[Additional File 1]

Differential Expression Analysis of PRCP-Associated Genes

The Wilcoxon rank-sum test was used to assess the differential expression of PRCP and PSMA4 in the control and experimental groups. Patients with ruptured aneurysms were categorized into high- and low-expression groups based on the median expression value of PRCP. The 'limma' package (1) was utilized to analyze the Differentially Expressed Genes (DEGs) between these groups for subsequent analysis. Statistical significance was set at $p < 0.05$, and $|Log2FC| > 0.5$ were utilized to determine statistical significance.

Enrichment Analysis

We utilized the 'clusterProfiler' R package(2) to perform Gene Ontology (GO) analysis on the DEGs, including Biological Processes, Molecular Functions (MF), and Cellular Components. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed. Additionally, the Reactome database was utilized for enrichment analysis, and the 'enrichplot' package in R was used for visualization, setting the significant enrichment threshold at $p < 0.05$. Further analysis involved obtaining the Hallmark gene set from the Molecular Signatures Database (MSigDB) and performing enrichment analysis using the 'fgsea' R package.

Immune Infiltration Analysis

We used five algorithms, including CIBERSORT (3), EPIC (4), ImmuneCellAI (5), MCPcounter (6), and xCell (7), to assess the scores of the various infiltrating immune cells in the dataset. Subsequently, Spearman's correlation analysis was used to determine the correlation between PRCP gene expression and the scores of diverse infiltrating immune cells.

Evaluation of the correlation between immune-related and metabolic-related genes and pathways with PRCP gene expression was performed.

Immune-related gene sets and pathways were downloaded from the ImmPort database (http://www.immport.org). The R package 'GSVA' was utilized to perform single-sample gene set enrichment analysis (ssGSEA) for the immune-related pathways. Subsequently, Spearman's correlation analysis was performed to evaluate the correlation coefficient (r) and corresponding p-values between PRCP gene expression and immune genes and related pathways. Genes and immune-related pathways with p-values < 0.05 were displayed. Metabolism-related genes and pathways were obtained from the KEGG database (https://www.genome.jp/kegg).

Protein-protein interaction network

GeneMANIA (https://genemania.org/) was used to predict potential mechanisms, pathways, and interacting genes involved in PRCP (8).

STRING (https://string-db.org) was used to construct the protein-protein interaction network of these proteins.

Statistical Analysis

The nonparametric Wilcoxon test was used to compare two groups of non-normally distributed, while the Student's t-test was applied for the analysis of normally distributed data. Statistical analyses were performed using R software version 4.2.2, with significance set at $p < 0.05$.

Enrichment Analysis of PRCP-Associated Genes

We thoroughly examined the expression profiles of PRCP and PSMA4 in the GSE15629 dataset. This investigation revealed a significant downregulation of PRCP gene expression within the disease cohort when employing two distinct grouping methodologies. In contrast, PSMA4 expression demonstrated minimal variability (Additional File 2; Fig S7 A). To further elucidate these observations, arterial aneurysm samples were categorized into two groups based on PRCP gene expression levels, facilitating extensive differential expression analyses. This comprehensive analysis identified 319 downregulated and 236 upregulated genes (Additional File 2; Fig S7 B). Supplementary Table 13 presents the comprehensive gene lists. Subsequent (GO functional enrichment analysis highlighted significant enrichment of differentially expressed genes in pathways associated with epithelial cell differentiation,

humoral immune response, steroid hormone reactions, reactive oxygen species, and endoplasmic reticulum function (Additional File 2; Fig S7 C). Additionally, KEGG enrichment analysis emphasized the involvement of these genes in pivotal signaling pathways, including PPAP, MAPK, PI3K-Akt signal transduction, steroid hormones, and N-glycan biosynthesis (Additional File 2; Fig S7 D). Using the Reactome database for enrichment analysis confirmed the involvement of these genes in signaling pathways, including estrogen-dependent nuclear events downstream of ESR-membrane signaling, retinoid metabolism and transport, and metabolism of fat-soluble vitamins (Additional File 2; Fig S7 E). Subsequently, we utilized the hallmark gene set from the MSigDB for functional Gene Set Enrichment Analysis (fGSEA). This analysis revealed positive enrichment in key signaling pathways such as TNFA-signaling-via-NFκB while demonstrating negative enrichment associated with protein secretion signaling pathways (Additional File 2; Fig S7 F). In summary, these results underscore the significant and multifaceted role of PRCP across a spectrum of immune responses, metabolic processes, and signal transduction pathways."

Correlation of PRCP Expression Levels with Immune and Metabolic-Related Indices

We utilized five prevalent algorithms for immune cell infiltration analysis to examine the correlation between PRCP gene expression and immune cell infiltration. The results indicated a significant positive correlation between PRCP gene expression and scores related to endothelial cells, cDC, and stromal cells, whereas a notable negative correlation was observed with Treg cells, NKT cells, and pericytes (Additional File 2; Fig S8 A). Subsequently, an analysis was performed to assess the correlation between PRCP and immune genes and their respective pathways. The findings demonstrated a positive correlation between the PRCP and immune genes, including CCL19, ACVRL1, IGK, and the TGF-β receptor family pathway. Conversely, a significant negative correlation was observed between immune genes, such as NFKBIZ, NR4A2, FOS, and interleukin pathways (Additional File 2; Fig S8 B). Furthermore, we explored the correlation between PRCP, metabolic genes, and their associated pathways. The results revealed a positive correlation between PRCP and metabolic genes such as EPHX1, MAN1A1, and ASPA and metabolic pathways such as histidine metabolism and valine, leucine, and isoleucine degradation. Conversely, a significant negative correlation was observed between the PRCP and metabolic genes, including PTGS2, ALDOB, MOGAT2, and the Glycosphingolipid biosynthesis - lacto and neolacto series pathways (Additional File 2; Fig S8 C).

Network Modeling

We utilized GeneMania to construct a gene-gene interaction network associated with PRCP (depicted in Additional File 2; FigS9 A), identifying 20 genes closely correlated with PRCP. Subsequently, using the STRING database, we obtained a Protein-Protein Interaction network (depicted in Additional File 2; Fig S9 B). All genes were then subjected to GO analysis, which revealed enrichment in the top 50 pathways for cellular components, biological processes, and molecular functions. Significant pathways included the protein activation cascade, regulation of the acute inflammatory response, and glycosaminoglycan binding, highlighting the association of PRCP with a wide range of protein synthesis, immune, and metabolic pathways (Additional File 2; Fig S9 C). Furthermore, we assessed the differential expression of these genes in the GSE15629 dataset, revealing significant differential expression only in IL6ST (depicted in Additional File 2; Fig S9 D).

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