

Transcriptome Data Reveal Geographic Heterogeneity in Gene Expression in Patients with Prostate Cancer

Yi Zheng^{#1}, Yang Wang^{#2}, Haitian He², Zhuping Zou³,
Huiling Lu³, Jinlong Li⁴, Jun Cai⁴, Kebing Wang^{*2,5}

Abstract

Background: The incidence of prostate cancer (PC) exhibits geographical heterogeneity. However, the metabolic mechanisms underlying this geographic heterogeneity remain unclear. This study aimed to reveal the metabolic mechanism of the geographic heterogeneity in the incidence of PC.

This study aimed to investigate the anti-cancer effects of different gum extracts on metabolic changes and their impact on gene expression in HT-29 cell.

Methods: Transcriptomic data from public databases were obtained and analyzed to screen geographic-differentially expressed genes and metabolic pathways. Associations between these differentially expressed genes and the incidence of PC were determined to identify genes that were highly associated with PC incidence. A co-expression network analysis was performed to identify geographic-specific regulatory pathways.

Results: A total of 175 differentially expressed genes were identified in four countries and were associated with the regulation of DNA replication and the metabolism of pyrimidine, nucleotides, purines, and galactose. Additionally, the expression of the genes *CLVS2*, *SCGB1A1*, *KCNK3*, *HHIPL2*, *MMP26*, *KCNJ15*, and *PNMT* was highly correlated with the incidence of PC. Geographic-specific differentially expressed genes in low-incidence areas were highly correlated with *KCNJ15*, *MMP26*, *KCNK3*, and *SCCB1A1*, which play a major role in ion channel-related functions.

Conclusions: This study suggests that geographic heterogeneity in PC incidence is associated with the expression levels of genes associated with amino acid metabolism, lipid metabolism, and ion channels.

Keywords: Differentially expressed genes, Geographical heterogeneity, Metabolic pathway, Prostate cancer.

Introduction

Prostate cancer (PC) is the second most common cancer and the fifth leading cause of cancer-related death in men worldwide (1). In 2020, it was estimated that approximately 1.4 million men will be diagnosed with PC and approximately 375,000 patients will die due to PC (2). A World Health Organization (WHO) report showed that there were

significant regional differences in the incidence of PC. The United States, Europe, and Oceania have the highest incidence rates whereas Asia has a relatively low incidence (3). The age-standardized incidence rate (ASIR, 1/105) of PC in Oceania and Northern America was higher than 70.0, and that in Latin America, the Caribbean, and Europe

1: Center for Medical Experiments (CME), University of Chinese Academy Sciences-Shenzhen Hospital, Shenzhen, China.

2: The Department of Urology of Shenzhen Qianhai Shekou Free Trade Zone Hospital, Shenzhen, China.

3: The Operating Room of Shenzhen Qianhai Shekou Free Trade Zone Hospital, Shenzhen, China.

4: TB Healthcare Co., Ltd, Foshan, China.

5: The Department of Urology of Huazhong University of Science and Technology Union Shenzhen Hospital, Shenzhen, China.

[#] The first and the second authors contributed equally to this work.

*Corresponding author: Kebing Wang; Tel: +86 0755 21606999; E-mail: kebingwang2005@163.com.

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was higher than 50, whereas those in Africa and Asia were lower at 29.7 and 13.6, respectively (4).

Prostate cancer (PC) is associated with age (5), sexual activity (6), and family genetic history (7). Owing to advances in research, the specific molecular mechanisms of PC are gradually becoming clear (8). Alterations in the androgen receptor signaling pathway have been identified as the main cause of PC (9). Additionally, six estrogen receptor α (ER α) mutations (E380Q, L536Q, Y537C, Y537S, Y537N, and D538G) were identified in the plasma of patients with advanced PC (10). Moreover, Yan et al. (11) have reported, using a TCGA (The Cancer Genome Atlas) analysis, that differentially expressed genes in PC were significantly correlated with drug metabolism, metabolism of xenobiotics by cytochrome P450, and chemical carcinogenesis. This in-depth understanding of the molecular mechanisms of the pathogenesis of PC has guided the treatment of PC as several novel diagnostic/prognostic markers have been discovered and used as targets for drug development. However, the mechanism underlying the geographic differences in ASIR

across countries remains unclear.

