses of the screened genes were performed using ConsensusPathDB.

Biological General Repository for Interaction Datasets (BioGRID) (http://thebiogrid.org), formed by the aggregation of a great number of individual proteins or genetic interactions and interactions of RNA, DNA and other molecule metabolites, is fundamental to understanding gene–phenotype relationships and cellular functions.[50]

Expression analysis of PABPC1 in pan-cancer

BioGPS (http://biogps.org/#goto=welcome) is an extensible and customisable gene annotation portal tool.[51] We used it to identify the expression of newly discovered PABPC1 gene in various normal human organs and tissues.[52]

Gene Expression Profile Interaction Analysis (GEPIA) (http://gepia2.cancer-pku.cn/) can analyse gene expression in tumour and normal samples from The Cancer Genome Atlas (TCGA) and Genotype Tissue Expression databases.[53] Pathological stages and ages of patients were selected to explore their association with PABPC1 expression. Patients were divided into pathological stages I, II, III and IV while 60 years old was set as a cut-off value to divide patients into two groups.

Human Protein Atlas (HPA) (https://www.proteinatlas.org/) (Protein Atlas V.21.0) is divided into three sections, those are, cell, tissue and pathology, which show expression of proteins in cells, normal tissues and cancerous tissues, respectively.[54–56]

TIMER V.2.0 (http://timer.cistrome.org/) uses six state-of-the-art algorithms to provide more reliable estimates of immune infiltration levels for tumour profiles provided by TCGA.[57 58] TIMER V.2.0 offers immune associations, cancer exploration and immune estimation to study association among immune infiltration, genetic and clinical characteristics.

Kaplan-Meier plotter (http://kmplot.com/analysis/) assesses the impact of 54 000 genes (mRNA, miRNA and protein) on survival in pan-cancer[59] and its main purpose is to discover and validate meta-analysis-based survival biomarkers. Overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI) were used to evaluate the relationship between PABPC1 expression and patient prognosis. Kaplan-Meier plotter was used to analyse the relationship between PABPC1 gene expression and survival rate of pan-cancer.[60 61]

UALCAN (http://ualcan.path.uab.edu) is a comprehensive and interactive web resource for analysing cancer OMICS data, which can provide access to publicly available cancer OMICS data. UALCAN was used to evaluate the expression of PABPC1 in normal and tumour samples.[62]

Tumour RNA-seq data (level 3) and clinical information were obtained from TCGA database (https://portal.gdc.com). Statistical analysis was performed using R software V.4.0.3 and immune correlations were assessed using immunedeconv.[57] We used Assistant for Clinical Bioinformatics (https://www.aclbi.com) to determine the association between PABPC1 and pan-cancer immune correlations or tumour mutation burden (TMB).[63]

Statistical analyses

The relationship of PABPC1 expression level between tumour and normal tissue was described by boxplots using TIMER V.2.0, UALCAN and GEPIA V.2, which were calculated based on the Wilcoxon testing. Immune infiltration score and PABPC1 gene expression in multiple tumour tissues were analysed by Spearman correlation analysis. Significance was set as *p<0.05, **p<0.01 and ***p<0.001. OS, DSS and PFI were characterised using COX regression analysis with 95% CI for the HR. Survival curves were constructed using the Kaplan-Meier method. Survival analysis compared HR and log-rank p value of PABPC1 expression in pan-cancer. P value<0.05 was considered statistically significant and all tests were two tailed.

RESULTS

Selection and identification of immunoreactive clones from sera of patients with TIA

Serum samples from 23 patients (online supplemental table 1) were subjected to 3 rounds of screening. The plaques were transferred to NC membranes, in which negative clones showed pale plaque shadows, whereas the positive clones showed a clear purple-blue colour (figure 1A). One hundred and ninety-one mono-clones were screened after the first (figure 1A–a), second (figure 1A–b) and third (figure 1A–c) screening rounds. After internal shearing, 191 pBluescript SK (+) plasmids carrying SEREX antigen gene fragments were obtained and the concentration of all 186 plasmid DNA was >50 µg/mL. *Eco*RI and *Xho*I double digestion was used to identify 181 plasmid vectors with target cDNA fragments (figure 1B).

A total of 181 plasmids were subjected to cDNA sequence detection using NCBI BLAST. Finally, 83 independent cDNA clones were obtained that showed high homology with known genes in RefSeq database (online supplemental table 2). Some of these 83 genes, such as BRAT1, WD repeat domain 1 (WDR1),[26] matrix metalloproteinase 1 (MMP1), chromobox homolog 1 (CBX1), chromobox homolog 5 (CBX5),[64] aldolase A (ALDOA) and fumarate hydratase (FH)[65] had been reported by our research team.

TIA-screened genes consistent with AS-related functions

We conducted GO and KEGG pathway functional enrichment analyses using DAVID and found that 83 TIA-screened genes were mainly associated with components of some AS and tumour related biological pathways, such as focal adhesion, cell–substrate junction and platelet alpha granule in cellular components (CC), cadherin binding and actin binding in molecular function and viral process and negative regulation of response to external stimulus in BP (figure 2A). KEGG suggested two important pathways, GLYCOLYSIS/GLUCONEOGENESIS KEGG and PYRUVATE METABOLISM KEGG pathway maps, which were key signals of two chronic metabolic diseases, cancers and AS[66–69] (figure 2B and C).

