# Discovering pathways in benign prostate hyperplasia: A functional genomics pilot study

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Received February 24, 2020; Accepted October 13, 2020

DOI: 10.3892/etm.2021.9673

**Abstract.** Benign prostate hyperplasia (BPH) is one of the well-known urological neoplasms common in males with an increasing number of associated deaths in aging males. It causes uncomfortable urinary symptoms, including urine flow blockage, and may cause bladder, urinary tract or kidney problems. The histopathological and clinical knowledge regarding BPH is limited. In the present study, an *in silico* approach was applied that uses genome-scale microarray expression data to discover a wide range of protein-protein interactions in addition to focusing on specific genes responsible for BPH to develop prognostic biomarkers. Various genes that were differentially expressed in BPH were identified. Gene and functional annotation clusters were determined and an interaction analysis with disease phenotypes of BPH was performed, as well as an RNA tissue specificity analysis. Furthermore, a molecular docking study of certain short-listed gene biomarkers, namely anterior gradient 2 (AGR2; PDB ID: 2LNT), steroid 5α-reductase 2 (PDB ID: 6OQX), zinc finger protein 3 (PDB ID: 5T00) and collagen type XII α1 chain (PDB ID: 1U5M), was performed in order to identify alternative Chinese herbal agents for the treatment of BPH. Data from the present study revealed that AGR2 receptor (PDB ID: 2LNT) and berberine (Huang Bo) form the most stable complex and therefore may be assessed in further pharmacological studies for the treatment of BPH.

## Introduction

Benign prostate hyperplasia (BPH), also known as prostate gland enlargement, is a genitourinary condition that is most prevalent in aging males, usually starting at 50-61 years of age (1), and causing lower urinary tract symptoms (LUTS), such as urine flow blockage as a result of the urethra being compressed by the enlarged gland. Other potential complications may include bladder, urinary tract or kidney problems (2). Most males have continued prostate growth throughout their life (2) After the age of 30 years, males exhibit a 1% drop in testosterone production per year and an increase in the level of dihydrotestosterone, possibly due to the age-related increase in 5α reductase (SRD5A2) activity. This rising level of dihydrotestosterone appears to increase prostate cell longevity and proliferation, leading to BPH (3).

BPH is a histological diagnosis that is specified by non-malignant hyperplasia of the stromal and glandular epithelial cells of the prostate, leading to an enlargement in its size (4). Studies and meta-analyses have revealed that BPH is associated with an increased risk of prostate and bladder cancers (5) due to their common pathophysiological driving factors (6). BPH arises mostly from the peripheral zone (70%), followed by growth in the transition zone (20%) and in the central zone (10%) of the gland. The public health burden of BPH is high due to the increased associated morbidity and treatment costs: As many as 33% of males older than 50 years, ~50% of those >60 years, 70% of those >70 years and 90% of those >85 years develop BPH (7). The risk for progressing into a cancerous state is small, but instead, the entire prostate gland grows uniformly, with small smooth, elastic and firm hyperplastic nodules. Common complications include urinary tract infections, bladder stones and chronic kidney problems (8). The symptoms maybe obstructive (weakened urine stream, strained or prolonged voiding, urinary hesitancy) or irritative (pain, nocturia, urge incontinence), or may produce a constant sense of incomplete bladder emptying after micturition that leads to the requirement of frequent urination (9).

The primary risk factors for BPH include age, family history, obesity and being of Afro-Caribbean descent. BPH is most common in western countries and affects >1 billion males all over the world (6). According to GLOBOCAN estimates, 1.2 million novel cases of prostate cancer were reported worldwide in 2018 (10). Diagnostic methods include physical and digital rectal examinations, prostate-specific antigen (PSA) level measurements, prostate biopsy, prostate ultrasound, urinalysis and urine culture (11,12). PSA, a

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*Key words:* benign prostate hyperplasia, gene expression analysis, drug interaction

glycoprotein enzyme produced by the epithelial cells of the prostate gland, is considered the mainstay for BPH prognosis and diagnosis. However, it is difficult to differentiate between the increase in PSA levels due to BPH and prostate cancer. Furthermore, the test fails to discriminate between low-risk and aggressive tumors (13). Major invasive and medical therapies are available for BPH treatment (14). Invasive therapies include microwave thermotherapy, prostate needle ablation, as well as surgical, laser and transurethral therapies; medical therapies include a-adrenergic blockers, 5a-reductase inhibitors, phosphodiesterase type-5 inhibitor therapy for BPH/LUTS and b-3-agonist therapy. Pharmaceutical treatments include finasteride (5-alpha reductase inhibitor) and alpha-1 antagonists. Finasteride shrinks the prostate gland by inhibiting the conversion of testosterone into dihydrotestosterone, resulting in urine flow obstruction relief (15), and alpha-1 antagonists (such as phenoxybenzamine) bind alpha-1 receptors of bladder-neck smooth muscle, causing its relaxation and allowing urine to pass (16).

The discovery of genomic mutations and development of high-throughput screening and microarray technologies have opened up possibilities for identifying gene biomarkers for the diagnosis, prognosis and treatment of BPH (17). Genomic functional networks may help reveal interactions between BPH-associated modules, genitourinary diseases and hyperglycemia, and identify pathway-specific interactions. Furthermore, as only a few drugs (with numerous side effects) are available for treating BPH (18), alternative drugs are required. Histopathology methods for BPH remain incomplete. Differentially expressed genes (DEGs) in BPH and normal prostate tissues are likely to reflect underlying pathogenic mechanisms involved in the development of the disease. Complementary DNA microarray technology may be used to identify genes associated with BPH. The present study focused on a specific set of genes responsible for BPH and performed protein-protein interaction analyses to disclose functional networks. Potential prognostic biomarkers were identified using *in silico* approaches, high-throughput microarray data and comprehensive protein-protein interaction analyses.