Therefore, in this study, we retrieved and analyzed transcriptome data of PC patients and healthy participants from China, South Korea, the United States, and France, which represent different ASIR levels, and screened genes associated with geographical differences in ASIR using differential expression and correlation analyses.

Materials and Methods

Dataset collection

All transcriptome datasets were retrieved from PCaDB (12), a public database that collects the transcriptomic expression data of PC. Only RNA-seq datasets containing both primary and normal groups were selected. Finally, four normalized datasets were retrieved; detailed information about each dataset is presented in Table 1. According to the PCaDB pipeline, all expression data in each dataset were normalized using the Trimmed Mean of M values (TMM) method implemented in the R package edgeR (13) and transformed into a log2 scale. The Ensembl identifier (<https://asia.ensembl.org/index.html>) was used to represent each gene in all datasets.

Table 1. Information on the datasets analyzed in this study. The datasets were retrieved from PCaDB (<http://bioinfo.jialab-ucr.org/PCaDB/>).

GEO ID	Country	Sample number	Primary	Normal	Platform	Reference
GSE114740	China	20	10	10	Illumina HiSeq 2000	unpublished
GSE104131	USA	32	16	16	Illumina HiSeq 2500	PMID: 29741809 (14)
GSE133626	France	60	30	30	Illumina HiSeq 2000	PMID: 34873462 (15)
GSE80609	South Korea	24	16	8	Illumina HiSeq 2000	PMID: 29383125 (16)

Differential expression analysis

For all four datasets, normal samples were treated as control samples. The R limma package version 3.52.4 (17) was used for the differential analysis of each dataset. The P value ($P < 0.05$) and fold-change, (log2 fold change) ≥ 1 , were used to determine differentially expressed genes (DEGs). After completion of

the differential analysis, the Ensembl identifier was compared to the data downloaded from Biomart (<http://asia.ensembl.org>), and only protein-coding genes were used for subsequent analysis.

All selected DEGs were considered risk factors for PC. Moreover, DEGs that were

differentially expressed in all four datasets were considered PC-common DEGs (cDEG whereas others that were only differentially expressed in one country were considered geographic-specific DEGs (sDEGs). A clustering heatmap was drawn using the pheatmap (1.0.12) package to show the expression patterns of DEG in all samples.

The age-standardized incidence rate (ASIR) correlation and co-expression analysis

The Pearson correlation coefficient was used to calculate the association between the fold-change of cDEGs and ASIR. A high correlation coefficient suggested that the geographic heterogeneity in prostate cancer incidence may be related to differences in the expression levels of cDEGs. All cDEGs with correlation coefficients > 0.8 were selected as potential correlates of geographic heterogeneity.

Additionally, the Pearson correlation coefficient was used to calculate the co-expression coefficient between each sDEG and cDEG based on their expression in the PC group. An association with a coefficient > 0.8 and a $P < 0.05$ was considered a co-expression relationship. Cytoscape (v3.9.0) (18) was used to construct a co-expression network.

Functional enrichment

Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were used for gene annotation. Functional enrichment analysis was performed using the R package clusterProfiler (v4.0.5) (19). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and GO terms with P values < 0.05

were considered significantly enriched based on the hypergeometric distribution.

Results

PC-common DEGs

We identified a total of 633, 839, 1626, and 3506 DEGs in the GSE114740, GSE104131, GSE133626, and GSE80609 datasets, respectively (Fig. 1 and Table 2). A total of 175 DEGs were differentially expressed in all four datasets and were considered PC-common DEGs (cDEGs) (Fig. 1E). Across the four datasets, all cDEGs had the same expression status in the cancer samples but with different levels of differential expression (Fig. 2).