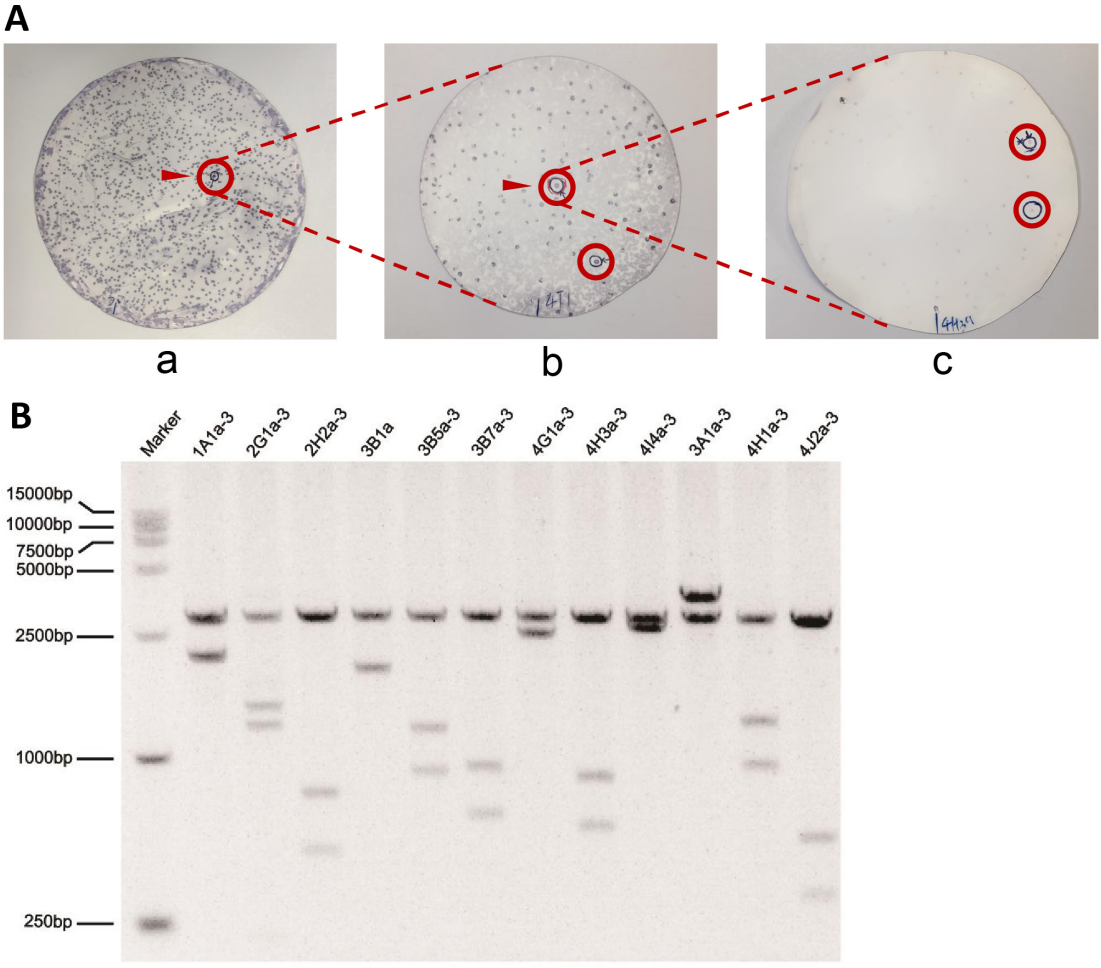

Figure 1 Immunoreactive clones were screened and identified. (A) a. Result of first screening of SEREX was shown (plate size 150 mm). Red circle in picture a was the positive monoclonal and was screened for the second round into picture b. (A) b. Results of second screening of SEREX was shown (plate size 100 mm). Red circles in picture b were the positive monoclonals and were screened for the third round into picture c. (A) c. Result of third screening of SEREX was shown (plate size 100 mm). (B) Digestion results of pBluescript plasmid were partially shown.

In addition, Metascape demonstrated that 83 genes showed abundant gene function expression that was consistent with AS and cancers, such as haemostasis, glycolysis/gluconeogenesis, toxin transport and cellular response to growth factor stimulus (figure 2D). Top-level GO BPs, including localisation, metabolic processes and response to stimulus, were also viewed (figure 2E). Each node represented an enriched term and was coloured first by gene function expression cluster ID (figure 2F) and then by p value (figure 2G).

Therefore, we confirmed that the genes screened from sera of patients with AS-related TIA were consistent with AS and cancer related functions, making solid preparations for further research on identifying AS biomarkers.

PABPC1 expression examined through gene–gene and protein–protein interactions

After above studies confirmed that the genes screened were closely related to AS, it was necessary to further understand whether there were marker genes that could be targeted for AS treatment among these 83 genes to explore therapeutic mechanism of AS. Therefore, we used multiple gene–gene and protein–protein interaction methods to simultaneously screen for biomarkers.

First, 3 advanced screenings of 83 genes were performed by Bisogenet. The resulting hub network consisted of 196 proteins in total, which included 5 screening proteins, that were EEF1A1, HDAC2, PABPC1, VIM and IFI16 (figure 3A).

Next, to simultaneously verify the reliability of 83 genes, we combined these genes as samples with 4 AS-related gene expression comprehensive datasets from GEO (GSE16561, GSE66724, GSE22255 and GSE58294), and the intersections between them represented coexistence genes. Among these, 64 genes in the centre existed in common positions in these 5 parts (figure 3B). The 64 genes were further processed by STRING and Cytoscape to present remaining genes with more centralised characteristics, including PABPC1, CBX1, MMP1, HDAC2, EEF1A1, VIM, ALDOA, IFI16 and CBX5 (figure 3C). To better use protein–protein interaction relationship

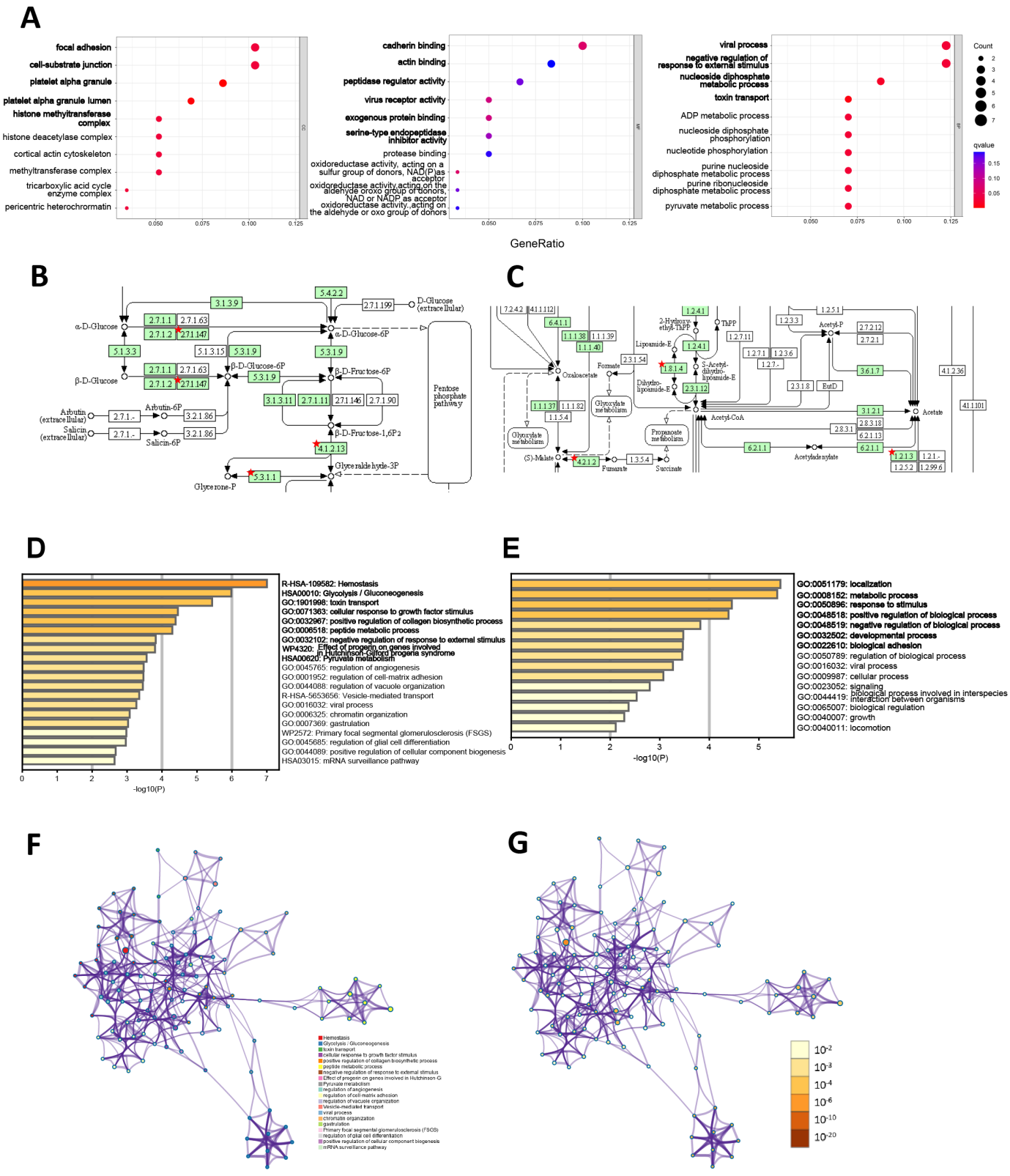

Figure 2 Pathway enrichment analyzes were performed on differential expressed genes in Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). (A) The Database for Annotation, Visualization and Integrated Discovery (DAVID) website was used to evaluate the enrichment of all genes in three aspects: cellular components (CC), molecular function (MF) and biological process (BP). (B) GLYCOLYSIS/GLUCONEOGENESIS pathway map was displayed by KEGG. (C) PYRUVATE METABOLISM pathway map was showed by KEGG. (D) The gene function expression was created using Metascape. (E) The top-level GO biological processes showed features of those genes. (F) Enriched terms were coloured by cluster ID. (G) Enriched terms were coloured by p value.