The objectives of the present study were to discover genes that are differentially expressed in BPH and normal prostate tissues, identify functional networks and look for potential alternative BPH agents in a list of Chinese herbs.

## Patients and methods

*Datasets*. The gene expression profiles of BPH patients from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) dataset with the accession number GSE6099 were used. For the analysis, seven samples were selected: A total of four human epithelium samples of BPH nodules (EPI_BPH) and three samples from human epithelium of individuals without a history of prostate disease (EPI_NOR). The epithelium is one of the basic types of animal tissue that lines the outer surface of the prostate gland. The pathological evidence for prostate diseases confirmed the neoplastic changes of the prostate epithelium.

*Identification of DEGs*. TheGEO2R web-based tool was used for basic processing, analysis of gene expression datasets and identification of DEGs in BPH. The GEO2R tool uses GEO query (19) and the R limma package (20) from the Bioconductor project (https://www.bioconductor.org/). Fold changes (FCs) were calculated as the ratio of the average expression values of each probe across the samples under normal and BPH conditions. Genes with logFC≥1.0 were considered as upregulated and those with logFC≤-1.0 as downregulated (20,21).

*Gene and functional annotation clustering*. Functional annotation clustering available in the DAVID tool (https://david.ncifcrf.gov/) was performed. Associations among the 'annotation terms' were measured based on their co-association genes in order to cluster similar, redundant and heterogeneous annotation content from the similar or different resources into annotation groups, based on the protocol by Huang *et al* (21) from 2009.

*Protein-protein interaction study*. The GeneMania database (https://genemania.org/) (22) was used to infer experimentally known physical interactions between proteins to predict pathways, protein functions and potential novel therapeutic targets.

*RNA tissue specificity analysis*. For the purpose of RNA tissue-specific analysis, the Human Protein Atlas (HPA) (23) was used. The HPA portal maps all the human proteins in cells, tissues and organs using integrated omics technologies. In addition, the distribution of proteins in the prostate was examined using the Tissue Atlas (one of the three major HPA projects).

*Molecular docking of active components of Chinese herbs to BPH receptor proteins*. A total of 10 Chinese herbs that have been cited as effective for treating BPH were selected (Table I). Using a systematic literature review, the phytochemicals potentially contributing to the effectiveness of these herbs were retrieved and listed in Table I.

*In silico extraction of phytochemical compounds*. The structures of the phytochemical compounds were obtained from various databases, such as ChEMBL (24), PubChem (25) and DrugBank (26).

*Molecular docking*. The structures of the relevant prostate receptor proteins and the phytochemicals selected for the study (cinnamonitride, astragaloside, cornuside, polyporenic acid C, berberine and alisol A monoacetate) were first converted into pdbqt files for docking. AutoDock Vina (27) was used with receptor proteins to perform blind flexible dockings.

*Interaction analysis*. The Protein-Ligand Interaction Profiler (PLIP) (28) was used to establish interactions between the docked complexes.

## Results and Discussion

The differentially expression genes (DEGs) in BPH were identified using the methodology described in the 'Identification of DEGs' section. Out of a variety of existing methods for identifying DEGs from microarray gene expression data, such as the FC (29) or t-test statistics (30), calculation of the log(FC) was

Table I. Phytochemical compounds occurring in Chinese medicinal herbs used to treat benign prostate hyperplasia.

| Chinese herb name | Phytochemical compound |
|---|---|
| *Chaun Shan Jia* | N-butyl tricosylamide, cyclo (L-seryl-L-tyrosyl) |
| *E Shu* | Curdioneandgermacrone |
| *Fu ling* | Polyporenic acid C |
| *Huang Bo* | Berberine |
| *Huang Qi* | Astragalosides |
| *Rou Gui* | Cinnamonitride |
| *Shan Zhu Yu* | Cornuside |
| *Shu Di Huang* | *Rehmania* polysaccharide, Jionoside |
| *Wang Bu Liu Xing* | Triterpene saponins |
| *Ze Xie* | Alisol A monoacetate |

chosen as one of the simplest ad-hoc methods for microarray analyses. The FC describes the change in expression of a gene between two observed samples, i.e., between normal and BPH tissues (31).

After selecting the gene expression datasets and evaluating them for differential expression analysis, a boxplot of the BPH and the normal samples was generated (Fig. 1). It was observed that the median of the two sample types (i.e., BPH vs. normal) was close to zero. However, there were significant variations in terms of their minimum, first quartile, third quartile and maximum values between BPH and normal groups, with BPH samples GSM141335 and GSM141337 showing lower values compared with those in normal samples GSM141338 and GSM141339 (Fig. 1).

The logFC statistics for the entire genome were computed. The logFC values of all the genes are presented in a scatter plot in Fig. 2. Genes with a logFC ≥1.0 were considered to be upregulated and those with logFC ≤-1.0 were considered to be downregulated in BPH tissues (32,33). Following conventional rules, a threshold of a two-fold change in gene expression (i.e., -1.0 ≤logFC ≥1.0), and P≤0.05 (5% significance level) were used to short-list DEGs in BPH. Table II lists the identified DEGs with their P-values and logFC values. Among the highest ranking identified DEGs are Zinc finger proteins (ZNF3; P<0.0001, logFC=3.0111), Acyl-CoA synthetase family member 3 (ACSF3; P<0.0001, logFC=1.5768), Fibrinogen like-1 (FGL1; P=0.0001, logFC=-1.4845), PMS1 homolog 1, mismatch repair system component (PMS1; P=0.0001, logFC=-1.4611), Forkhead box P2 (FOXP2; P<0.0001, logFC=-1.3491), anterior gradient 2 (AGR2; P<0.0001, logFC=-1.3156) andRing finger protein 135 (RNF135; P=0.0001, logFC=1.2748). In addition, profile graphs of identified DEGs were plotted to obtain a graph of DEG expression across the different samples, as presented in Fig. 3. The profile graphs point to differential expression behaviours in the BPH and normal prostate samples.