Upregulated cDEGs were mainly enriched in RNA polymerase, inositol phosphate metabolism, and platinum drug resistance pathways and were also related to negative regulation of DNA-templated DNA replication, DNA replication checkpoint signaling, regulation of the endoplasmic reticulum-associated protein degradation (ERAD1) pathway, negative regulation of DNA replication, nuclear DNA replication, and cell cycle DNA replication. Downregulated cDEGs were associated with the metabolism of pyrimidine, nucleotide, purine, and galactose, as well as the biosynthesis of ubiquinone, glycosaminoglycan, and glycosphingolipid. Additionally, downregulated cDEGs were associated with the regulation of peptidase activity, endopeptidase activity, intrinsic apoptotic signaling pathway, and cysteine-type endopeptidase activity. The expression imbalance of these functions may be one of the causes of PC pathogenesis.

Table 2. The number of differentially expressed genes in the four datasets. The datasets were retrieved from PCaDB (<http://bioinfo.jialab-ucr.org/PCaDB/>).

GEO ID	DEG Number	Up-regulated	Down-regulated
GSE114740	633	211	422
GSE104131	839	351	488
GSE133626	1626	519	1107
GSE80609	3506	1180	2380

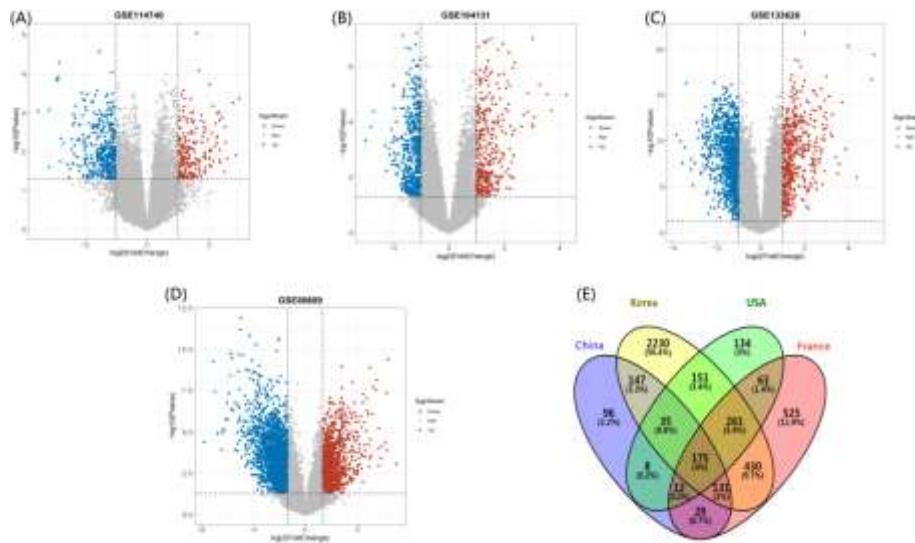


Fig. 1. Differentially expressed genes across the four datasets. The datasets were retrieved from PCaDB (<http://bioinfo.jialab-ucr.org/PCaDB/>). (A)-(D) Volcano plots of GES114740, GSE104131, GSE133626, and GSE80609 datasets, respectively. (E) Venn diagram of the numbers of differentially expressed genes in the datasets.