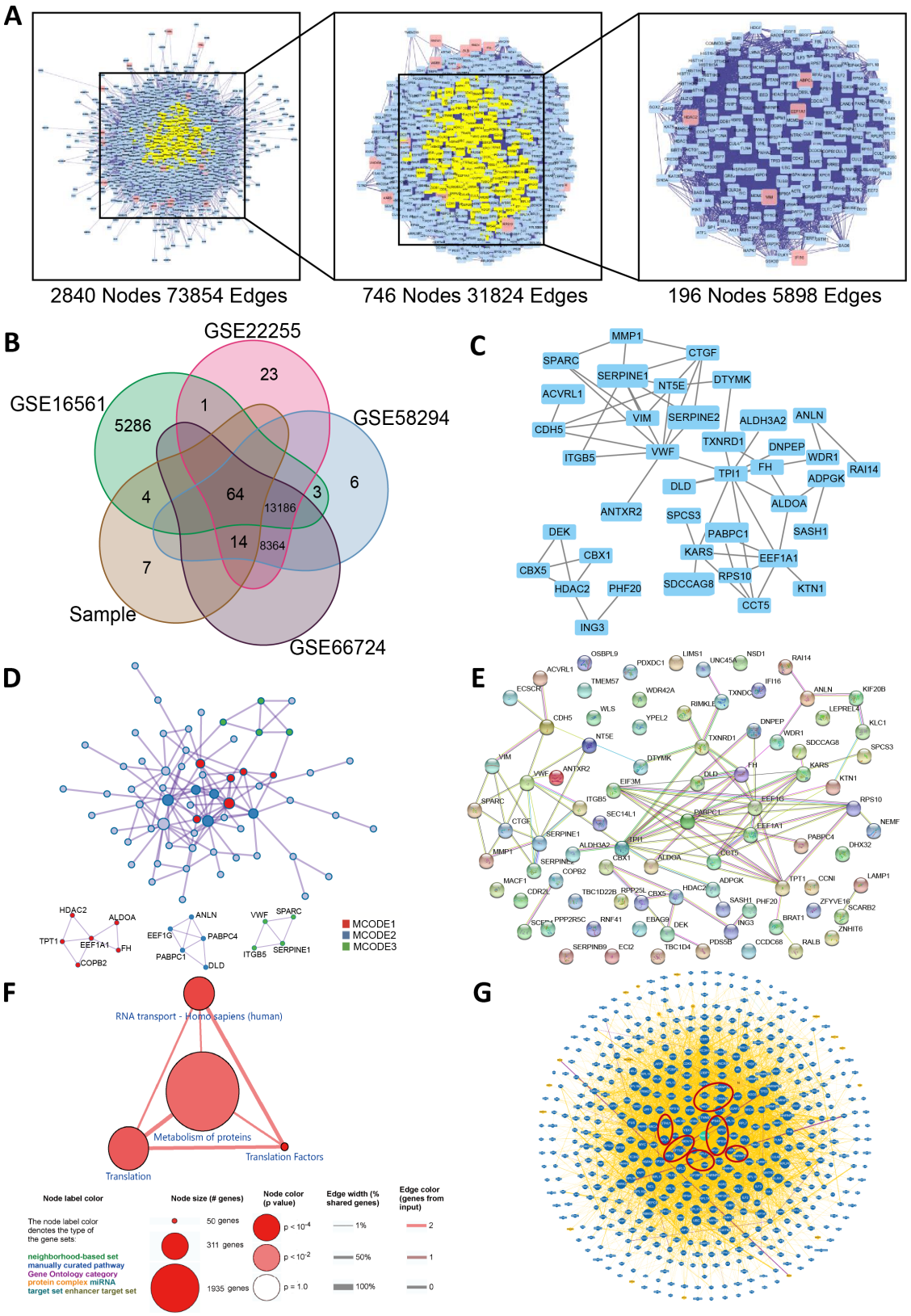

Figure 3 The selected genes were used for gene–gene and protein–protein interactions. (A) A total of 3 advanced screenings of the 83 genes for gene–gene interactions used the Bisogenet network. (B) Venn diagram was made using four atherosclerosis-related databases in GEO interact with the 83 gene samples. (C) The 64 genes screened using GEO were combined with STRING and Cytoscape. (D) Protein–protein interactions and links of significant intersections were showed by Metascape. (E) Protein–protein interactions of 83 genes was analyzed using STRING. (F) Interaction and functional expression of PABPC1, EEF1A1 and HDAC2 genes was analyzed using ConsensusPathDB. (G) An independent biological interaction network centred on PABPC1 was showed by BioGRID, with cancer-related genes circled in red.

to clarify marker genes of AS, we used Metascape and STRING again to respond to the corresponding proteins of these 83 genes (figure 3D and E).

Through intersection of above methods, we found that PABPC1, EEF1A1 and HDAC2 coexisted in various screening results and were likely to be marker genes of AS. Using ConsensusPathDB to conduct interaction and functional expression of these three genes, we found that their common intersection existed in RNA transport, metabolism of proteins, translation factors and translation (figure 3F). Combined with known research reports, we found that EEF1A1 and HDAC2 were common marker genes in cardiovascular diseases and various cancers,[70–73] which further verified the reliability of our methods for screening AS and pan-cancer common biomarkers. Finally, we focused on the expression of PABPC1 and used BioGRID to produce an independent biological interaction network centred on it. We found that among the most related and adjacent genes around PABPC1, many genes had adverse effects on the occurrence and development of cancers, such as SNRNP70, LUC7L2, RPS3 and STAU1, which were circled in red (figure 3G). Therefore, we hypothesised that PABPC1 might play a role in the pan-cancer disease mechanism.

To confirm the reliable expression of PABPC1 in AS-related diseases, we performed reverse validation using GSE58294. In group comparison, there was a particularly significant statistical difference in the expression of PABPC1 between control group and stroke samples, ***p<0.001 (figure 4A). In the receiver operating characteristic curve, area under the curve was 0.771 and 95% CI was 0.666 to 0.875, p<0.001 (figure 4B). In addition, in order to further verify the difference of PABPC1 expression between AS and healthy people with external cohorts, the GSE230214 dataset was added and PABPC1 expression was indeed significantly increased in the AS group (figure 4C).

The above results showed that we screened the AS disease marker gene PABPC1 using a variety of gene–gene and protein–protein interaction methods.

PABPC1 exhibited significant performance in pan-cancer

After identifying PABPC1 as an AS-related target gene, we were considered its expression in different contexts. First, we aimed to understand the mRNA expression of PABPC1 in various organs, tissues, and cell types of human body by BioGPS. BioGPS analysis showed that PABPC1 was expressed in a variety of organs and tissue cells (figure 5A). Among these, PABPC1 exhibited significantly higher expression levels in lymphoma burkitts. PABPC1 also exhibited a relatively high expression of clear aggregation in $CD34^+$ and $CD105^+$ endothelial cells, B lymphoblasts, $CD19^+$ B cells, dendritic cells, $CD8^+$ T cells, $CD4^+$ T cells and so on.

Because the inflammatory response, immune response and endothelial activation were all closely related to cancer, and these were also the responses shared by AS and cancer, the results of BioGPS suggested that we should further explore whether AS-related PABPC1 also had a predictive role in pan-cancer. Therefore, we applied various methods to explore the performance of PABPC1 in pan-cancer analysis. Differences in PABPC1 expression between all TCGA tumours and normal tissues were analysed and identified using the TIMER V.2.0 database (figure 5B). Relative to adjacent normal healthy tissue, PABPC1 was highly expressed in breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), oesophageal carcinoma (ESCA), etc.