The list of identified DEGs was validated against the published literature in order to find evidence for their involvement in BPH or other prostatic conditions. It was revealed that, for instance, the association of the genes

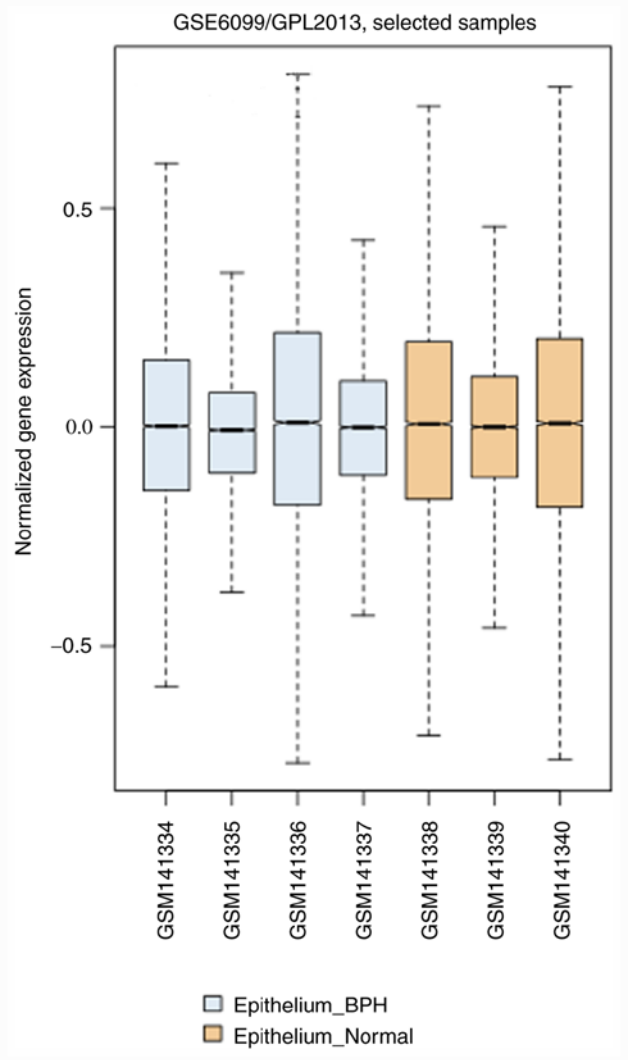


Figure 1. Boxplot of gene expression between BPH samples and normal samples. The x-axis represents samples, whilst the y-axis represents the normalized gene expression of a sample. All the samples show fairly consistent medians across all the samples, however, a smaller interquartile range is observed in BPH samples (GSM141335 and GSM141337) compared to all the three normal samples. BPH, benign prostate hyperplasia.

ADAM metallopeptidase with thrombospondin type 1 motif 1 (ADAMTS1) (34), folate hydrolase 1 (FOLH1) or Prostate-specific membrane antigen (35) and insulin like growth factor binding protein 5 (IGFBP5) (36) with BPH was listed in the DisGeNET database (37). Modified ADAMTS1 expression results in markedly changed blood vessel morphology and altered thrombospondin-1(TSP1) levels in tumors. Loss of ADAMTS1 is associated with small-diameter vessels that are consistent with more aggressive prostate tumors (38). These results suggest that ADAMTS1 is an important regulatory factor of tumor growth and angiogenesis during prostate cancer progression. According to the Human Protein Atlas (https://www.proteinatlas.org/), ADAMTS1RNA expression is significantly enhanced in prostate tissue. The DisGeNET database reports that PMS1 is a biomarker of malignant prostate neoplasms (39,40), and anterior gradient 2(AGR2) is highly associated with prostate neoplasms (41-44) and prostate carcinoma (44). Zinc finger proteins (ZNF) ZNF91 (45), ZFX (46), ZNF185 (47), ZNF132 (48) and myc