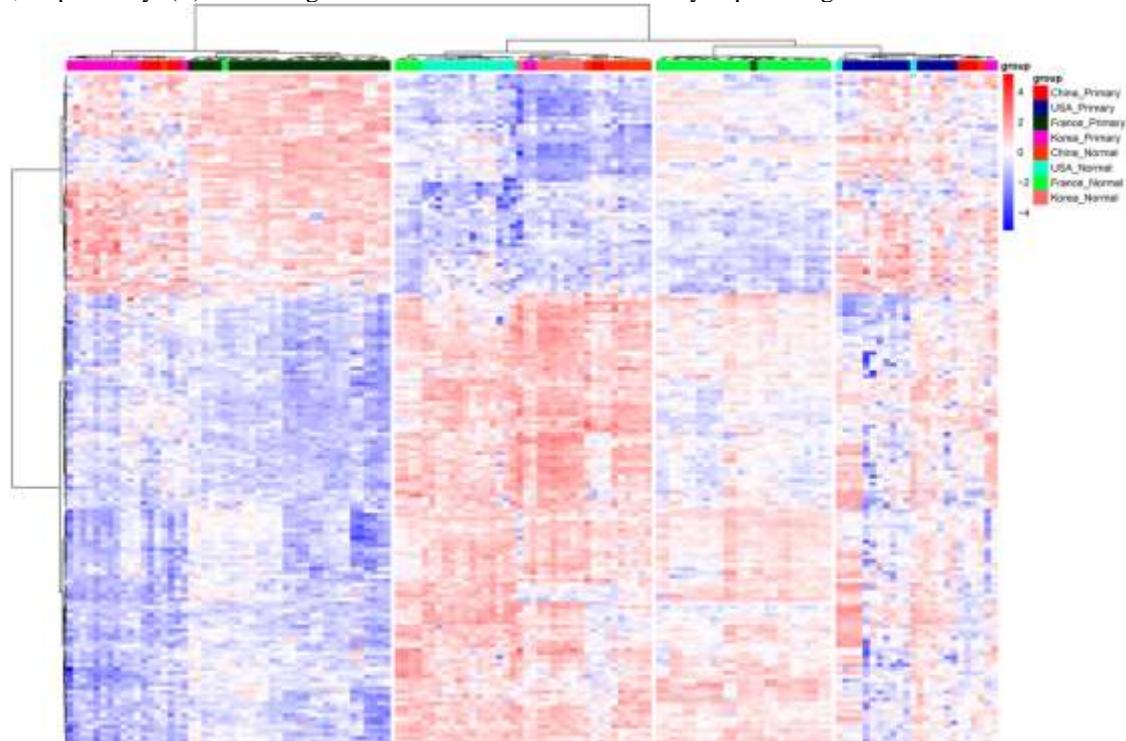


Fig. 2. Clustering heatmap of the expression of the 175 common differentially expressed genes in all samples.

Pathways associated with sDEGs

To determine the function of the sDEGs in the four countries, a KEGG pathway enrichment analysis was performed. The results showed that the sDEGs of the different countries were enriched in 24 common pathways (Fig. 3). The distribution of the sDEG-related KEGG pathway types is shown in Table 3. Amino acid

metabolism, carbohydrate metabolism, glycan biosynthesis and metabolism, and lipid metabolism were the main enriched KEGG pathways associated with sDEGs. Among the 24 common pathways, many were related to PC, such as tyrosine metabolism (20), tryptophan metabolism (21), MAPK signaling pathway (22), and PI3K-Akt signaling

pathway (23). In addition, other glycan degradations were specific to the sDEGs of China whereas the citrate cycle (TCA cycle), mismatch repair, and base excision repair were specific to the sDEGs of France. Among the 31

specific pathways in Korea, four pathways were related to glycan biosynthesis and metabolism, and two were related to lipid metabolism, which has been previously reported to be related to PC.