Moreover, we applied UALCAN to perform simultaneous pan-cancer analysis of normal and tumour samples. We observed that the expression of PABPC1 in tumour tissues was significantly upregulated including breast cancer, colon cancer and ovarian cancer compared with normal tissues (figure 5C).

Simultaneously, we used GEPIA V.2 to generate PABPC1 expression profile of all tumour samples and paired normal tissues. We found that PABPC1 was significantly expressed in COAD, lymphoid neoplasm diffuse large b-cell lymphoma, ESCA, etc (shown in red font) (figure 5D).

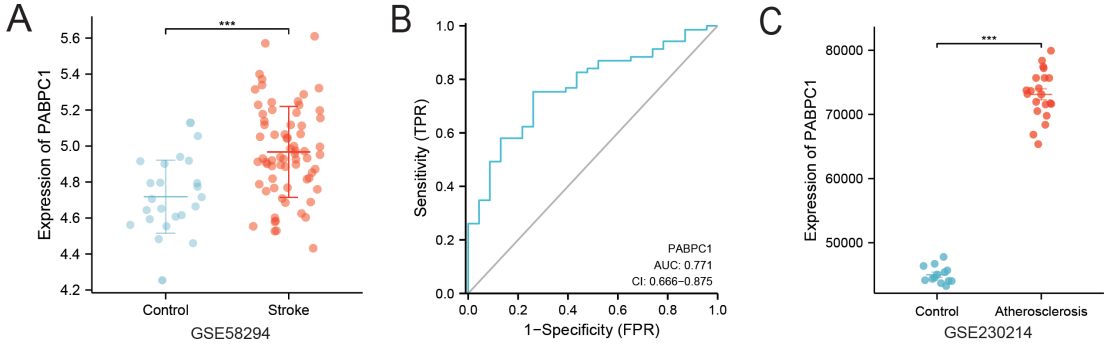

Figure 4 Poly(A) binding protein cytoplasmic 1 (PABPC1) was high expressed in atherosclerosis (AS)-related diseases. (A) PABPC1 expression levels between controls and patients with stroke was different in GSE58294. ***p<0.001. The sample numbers of control and stroke were 23 and 69, respectively. (B) Receiver operating characteristic curve analysis of PABPC1 had predicted stroke in GSE58294. The area under the curve was 0.771 and 95% CI was 0.666 to 0.875, p<0.001. (C) PABPC1 expression between AS disease and healthy individuals had differences in GSE230214.

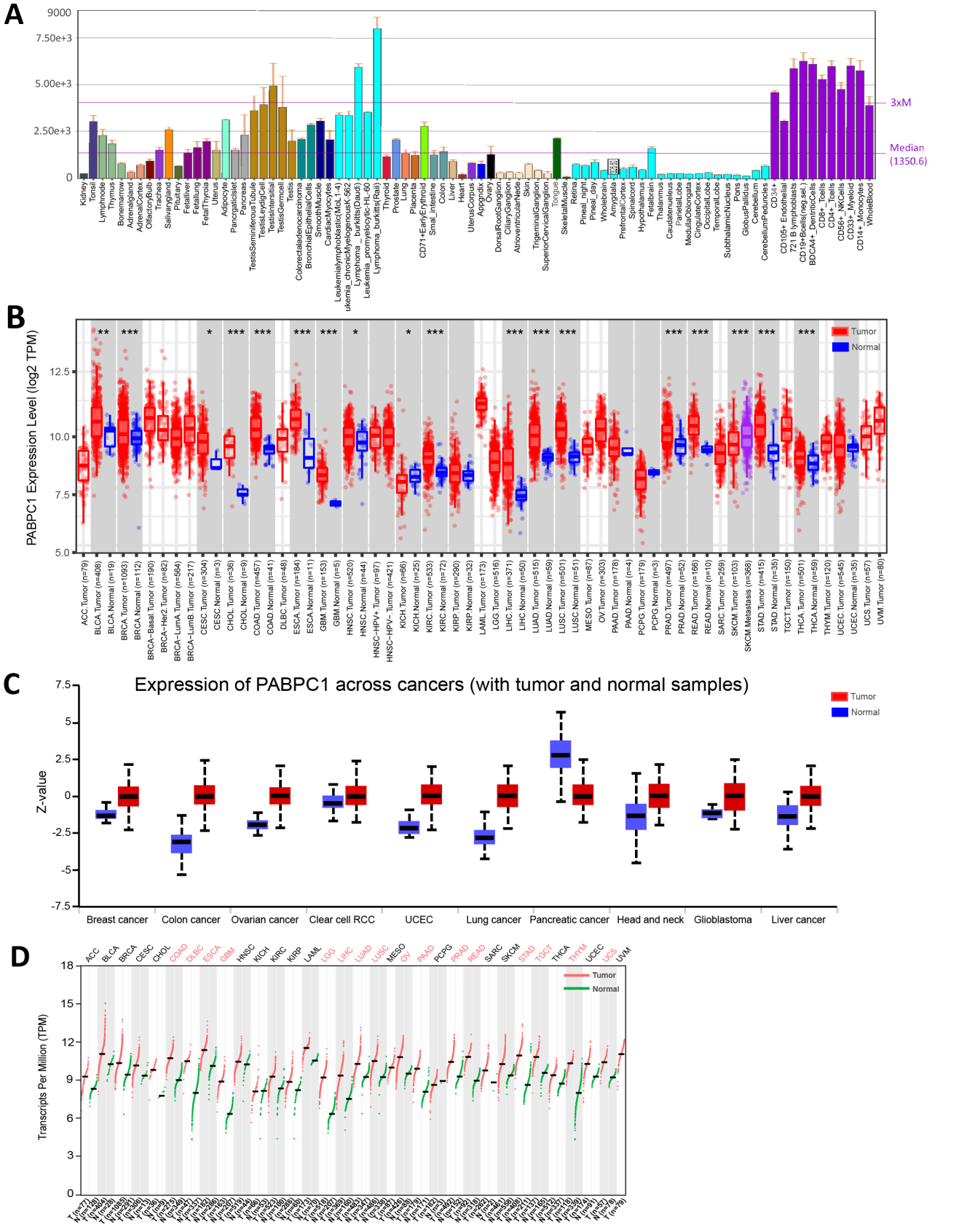

Figure 5 Poly(A) binding protein cytoplasmic 1 (PABPC1) exhibited significant performance in pan-cancer. (A) PABPC1 was expressed to an extent in various organs and tissue cells. (B) Differences in PABPC1 expression between The Cancer Genome Atlas (TCGA) tumours and normal tissues were analysed using TIMER V.2.0. *P<0.05, **p<0.01 and ***p<0.001. (C) Pan-cancer analysis with normal and tumour samples used UALCAN. (D) GEPIA V.2 was used to generate PABPC1 gene expression profiles of all tumour samples and paired normal tissues, with each point of the dot plot representing the expression of the sample.

These results suggested that we used numerous tumour databases and networks to determine the relationship between PABPC1 and pan-cancer, and high expression of PABPC1 in most cancers indicated a significant risk of carcinogenesis.