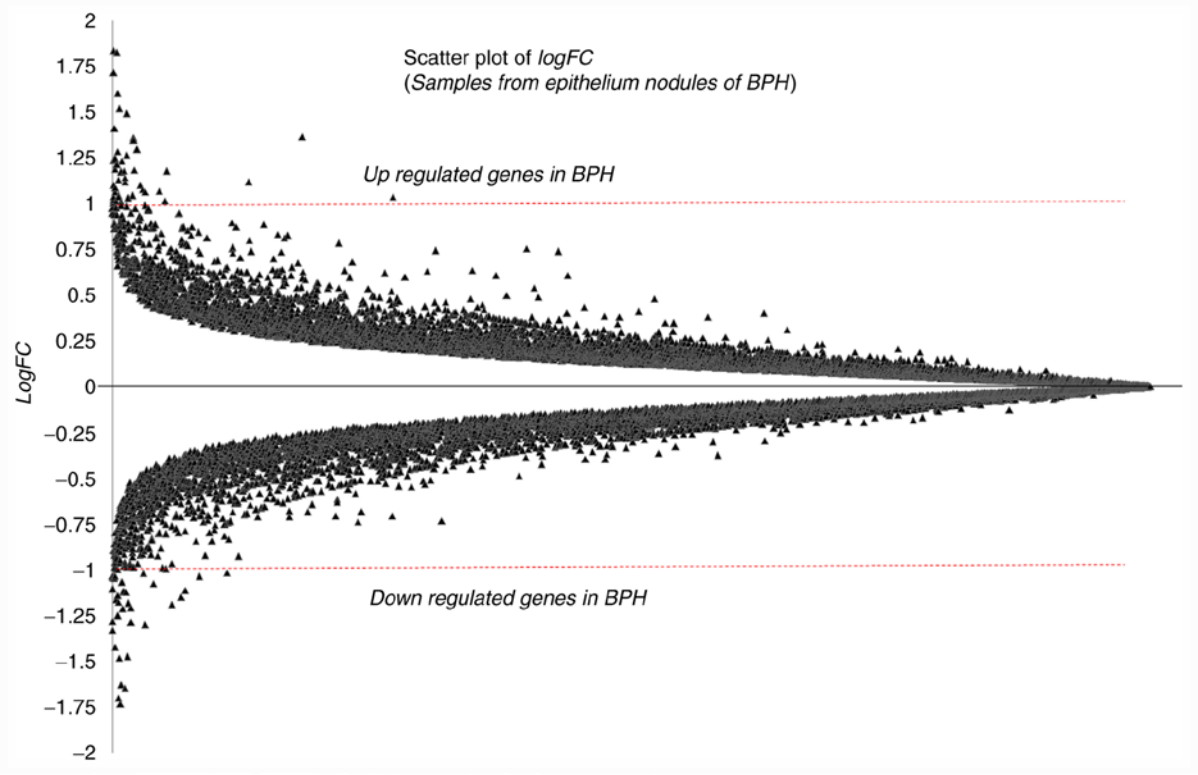


Figure 2. Scatter plot showing the logFC of differentially expression genes in BPH. The x-axis represents genes whereas the y-axis represents the logFC values. BPH, benign prostate hyperplasia; FC, fold change.

associated zinc finger protein (49), as the family of ZNF3, have been associated with prostate pathology, prostate cancer progression and prostate cancer pathogenesis. The association of zinc finger proteins with BPH and prostate cancer was reviewed in Rahman (50) in 2016. Fibrinogen like-1 (FGL1) has been associated with prostate cancer and high-grade prostatic intraepithelial neoplasia (HGPIN) (51). Collagen type XII α1 chain (COL12A1) is upregulated in BPH (52-54). Similarly, other identified DEGs are involved in different prostate diseases. One of the key biomarkers involved in BPH, but not in prostate cancer, is Steroid 5α-reductase 2 (SRD5A 2) (55-58). It was not identified in the differential expression analysis of the present study, but it was considered for drug interaction studies.

Another gene identified in this analysis was FOLH1, also known as prostate-specific membrane antigen. It encodes a type II transmembrane glycoprotein expressed in a number of tissues, including the prostate. In the prostate, FOLH1 is upregulated in cancerous cells, has been used as a diagnostic and prognostic marker for prostate cancer (59) and was also proposed as a possible marker for neurological disorders such as Alzheimer's and Huntington's disease (60). According to GeneCards, the Human Gene Database (https://www.genecards.org), FOLH1 is involved in prostate tumor progression (61,62). Finally, insulin-like growth factor binding proteins (IGFBPs) exhibit abnormalities in prostatic stromal cells in BPH (36,63).

*Gene and functional annotation clustering.* Clustered annotations make the functional analyses more clear and focused. Clustering algorithms rely on the hypothesis that similar annotations should have similar gene members (64). The functional annotation clustering of the present study used Kappa statistics to estimate the degree of the common genes between two annotations, and fuzzy heuristic clustering was used to classify the groups of similar annotations based on kappa values. Hence, common gene annotations have a high chance of being grouped together. This eases the biological analysis and interpretation at the group level.

After gene clustering and functional annotation clustering, only two gene clusters were obtained with the 'lowest' classification stringency. The first cluster contained five genes, namely bromo domain adjacent to zinc finger domain 2B, TEA domain transcription factor 1 (TEAD1), erythroid differentiation regulatory factor 1, ZNF3 and forkhead box P2with an enrichment score of 0.68 and the second cluster contained only three genes, namelyβ-secretase 2, ADAMTS1 and FOLH1 with an enrichment score of 0.47. Fig. 4 presents a 2D view of clustered genes with their associated gene terms.

For the functional annotation clustering analysis, a 'high' classification stringency was selected and 4 annotation clusters were obtained, as presented in Table III and Fig. 5. Out of the 4 clusters (Table III and Fig. 5), the first 3 were significantly enriched (enrichment scores of 0.95, 0.70 and 0.52). The P-values of functional Gene Ontology terms of these 3 significantly enriched clusters are also reasonably acceptable. Each of the four terms within cluster 1 was associated with both overlapping as well as differing genes (Fig. 5B). The terms of clusters 2 and 4 were only associated with overlapping genes.

*Protein interaction analysis.* Protein-protein interactions (PPIs) have a crucial role in cells and control essential

Table II. List of differentially expressed genes in BPH and predicted scores.