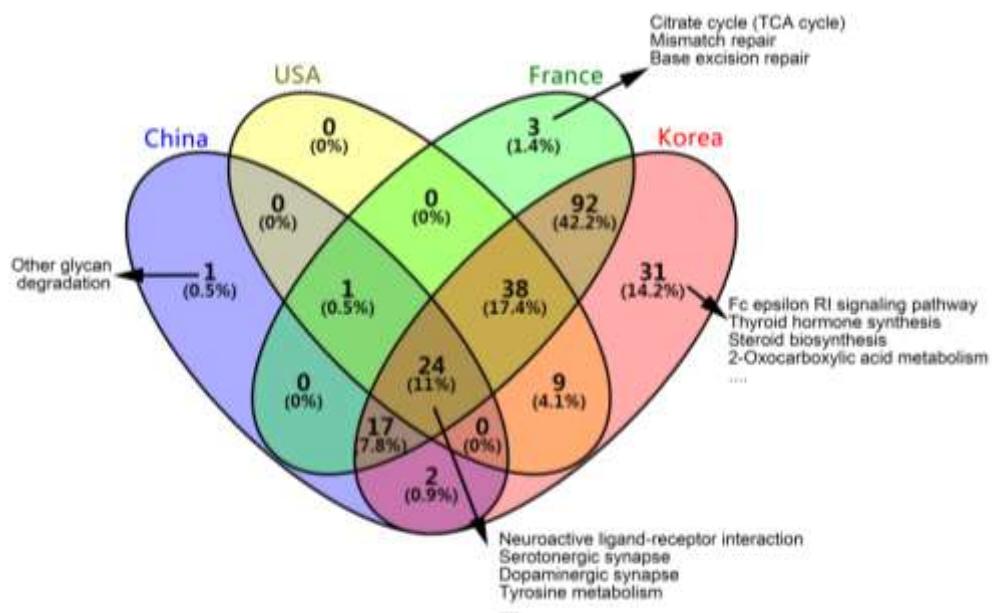


Fig. 3. Venn diagram of the Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis.

Table 3. Types of sDEGs-related the Kyoto Encyclopedia of Genes and Genomes pathways.

Pathway type	Number of pathways
Amino acid metabolism	11
Carbohydrate metabolism	14
Energy metabolism	3
Glycan biosynthesis and metabolism	14
Lipid metabolism	13
Metabolism of cofactors and vitamins	6
Metabolism of other amino acids	4
Metabolism of terpenoids and polyketides	1
Nucleotide metabolism	2
Xenobiotics biodegradation and metabolism	3

Correlation analysis of gene pattern and incidence

According to a previous study (4), the ASIR in 2020 for China, the USA, France, and South Korea was 10.2, 72.0, 99.0, and 27.2, respectively. Therefore, we calculated the Pearson correlation coefficient between the fold-

change (FC) of cDEGs and ASIR. Seven cDEGs were selected under this threshold (Table 4). The correlation analysis of each cDEG with ASIR showed the fold-change of five cDEGs was positively correlated with ASIR whereas that of the remaining two was negatively correlated with

ASIT. Among the seven cDEGs, KCNK3 and KCNJ15 were mainly associated with functions related to ion channels, such as potassium ion transmembrane transport, potassium ion transport, voltage-gated potassium channel

activity, and potassium channel activity (Fig. 4). SCGB1A1 was related to immune functions, such as interleukin-5 production, interleukin-13 production, and regulation of inflammatory responses (Fig. 4).

Table 4. Correlation analysis of age-standardized incidence rate (ASIR) and common differentially expressed genes (cDEGs).

ID	cDEG name	FC in China	FC in USA	FC in France	FC in Korea	Correlation with ASIR
ENSG00000146352	CLVS2	-1.18	-1.61	-1.77	-1.51	-0.9244
ENSG00000149021	SCGB1A1	-3.49	-2.01	-1.64	-4.66	0.8555
ENSG00000171303	KCNK3	-2.05	-1.01	-1.06	-2.58	0.8547
ENSG00000143512	HHIPL2	-2.32	-1.44	-1.53	-2.71	0.8523
ENSG00000167346	MMP26	2.98	2.18	2.39	3.13	-0.8481
ENSG00000157551	KCNJ15	-1.96	-1.13	-1.1	-2.55	0.834
ENSG00000141744	PNMT	-2.87	-1.36	-1.2	-4.39	0.8019

Note: FC represented the fold-change in differential expression analysis.