PABPC1 played a role in pan-cancer through tumour immune infiltration

Because existing studies had suggested a possible impact of cardiovascular disease on tumour immune infiltration, we aimed to further explore whether AS-associated PABPC1 was involved in cancer infiltration. TIMER V.2.0 was used to demonstrate the association of PABPC1 with various immune infiltrations in human tumours (figure 6A). It was significantly positively correlated with immune infiltration levels of several immune infiltrates, such as B cells, $CD4^+$ T cells, neutrophils and mast cells. The positive correlation of PABPC1 in monocytes and myeloid dendritic cells was relatively low, and there was no special expression in $CD8^+$ T cells, endothelial cells, macrophages, etc. However, abundance was negatively correlated with natural killer and plasmacytoid dendritic cells. It is noteworthy that tumours, including CHOL, kidney renal papillary cell carcinoma (KIRP) and kidney chromophobe (KICH), showed a positive correlation trend in almost every immune infiltration situation.

Subsequently, to clarify whether there was a correlation between expression level of PABPC1 and TMB, which had an essential connection with immune checkpoint, we studied the role of PABPC1 in pan-cancer. The results showed that in prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD) and adrenocortical carcinoma (ACC), etc, the expression of PABPC1 was significantly positively correlated with TMB (figure 6B). Correspondingly, COAD, ovarian serous cystadenocarcinoma (OV), uterine carcinosarcoma (UCS), etc, had significant negative correlation with it. figure 6C illustrated the correlation between PABPC1 expression levels and immune checkpoint expression levels, such as TIGIT. A significant negative correlation was found between PABPC1 and skin cutaneous melanoma (SKCM), Sarcoma (SARC) and so on, whereas a positive correlation was found with uveal melanoma (UVM), KIRP, etc.

Figure 7 showed the relationship between PABPC1 and tumour microenvironment across pan-cancer. PABPC1 was negatively associated with immune scores in ACC, bladder cancer (BLCA), SARC and so on, while it was positively correlated with BRCA, CHOL, glioblastoma (GBM), KIRP, etc.

These findings suggested that AS disease-related PABPC1 played a role in cancer immune infiltration and affected TMB.

Association between PABPC1 expression and clinical features of patients

In the HPA, we observed significant differences in tissue expression of PABPC1 in several cancers (figure 8A). PABPC1 was found to express at low levels in glomerular cells and moderately expressed in renal tubular cells in normal kidney tissue. However, for renal cancer, the number of cytoplasmic/membranous staining was generally greater than 75%. For liver, PABPC1 was low expressed in normal tissues and moderately expressed in tumour tissues. For colon and testis conditions, PABPC1 was expressed at low levels in normal tissues but expressed at high in tumour ones.

In addition, pathological stages and age of patients were selected to explore their association with PABPC1 expression. Patients were divided into four groups: I, II, III and IV of pathological stages (figure 8B). PABPC1 was significantly correlated with pathological stages in four cancer types, including COAD, KICH, KIRP and lung adenocarcinoma. In KIRP, PABPC1 expression increased as the pathological stage increased. In COAD and KICH, significant differences in PABPC1 expression were observed in stage IV.

Moreover, 60 years of age was set as a cut-off value to divide patients into two groups (figure 8C). PABPC1 was found to be highly expressed in patients more than 60 years old in BRCA, COAD, KIRP, liver hepatocellular carcinoma (LIHC), OV and SKCM, as well as highly expressed in less than 60 in PRAD.

The effect of different PABPC1 expression on the survival of patients with pan-cancer

Finally, knowing that PABPC1 had widespread effects on pan-cancer through immune infiltration and TMB, we studied its impact on the survival of cancer patients. Kaplan-Meier plotter was used to analyse the survival curves of patients with various types of cancer. We investigated the role of PABPC1 in pan-cancer prognosis using the COX model and found that PABPC1 played various prognostic roles in OS (figure 9A). PABPC1 acted as a protector in brain lower-grade glioma and Thymoma, and it was a detrimental prognostic factor of ACC, LIHC, KIRP, etc. For DSS, PABPC1 played a detrimental prognostic role in ACC, KIRP and SARC. In PFI, PABPC1 predicted poor outcomes in ACC, KICH, KIRP, etc.

The survival curve showed that many tumours shared one characteristic, that was, compared with low expression of PABPC1, high expression made downward trend of survival curve steeper (figure 9B). HR was a ratio of 2 mortality risk rates in patients with high and low PABPC1 expression per unit time. All the PABPC1-expressing cancer survival curves above had HR between 1.23 and 5.75.

The above studies supported that high expression of PABPC1 increased the risk of death in patients with several different cancers and that the PABPC1 gene had a clear role in pan-cancer and AS.

DISCUSSION

Cardiovascular diseases and cancers were the two most important classes of diseases in the world, and they shared characteristics of metabolism and risk factors. Therefore,