| ID | P-value | T | logFC | Gene symbol | Gene name | Gene function | Uniprot ID |
|---|---|---|---|---|---|---|---|
| Hs6-3-3-1 | <0.0001 | 11.0053 | 3.0111 | ZNF3 | Zinc finger protein 3 | Involved in cell differentiation and/or proliferation. | https://www.uniprot.org/uniprot/P17036 |
| Hs6-11-20-9 | <0.0001 | 7.0481 | 1.5768 | ACSF3 | Acyl-CoA synthetase family member 3 | Catalyzes the initial reaction in intramitochondrial fatty acid synthesis into respective CoA thioester, by activating malonate and methylmalonate. | https://www.uniprot.org/uniprot/Q4G176 |
| Hs6-30-1-12 | 0.0001 | -6.6404 | -1.4845 | FGL1 | Fibrinogen like 1 | Upregulated in various cancers, including lung cancer, prostate cancer, melanoma and colorectal cancer. | https://www.uniprot.org/uniprot/Q08830 |
| Hs6-32-17-24 | 0.0001 | -6.2384 | -1.4611 | PMS1 | PMS1 homolog 1, mismatch repair system component | Repair of DNA mismatch. | https://www.uniprot.org/uniprot/P54277 |
| Hs6-18-1-13 | <0.0001 | -6.4221 | -1.3491 | FOXP2 | Forkhead box P2 | A transcriptional repressor that participate in the specification and differentiation of the lung epithelium, development of neural, gastrointestinal and cardiovascular tissues. | https://www.uniprot.org/uniprot/O15409 |
| Hs6-13-2-18 | <0.0001 | -7.5867 | -1.3156 | AGR2 | Anterior gradient 2, protein disulphide isomerase family member | Catalyze protein folding and thiol-disulfide interchange reactions. | https://www.uniprot.org/uniprot/O95994 |
| Hs6-11-16-9 | 0.0001 | 5.9628 | 1.2748 | RNF135 | Ring finger protein 135 | Acts as an E2-dependent E3 ubiquitin-protein ligase, involved in innate immune defense against viruses. | https://www.uniprot.org/uniprot/Q8IUD6 |
| Hs6-20-10-21 | 0.0249 | 2.5918 | 1.2741 | HECTD4 | HECT domain E3 ubiquitinprotein ligase 4 | Transfers the ubiquitin to targeted substrates directly. | https://www.uniprot.org/uniprot/Q9Y4D8 |
| Hs6-31-19-17 | 0.0001 | -6.0425 | -1.2109 | BAZ2B | Bromodomain adjacent to zinc finger domain 2B | May play a role in transcriptional regulation interacting with imitation SWI. | https://www.uniprot.org/uniprot/Q9UIF8 |
| Hs6-8-2-2 | 0.0018 | 3.8935 | 1.1828 | NCAPD3 | Non-smooth muscle cellcondensin II complexsubunit D3 | Mitotic chromosome assembly and segregation. | https://www.uniprot.org/uniprot/P42695 |
| Hs6-11-17-23 | 0.0112 | -2.9520 | -1.1392 | PDK4 | Pyruvate dehydrogenase kinase 4 | A member of the PDK protein kinase family that encodes a mitochondrial protein with a histidine kinase domain. | https://www.uniprot.org/uniprot/Q16654 |

Table II. Continued.

| ID | P-value | T | logFC | Gene symbol | Gene name | Gene function | Uniprot ID |
|---|---|---|---|---|---|---|---|
| Hs6-23-18-13 | 0.0087 | -3.0793 | -1.1176 | ADAMTS1 | ADAM metallopeptidase with thrombospondin type 1 motif 1 | Angiogenic inhibitor activity; has a critical role in follicular rupture; associated with various inflammatory processes and development of cancer cachexia. | https://www.uniprot.org/uniprot/Q9UHI8 |
| Hs6-20-19-25 | 0.0040 | 3.9720 | 1.0868 | CDC45 | Cell division cycle 45 | Required for initiation of chromosomal DNA replication. | https://www.uniprot.org/uniprot/O75419 |
| Hs6-19-25-7 | 0.0024 | -3.7587 | -1.0450 | FOLH1 | Folate hydrolase (prostate-specific membrane antigen) 1 | Folate hydrolase and N-acetylated-alpha-linked-acidic dipeptidase activity; associated with prostate tumor progression. | https://www.uniprot.org/uniprot/Q04609 |
| Hs6-28-5-13 | 0.0014 | -4.5094 | -1.0398 | CXXC4 | CXXC finger protein 4 | Represses Wnt signaling pathway via interaction with DVL1; binds to DNA containing CpG dinucleotides over CpH (H=A, T, and C), hemimethylated-CpG and hemimethylated-hydroxymethyl-CpG. | https://www.uniprot.org/uniprot/Q9H2H0 |
| Hs6-10-24-14 | 0.0012 | 4.6071 | 1.0299 | KCTD13 | Potassium channel tetramerization domain containing 13 | Substrate-specific adapter of a BCR E3 ubiquitin-protein ligase complex required for synaptic transmission; mediates the ubiquitination of RhoA, leading to its degradation by the proteasome. | https://www.uniprot.org/uniprot/Q8WZ19 |
| Hs6-15-23-4 | 0.0049 | -3.3798 | -1.0297 | ARHGAP42 | Rho GTPase activating protein 42 | May influence blood pressure by functioning as a GTPase-activating protein for RhoAin vascular smooth muscle. | https://www.uniprot.org/uniprot/A6NI28 |
| Hs6-11-11-1 | 0.0052 | -3.3483 | -1.0290 | TEAD1 | TEA domain transcription factor 1 | Plays a key role in the Hippo signaling pathway, a pathway involved in organ size control, and tumor suppression by restricting proliferation and promoting apoptosis; cardiac development; binds to the M-CAT motif. | https://www.uniprot.org/uniprot/P28347 |
| Hs6-28-7-19 | 0.0015 | 4.0068 | 1.0283 | COL12A1 | Collagen type XII alpha 1 chain | Interacts with type I collagen-containing fibrils, the COL1 domain could be associated with the surface of the fibrils, and the COL2 and NC3 domains may be localized in perifibrillar matrix. | https://www.uniprot.org/uniprot/Q99715 |
| Hs6-16-23-19 | 0.0014 | -4.5432 | -1.0194 | EDRF1 | Erythroid differentiation regulatory factor 1 | A transcription factor involved in erythroid differentiation; transcriptional activation of the globin gene. | https://www.uniprot.org/uniprot/Q3B7T1 |

Table II. Continued.

| ID | P-value | T | logFC | Gene symbol | Gene name | Gene function | Uniprot ID |
|---|---|---|---|---|---|---|---|
| Hs6-32-13-1 | 0.0003 | 4.7861 | 1.0155 | IGFBP5 | Insulin-like growth factor binding protein 5 | IGF-binding proteins prolong the half-life of the IGFs and either inhibit or stimulate the growth-promoting effects of the IGFs in cell culture; alter the interaction of IGFs with their cell surface receptors. | https://www.uniprot.org/uniprot/P24593 |
| Hs6-20-10-20 | 0.0014 | -4.5268 | -1.0133 | BACE2 | Beta-site APP-cleaving enzyme 2 | Proteolytic processing of the APP; cleaves APP, between residues 690 and 691, leading to the generation and extracellular release of beta-cleaved soluble APP, and a corresponding cell-associated C-terminal fragment. | https://www.uniprot.org/uniprot/Q9Y5Z0 |
| Hs6-27-5-1 | 0.0298 | -2.4615 | -1.0002 | TF | Transferrin | Iron binding transport proteins, bind two $Fe^{3+}$ ions in association with the binding of an anion, usually bicarbonate; responsible for the transport of iron from sites of absorption and heme degradation to those of storage and utilization; stimulating. cell proliferation. | https://www.uniprot.org/uniprot/P02787 |

FC, fold change; APP, amyloid precursor protein; BCR, BTB-CUL3-RBX1; DVL-1, Dishevelled-1.