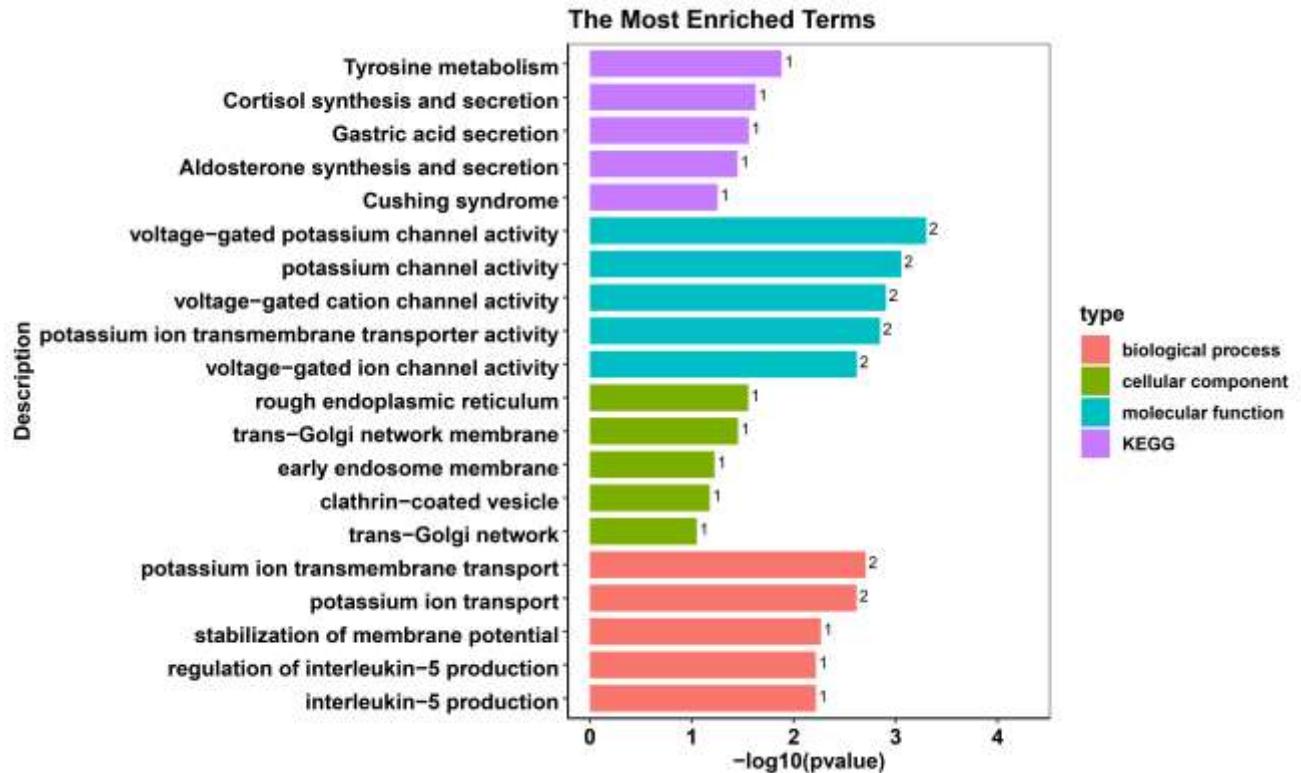


Fig. 4. Functional enrichment of common differentially expressed genes (cDEGs) with a high correlation coefficient.

Co-expression network

The co-expression relationship of each sDEG and the seven cDEGs correlated with ASIR were investigated. We identified 18, 10, 4, and 104 co-expression associations in China, the USA, France, and Korea, respectively, indicating a clear regional concentration trend (Fig. 5). Moreover, sDEGs in low-ASIR countries were highly related to KCNJ15,

MMP26, KCNK3, and SCCB1A1 whereas sDEGs in high-ASIR countries were preferentially co-expressed with HHIPL2, PNMT, and CLVS2 (Fig. 5). Most of the co-expression correlations were positive. These results suggest that the four genes (KCNJ15, MMP26, KCNK3, and SCCB1A1) are highly associated with geographic heterogeneity in PC.