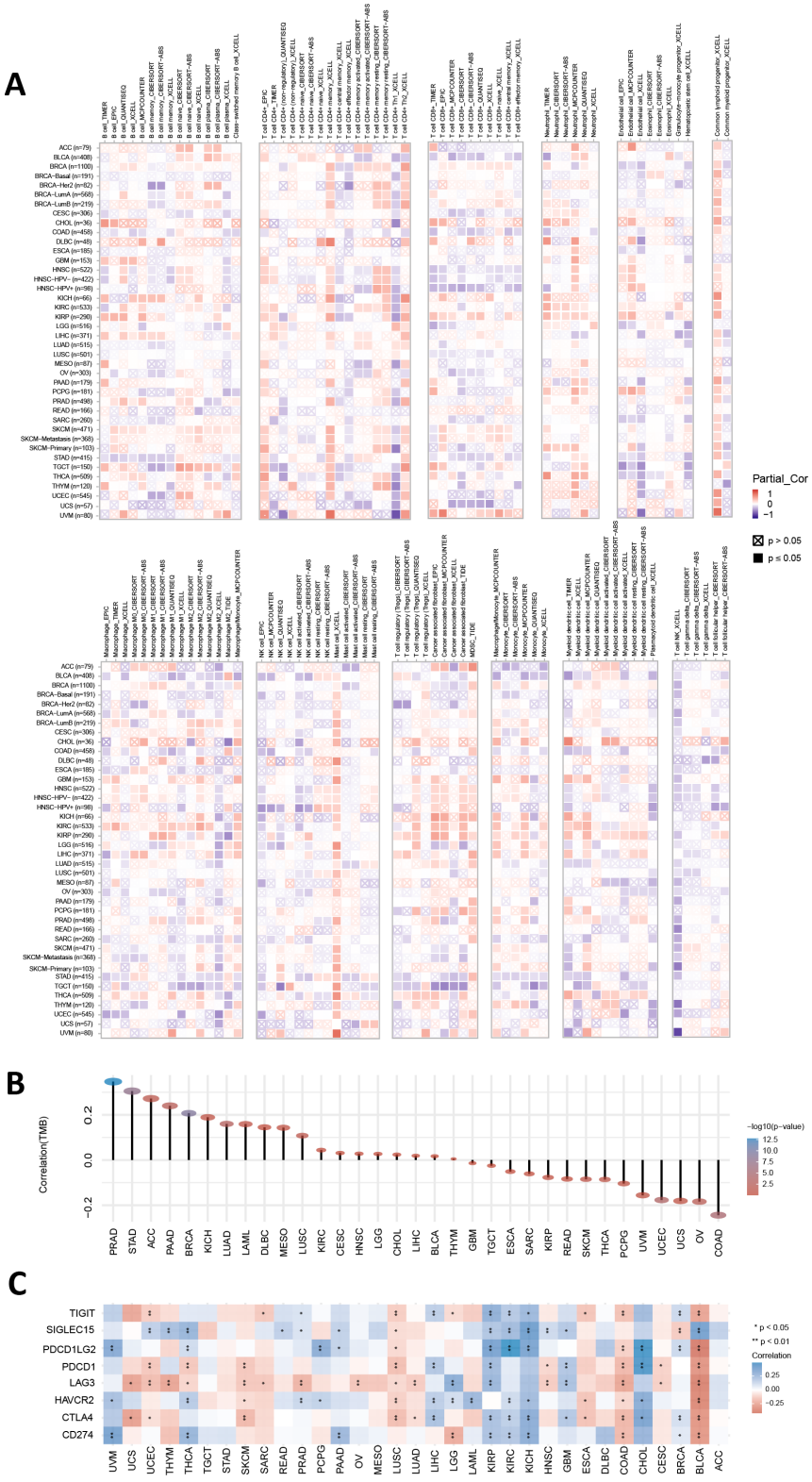

Figure 6 Poly(A) binding protein cytoplasmic 1 (PABPC1) functioned in pan-cancer through tumour immune infiltration. (A) The immune correlation of PABPC1 with various immune infiltrates in human tumours was demonstrated using TIMER V.2.0. Different colours represent correlation coefficients. Negative values indicated negative correlation, positive values indicated positive correlation, and the darker the colour, the stronger the correlation was. (B) The relationship between tumor mutation burden (TMB) and PABPC1 expression was represented by Spearman's correlation analysis. The vertical axis represented the correlation coefficient between genes and TMB, and the horizontal axis represented various tumours. The size of the dots represented the size of the correlation coefficient. (C) Immune checkpoint-related genes were in different tumour tissues. Each box represented the correlation analysis between expression of the selected genes and expression of immune checkpoint-related genes in corresponding tumours.

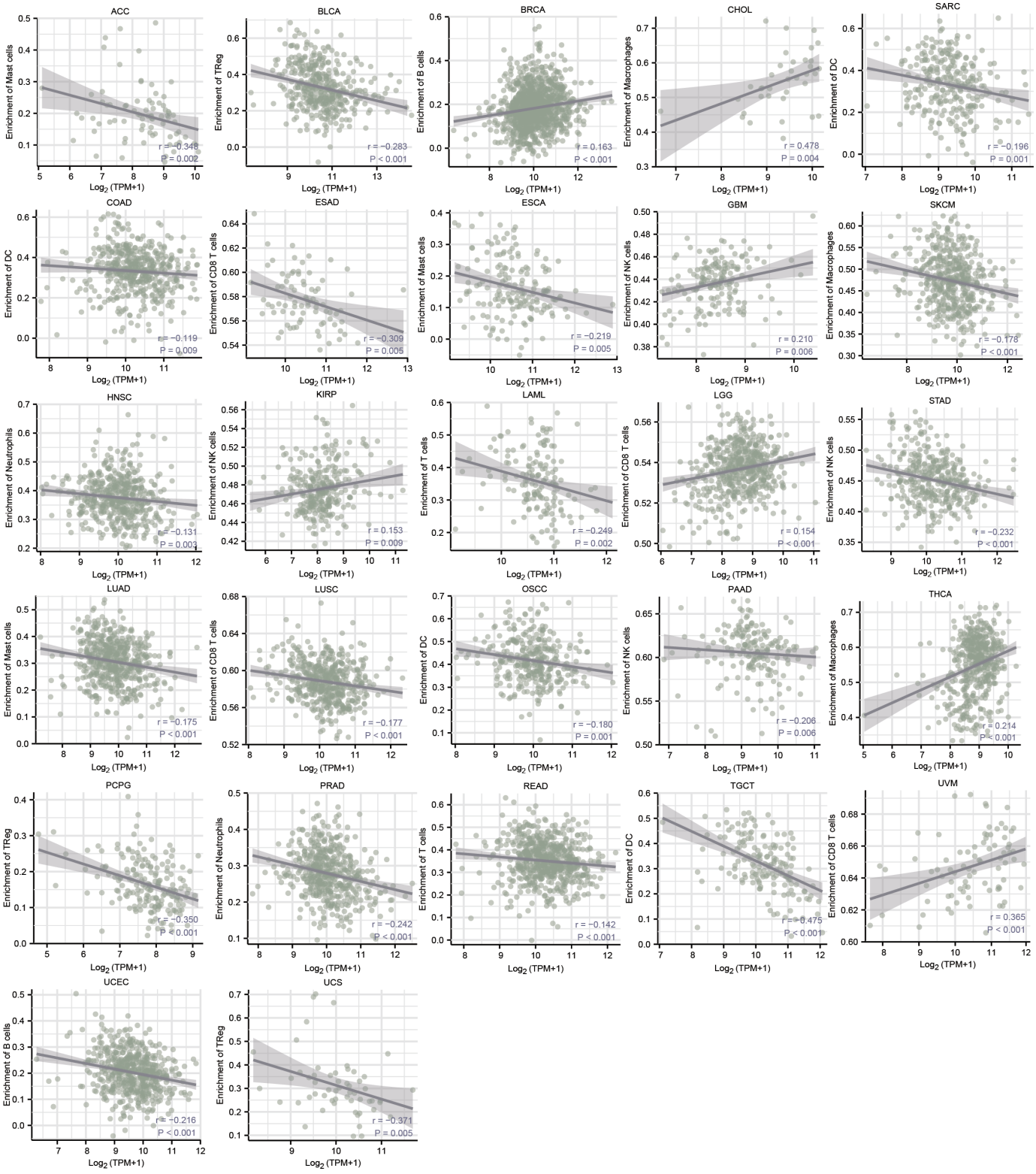

Figure 7 Poly(A) binding protein cytoplasmic 1 (PABPC1) had different Immune infiltration signature in various tumors. Pearson correlation coefficient r>0 (p<0.05) indicated that there was a positive correlation between PABPC1 and the corresponding immune cells. r<0 (p<0.05) indicated that there was a negative correlation between PABPC1 and the corresponding immune cells.

it could be speculated that biomarkers of AS, which were representative of cardiovascular diseases, might also be used in tumour prediction strategies. We performed SEREX screening using serum from 23 TIA patients and identified 83 AS-related genes (figure 1). Functional enrichment analysis of these genes showed that their metabolic pathways were consistent with common pathways of AS and tumours, indicating reliability of them