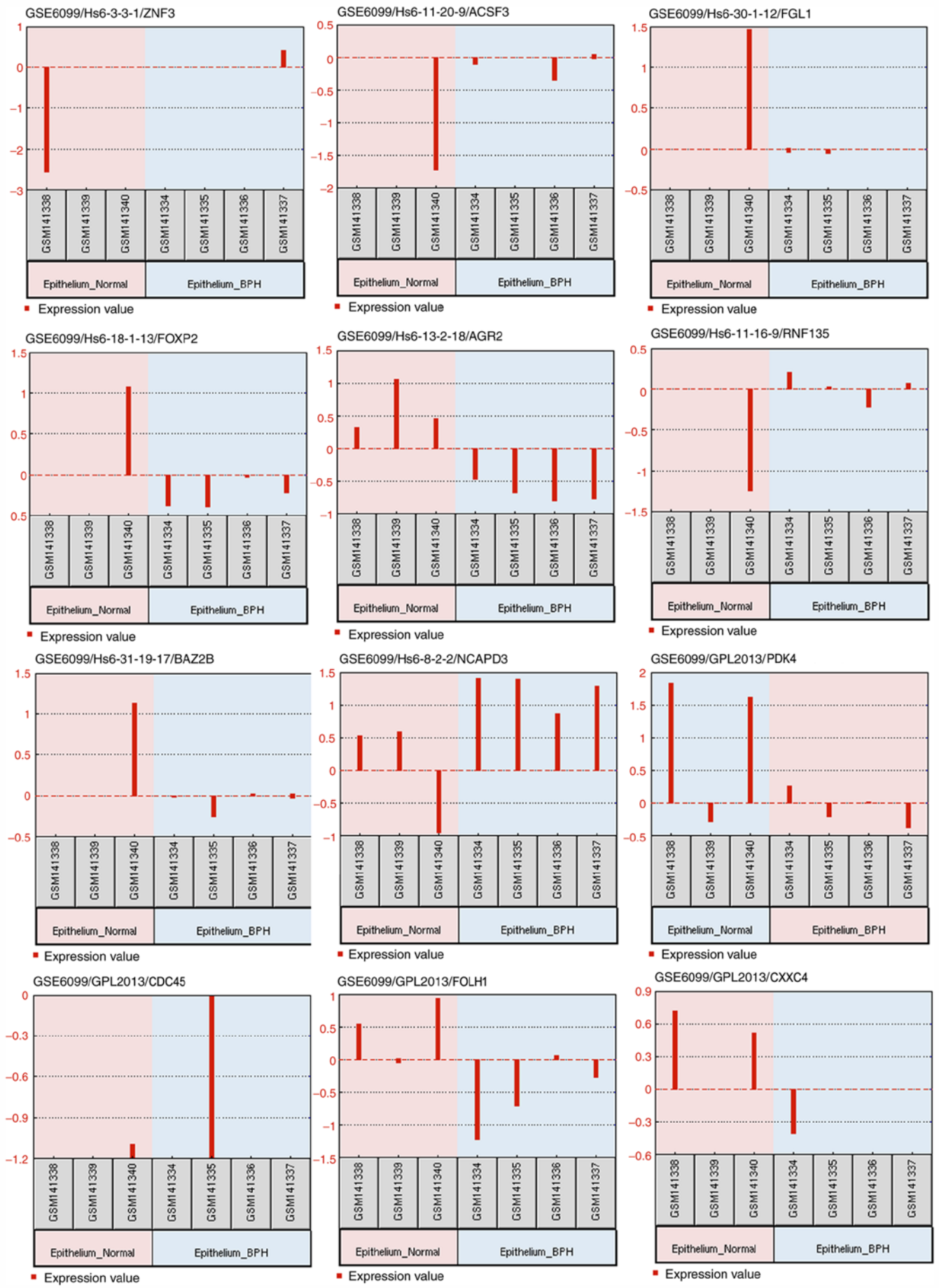


Figure 3. Continued.