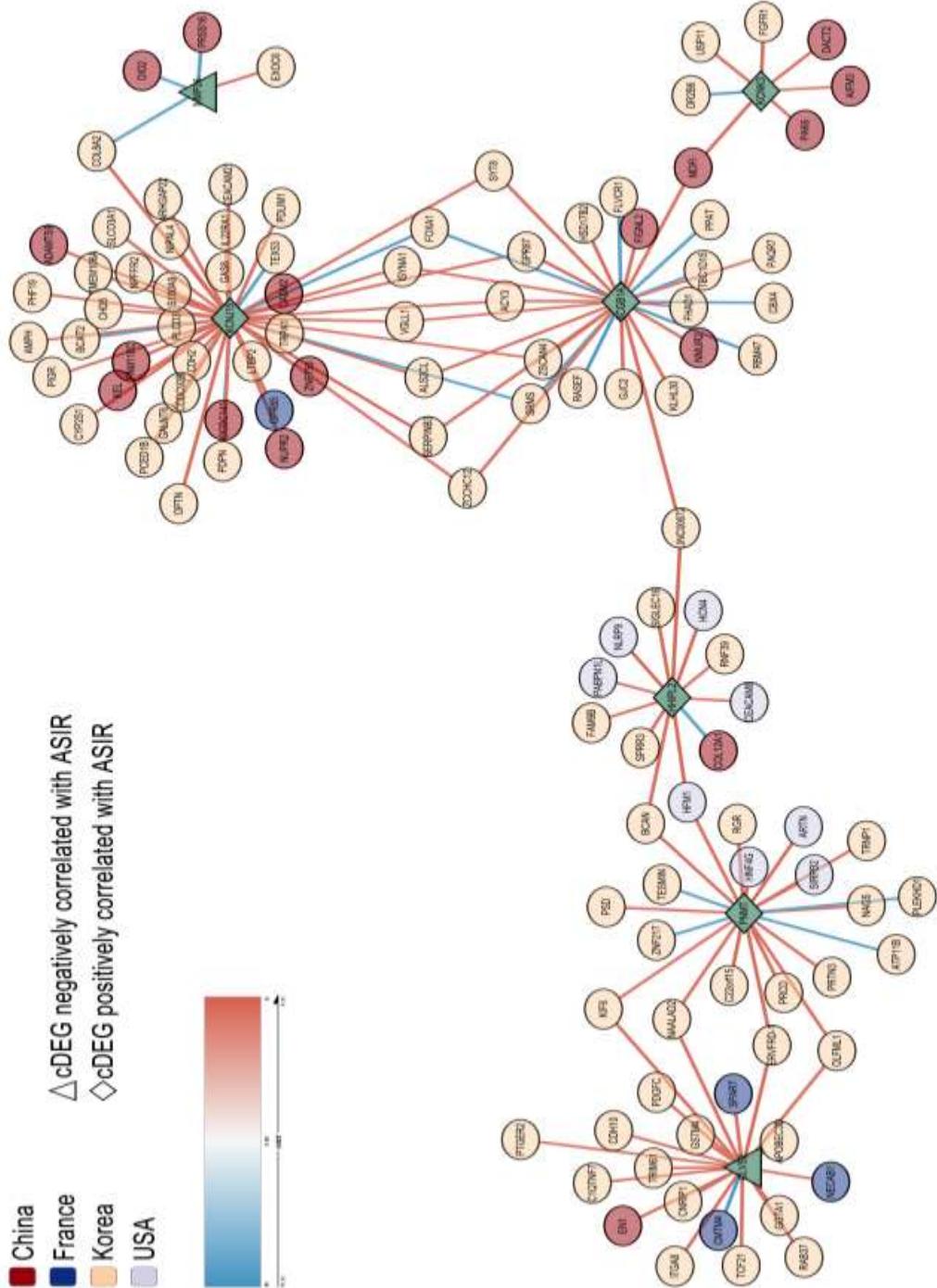


Fig. 5. Co-expression network of specific and common differentially expressed genes.