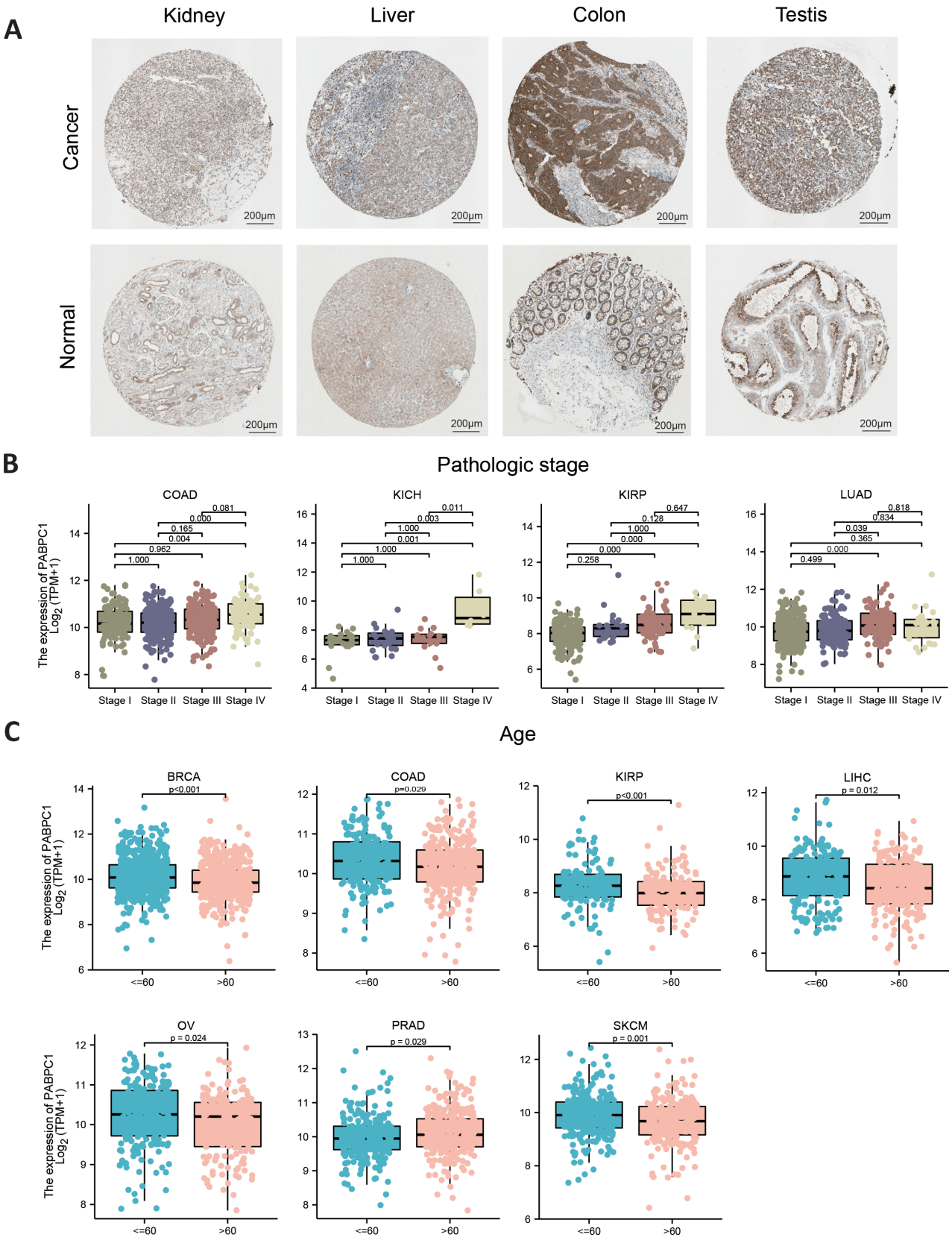

Figure 8 Poly(A) binding protein cytoplasmic 1 (PABPC1) expression was related to pathological and clinical features of patients. (A) Immunohistochemical tissue expression of PABPC1 in several cancers differed in Human Protein Atlas. (B) The expression level of PABPC1 affected pathologic stage. (C) The expression level of PABPC1 was showed in age. BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; KICH, kidney chromophobe; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; OV, ovarian serous cystadenocarcinoma; PRAD, prostate adenocarcinoma; SKCM, skin cutaneous melanoma.

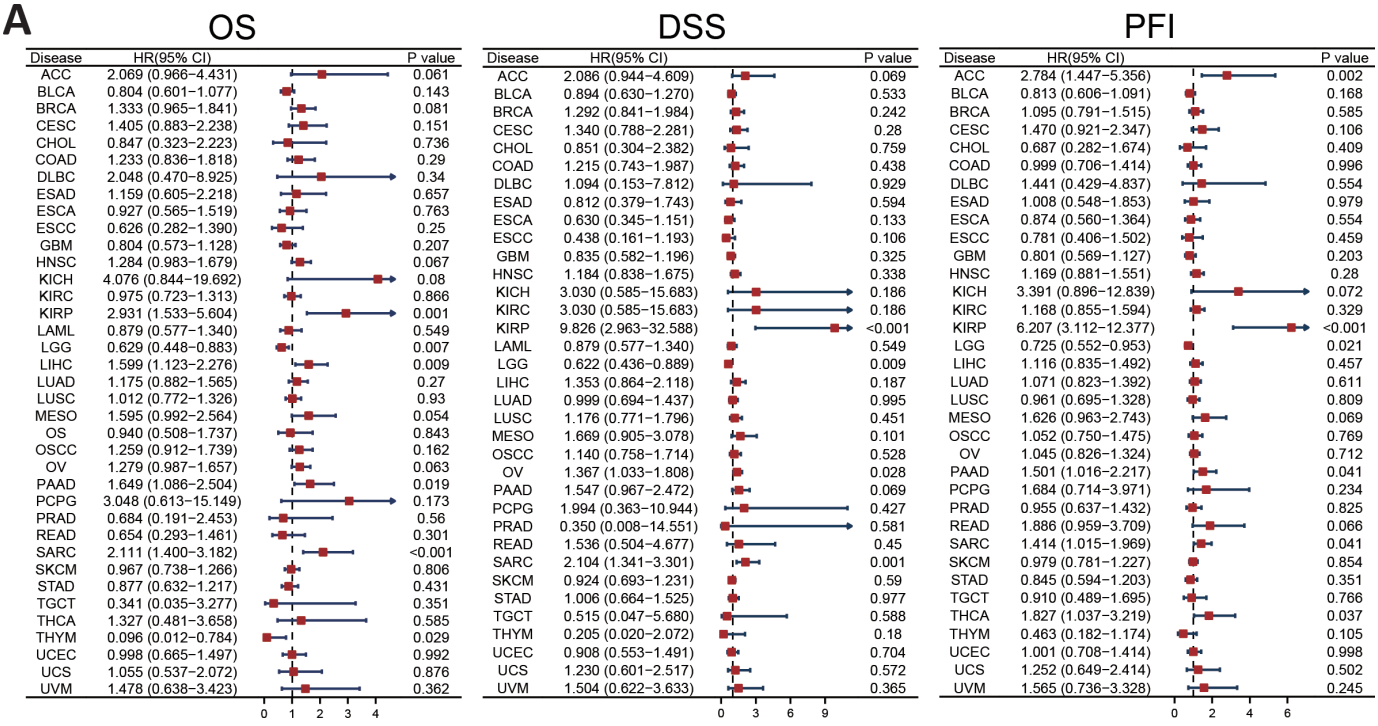

Figure 9 Different poly(A) binding protein cytoplasmic 1 (PABPC1) expression levels affected the survival of patients with pan-cancer. (A) Overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI) in patients with PABPC1 had different manifestations in pan-cancer. (B) Patients with high or low expression of PABPC1 had different overall survival curves in different type of tumors.

for screening as common markers of AS and cancers (figure 2). We then used gene–gene and protein–protein interaction methods, such as Bisogenet and STRING, to screen out biomarker PABPC1 (figures 3 and 4). We observed the expression of PABPC1 in different organs, tissues, and cells of normal human body, and found that it

was high in relation to inflammation and immunity. Since inflammation, immunity and endothelial activation were closely related to AS and cancers, this result prompted us to further use bioinformatics pan-cancer database analysis methods to explore the role of AS-related PABPC1 in pan-cancer and find its high oncogenic expression in the majority of cancers (figure 5). Knowing the possible impact of cardiovascular disease on TMB, we explored the expression of PABPC1 in cancer infiltration and showed that it was significantly positively correlated with immune infiltration level of various immune cells and TMB (figures 6 and 7). We then determined the association between PABPC1 expression and clinical features of patients (figure 8). Finally, the tumour-associated OS, DSS and PFI of PABPC1 were shown, and high expression of PABPC1 compared with low expression made downward trend of survival curve steeper and increased risk of death in patients with cancers (figure 9). Therefore, based on the results of above researches, it was hypothesised that AS-related PABPC1 could be used as a predictive biomarker for pan-cancer.