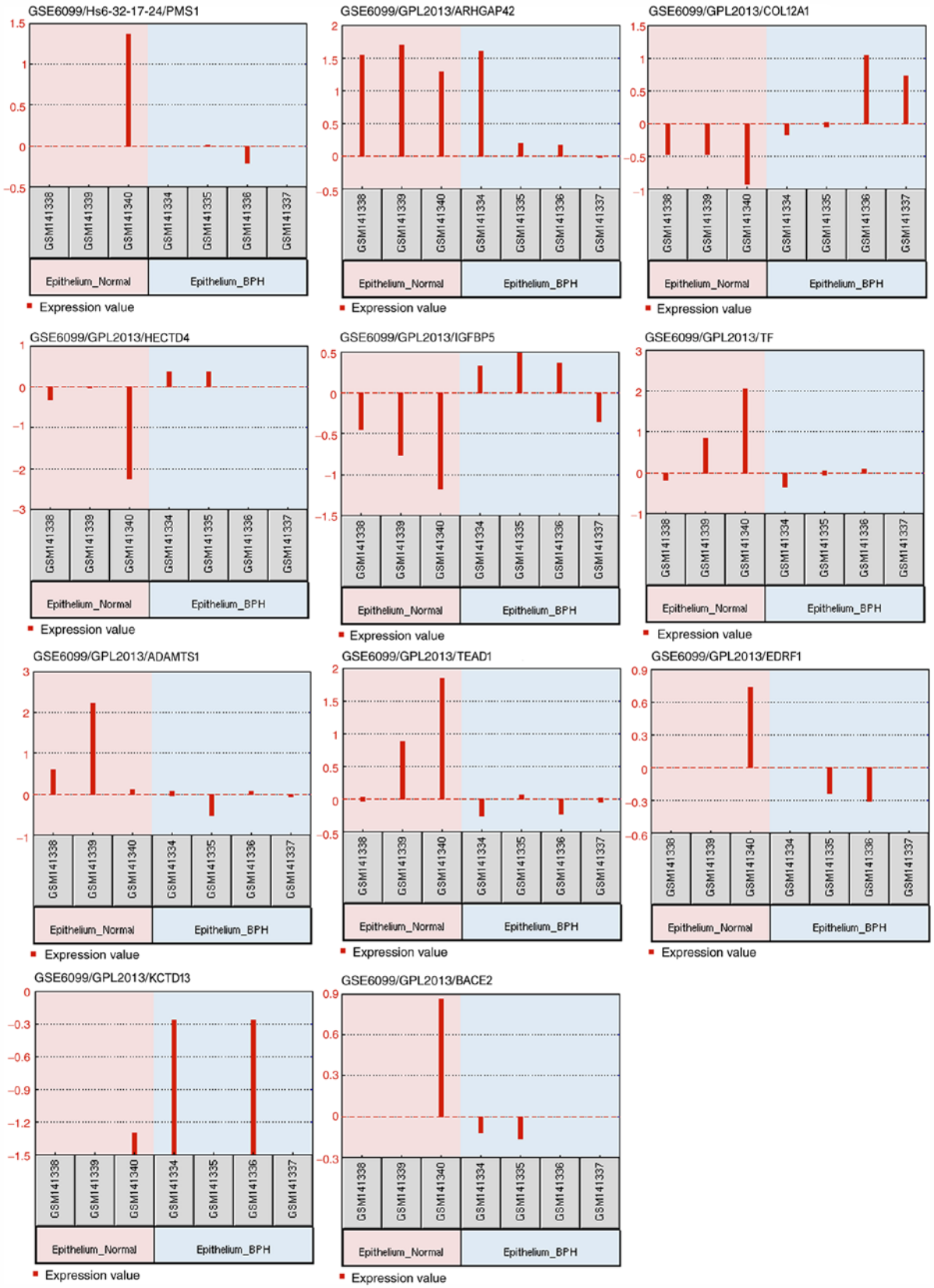


Figure 3. Profile graphs of differentially expressed genes, namely ZNF3, ACSF3, FGL1, FOXP2, AGR2, RNF135, BAZ2B, NCAPD3, PDK4, CDC45, FOLH1, CXXC4, PMS1, ARHGAP42, COL12A1, HECTD4, IGFBP5, TF, ADAMTS1, TEAD1, EDRF1, KCTD13 and BACE2. BPH, benign prostate hyperplasia; ZNF3, Zinc finger protein 3; ACSF3, Acyl-CoA synthetase family member 3; FGL1, Fibrinogen like 1; FOXP2, Forkhead box P2; AGR2, Anterior gradient 2; RNF135, Ring finger protein 135; BAZ2B, Bromodomain adjacent to zinc finger domain 2B; NCAPD3, Non-smooth muscle cell condensin II complex subunit D3; PDK4, Pyruvate dehydrogenase kinase 4; CDC45, Cell division cycle 45; FOLH1, Folate hydrolase (prostate-specific membrane antigen) 1; CXXC4, CXXC finger protein 4; PMS1, PMS1 homolog 1, mismatch repair system component; ARHGAP42, Rho GTPase activating protein 42; COL12A1, collagen type XII α1 chain; HECTD4, HECT domain E3 ubiquitin protein ligase 4; IGFBP5, Insulin-like growth factor binding protein 5; TF, Transferrin; ADAMTS1, ADAM metallopeptidase with thrombospondin type 1 motif 1; TEAD1, TEA domain transcription factor 1; EDRF1, Erythroid differentiation regulatory factor 1; KCTD13, Potassium channel tetramerization domain containing 13; BACE2, Beta-site APP-cleaving enzyme 2.