The green nodes of the triangle represent cDEGs that were positively correlated with ASIR, and the green nodes of the diamonds represent a negative correlation. Red and blue nodes represent the sDEGs in low and high-ASIR countries, respectively. The red line represents the level of correlation. sDEGs, specific and common differentially expressed genes; cDEGs, common differentially expressed genes; ASIR, age-standardized incidence rate.

Discussion

PC, one of the most common cancers worldwide, has become a deadly killer for men. The incidence of PC exhibits geographic heterogeneity, with a higher incidence in Western countries. In the present study, we collected transcriptome data from four different countries to identify factors related to geographic heterogeneity at the gene expression level. We identified DEGs shared between the four countries, which were considered inherent risk factors for PC, and other DEGs that were specific to each country, which were considered environmental factors that affect the incidence of PC.

We identified seven cDEGs that were highly correlated with ASIR. These sDEGs mainly play important roles in ion channels and immune-related functions, which is consistent with numerous studies reporting that ion channels affect PC cells (24-27). The co-expression network of the sDEGs and cDEGs also showed an obvious regional clustering phenomenon; the sDEGs of the low-ASIR countries were more highly co-expressed with ion channel-related genes than those of the high-ASIT countries. Therefore, our results suggest these sDEGs may have synergistic or inhibitory effects with cDEGs that are highly related to ASIR, resulting in the differential expression of the cDEGs and geographic differences in PC incidence.

Furthermore, although we identified region-specific DEGs, most of these DEGs were enriched in the same pathways; five sDEGs were enriched in the tryptophan metabolism

pathway, the synthesis of different metabolites, and different lipid metabolism pathways such as arachidonic acid metabolism, fatty acid biosynthesis, and steroid hormone biosynthesis. Amino acid metabolism-related pathways are closely related to PC (28). For example, tyrosine and tryptophan are potential diagnostic and prognostic biomarkers for PC (29). Tryptophan catabolism is a known mechanism of immune system modulation (30), and the ability of cancer to escape the immune response is an important factor that drives the development of aggressive forms of tumors (31). Tryptophan consumption is also a critical factor in cancer progression (32). In addition, androgens and androgen receptor signaling play an important role in lipid metabolism and are established drivers of PC progression and castration resistance (33). Therefore, our results indicate that there are specific environmental factors in each region, which indirectly affect the incidence of PC through different regulations of the same pathway.

In our study, we observed that Korea had the highest number of DEGs compared to the other three countries. This could be attributed to inconsistency in the selection of control samples for the dataset. For France, the United States, and China, datasets for adjacent normal tissues were analyzed whereas, for Korea, the benign prostatic hyperplasia (BPH) tissue was analyzed. This result indicates that future in-depth studies should carefully select sample sources. Moreover, cross-regional exploration experiments should be consistent with various environmental factors to improve their verifiability and accuracy.

This study has some limitations, including the small number of samples in the dataset collected, lack of clinical information, and differences in sample types. In future studies, PC datasets from more countries should be added to obtain more accurate results. Furthermore, the results of this study cannot be verified yet, and future international cooperation can help reveal the underlying mechanisms of the geographical differences in

the incidence of PC and to develop effective treatment plans for PC.

In a conclusion, through the analysis of transcriptome data, our study mainly aimed to discovery the reasons for the geographical differences in the incidence of PC and uncovered core differences at the molecular level. The result also provided effective theoretical support for the personalized treatment plan of PC.

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Conflict of Interest

All authors declare no conflict of interest.

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