SEREX was proposed by Professor Sahin, and could isolate and identify autoantigens that induced IgG antibody production at molecular level (figure 1).[74] AS-related disease TIA serum was selected as the SEREX screening sample in our study. TIA was the early group of AS-related diseases with simple disease background, whose patients with no other obvious underlying diseases. It was speculated that SEREX antigen-antibody reaction was relatively concentrated in TIA, which could clarify the specificity of AS-related diseases. The purpose of our study was to screen for early diagnosis warning markers and TIA samples best met the requirements of AS-related diseases. In earlier studies, our group had published some of the AS-associated genes identified using SEREX. Our collaborators further assessed serum antibody levels and identified LRPAP1, which was positively correlated in solid cancers, such as colorectal cancer, as well as some AS-related diseases, such as diabetes.[75] Other partners identified BRAT1 and WDR1 as potential AS markers and found that BRAT1 was a common antibody biomarker for diagnosis of AS and gastrointestinal cancers.[26] In addition, ALDOA, FH, MMP1, CBX1 and CBX5 were expected to become great diagnostic and predictive tools in AS-related diseases.[64 65] Researches and development of these genes by our research group showed that using SEREX to screen AS-related genes from sera of TIA patients was reliable.[76]

We performed a functional enrichment analysis with 83 genes (figure 2). The series of functions, namely focal adhesion, cell–substrate junction, platelet alpha granulation, etc, directly pointed to AS and tumour related pathological mechanisms. In AS, unstable plaques on the vascular surface ruptured and fell off, forming microemboli that flowed distally. Hypoxia and ischaemia led to transient dysfunction in the corresponding areas of blood supply. AS was an autoimmune and inflammatory disease of arteries, in which vascular smooth muscle cells (VSMCs) shared immune effects with vascular endothelial cells, immune cells in sclerotic plaques and resident cells in the adventitia.[77] In contrast, malignant tumours and haemostasis and coagulation systems interacted with each other. Cancer cells activated coagulation system, and haemostatic factors played a role in tumour progression. In cancer patients, phenomenon of coagulation abnormalities provided background for increased propensity for thrombosis and bleeding.[78] Tissue factor-mediated thrombin produced by cancer cells explained local activation of plasma coagulation.[79] In addition, viral processes and toxin transport clearly pointed to the issues of inflammation and immunity. The GO definition of the viral process included the infection of host cells, replication of viral genomes and assembly of progeny viral particles. For toxin transport, although viruses and toxins were evolutionarily distinct toxic substances, their supportment for cellular location of disassembly, host factors selected, conformational changes and physiological function served by disassembly was strikingly conserved.

KEGG proposed two important pathways, namely GLYCOLYSIS/GLUCONEOGENESIS and PYRUVATE METABOLISM, which further verified the accuracy of the SEREX method combined with bioinformatics for screening AS and cancer markers (figure 2). Abnormal tumour glucose metabolism was an important part of tumour metabolic reprogramming and this metabolic change was beneficial for malignant proliferation of tumours and adaptation to unfavourable living environments.[66 67] Increased glycolysis was involved in the proliferation and migration of VSMCs.[68 80 81]

Through screening, intersection and exclusion, we finally focused on PABPC1, which could be used as a cobiomarker for AS and cancers (figures 3 and 4). PABPC1 encoded a poly(A)-binding protein that bound to poly(A) in order to promote ribosome recruitment and translation initiation.[82] The specific expression of PABPC1 in AS was not discovered by researchers, but some people had found that PABPC1 played an important role in cardiovascular related diseases. Tan *et al* focused on function of lncRNA NONRA TT011842 in VSMCs during hypertension and confirmed that NONRA TT011842 could interact with PABPC1 to regulate the functions of VSMCs.[83] Meanwhile, many researchers had reported the situation of PABPC1 in different tumours, further confirming its significant role in pan-cancer. YuFeng *et al* found that high expression of PABPC1 was correlated with worse OS for hepatocellular carcinoma.[84]

We explored the expression of PABPC1 in normal organs and tumour tissues (figure 5). Among the mRNA of embryonic tissues and stem cells, PABPC1 was mostly expressed in the liver and testis, whereas it was overexpressed in lymph nodes in normal human tissues and organs. PABPC1 was significantly highly expressed in lymphoma burkitts, which was highly associated with the Epstein-Barr virus and was a highly malignant B-cell tumour that might originate from follicular germinal centre cells.[85] In GEPIA V.2, mean expression of PABPC1

was higher in all listed cancers than in normal tissues. Using Spearman's correlation analysis, it was found that PABPC1 showed a significant positive or negative correlation with different tumour immune cell infiltrations. Cancers CHOL and KIRP showed positive correlation trends in nearly all immune infiltration situations, which could be caused by different immune infiltration rates in different cancers. In UVM, there was a positive trend in $CD8^+$ T-cell and T-cell follicular helper immune infiltration, which might be associated with the role of different tumour microenvironments (figures 6 and 7).

Moreover, immunohistochemical expression of PABPC1 was particularly evident in pathological sections of tumour and normal tissues. Compared with normal tissues, tumours with high expression of PABPC1 had a disordered tissue texture and damaged structure. PABPC1 was associated with tumour stages in four cancer types and with ages in seven cancer types. The expression of PABPC1 in different pathological stages and at different ages suggested that it had potential to become an effective tumour marker (figure 8).

PABPC1 played a significant detrimental prognostic role among OS, DSS and PFI in ACC, KIRP and SARC, indicating that the effect of PABPC1 was extensive in them. Compared with low expression of PABPC1, the survival rate of patients with high expression of it showed a downward trend. The log-rank test gave greater weight to the long-term differences in outcome events, meant that it was sensitive to long-term differences (figure 9).

CONCLUSION

Through the findings of SEREX and bioinformatics pan-cancer analysis, we concluded that PABPC1 might serve as a potential cobiomarker for prediction and diagnosis of AS and pan-cancer. The study of PABPC1 might develop new serum markers and provide new indicators for further clinical liquid biopsy as well as promote prevention for AS and tumours to a certain extent.

Author affiliations
[1]Stroke Center, The First Affiliated Hospital, Jinan University, Guangzhou, China
[2]Department of Anesthesiology, The First Affiliated Hospital, Jinan University, Guangzhou, China
[3]The Biomedical Translational Research Institute,Faculty of Medical Science, Jinan University, Guangzhou, China
[4]Department of Radiology, Medical Imaging Center, The First Affiliated Hospital, Jinan University, Guangzhou, China
[5]Department of Biochemistry and Genetics, Graduate School of Medicine, Chiba University, Chiba, Japan
[6]Department of Neurology, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China
[7]Department of Neurology, Chiba Rosai Hospital, Chiba, Japan
[8]Department of Neurology, Chibaken Saiseikai Narashino Hospital, Chiba, Japan
[9]Department of Neurological Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan

Contributors All authors have contributed to the manuscript and approved the submitted version. ML, LH, SS, JL, YL, YX, HC, KS, HW and AX contributed to the project development, researching and manuscript writing. JL, HW and AX edited the manuscript. KS, IK, TH and HW collected the serum samples. HW, LH, ML and AX contributed toward the statistical analysis of this work. ML interpreted the data. HW was the guarantor to this manuscript.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants. These studies involving human participants and recombinant DNA were approved by the Ethics Review Committee of Chiba University (No. 2012-438, No. 2014-44, No. 2016-86, No. 2017-251, No. 2018-320 and No. 2020-1129) and the Human Research Ethics Committee of Jinan University (JNUKY-2021-045, JNUKY-2022-027) and were conducted in conformity with the rules of the Japanese and Chinese governments. This study was conducted in accordance with the principles of the Declaration of Helsinki. All patients understood the purpose and steps of the experiments and agreed to participate in the study before serum collection. Written informed consent was obtained from all participants, thereafter, their serum was collected.

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Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information. All data generated or analyzed during this study are included in this published article and its supplemental information files, and are available from the corresponding author on reasonable request.

ORCID iDs
Hao Wang http://orcid.org/0000-0003-2169-1712
Anding Xu http://orcid.org/0000-0003-3154-0985

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