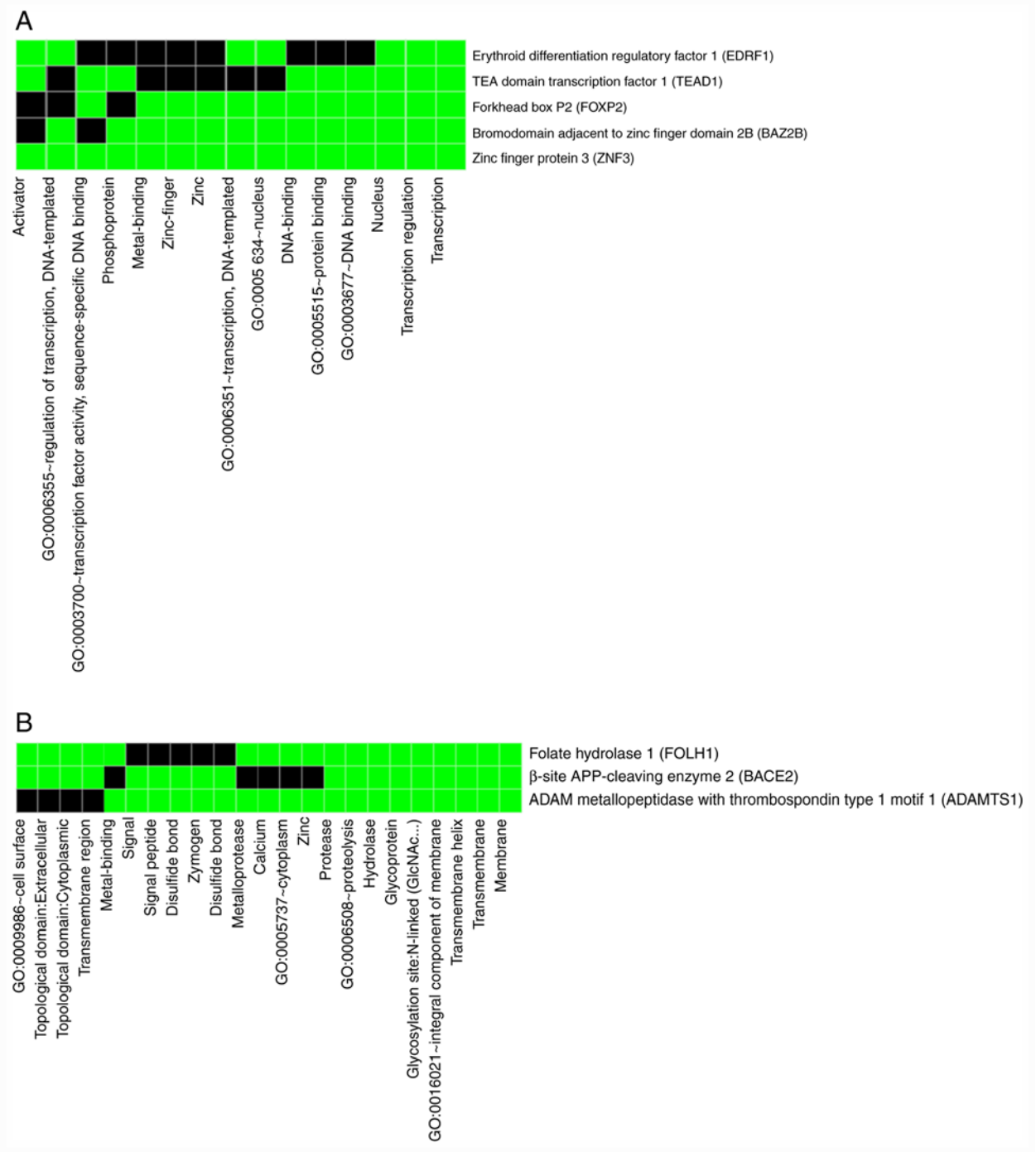


Figure 4. Two-dimensional view of clustered genes with their associated GO gene-terms. (A) Gene cluster 1; (B) gene cluster 2. GO, gene ontology.

cellular and biological processes. Any disease-causing mutations affecting PPIs may lead to disruption of protein-DNA interactions, protein misfolding and new undesirable interactions (65). A better understanding of possible PPIs allows for the prediction of pathways, protein functions and potential novel therapeutic targets. In the present study, gene interactions were predicted using the GeneMania tool with customized gene-gene interaction parameters, such as physical interactions and gene co-expression interactions only, as presented in Fig. 6. The physical interactions are depicted in pink, while co-expression interactions are displayed in purple. The maximum resultant genes and maximum resultant attributes were set to default, i.e., 20 and 10, respectively. The genes that do not interact with any other genes under these parameter settings were removed from the network (Fig. 6). This way, 3 different interaction networks were obtained (Fig. 6). The size of the nodes represents the gene score, i.e., the degree with which GeneMania predicted the gene-gene association. Similarly, the thickness of the edges represents the strength of the interaction. The topological analysis of gene-gene interactions suggested that the genes IGFBP5, TEAD1 and transferrin are hub genes that have direct or indirect interactions with other DEGs identified. Most of these interactions are physical interactions with good strengths (pink color). This suggests that these DEGs may be used for the rapeutic strategies and as drug targets (66,67).

Table III. Results of functional annotation clustering analysis.

A, Annotation cluster 1 (enrichment score: 0.95)

| Category | Term | Count | P-value | Genes | Benjamini | FDR |
|---|---|---|---|---|---|---|
| UP_KEYWORDS | Secreted | 6 | 0.0526 | TF, COL12A1, ADAMTS1, FGL1, AGR2, IGFBP5 | 0.9020 | 44.1446 |
| UP_KEYWORDS | Signal | 8 | 0.1386 | TF, CDC45, BACE2, COL12A1, ADAMTS1, FGL1, AGR2, IGFBP5 | 0.8822 | 79.9935 |
| UP_SEQ_FEATURE | Signal peptide | 7 | 0.1472 | TF, BACE2, COL12A1, ADAMTS1, FGL1, AGR2, IGFBP5 | 1.0000 | 85.0691 |
| UP_KEYWORDS | Disulfide bond | 7 | 0.1475 | TF, BACE2, COL12A1, ADAMTS1, FGL1, AGR2, IGFBP5 | 0.8592 | 82.1005 |

B, Annotation cluster 2 (enrichment score: 0.70)

| Category | Term | Count | P-value | Genes | Benjamini | FDR |
|---|---|---|---|---|---|---|
| UP_KEYWORDS | Protease | 3 | 0.1139 | FOLH1, BACE2, ADAMTS1 | 0.9257 | 72.8568 |
| GOTERM_BP_DIRECT | GO:0006508~ proteolysis | 3 | 0.1283 | FOLH1, BACE2, ADAMTS1 | 0.9987 | 80.3993 |
| UP_KEYWORDS | Hydrolase | 3 | 0.5431 | FOLH1, BACE2, ADAMTS1 | 0.9810 | 99.9785 |

C, Annotation cluster 3 (enrichment score: 0.52)

| Category | Term | Count | P-value | Genes | Benjamini | FDR |
|---|---|---|---|---|---|---|
| UP_KEYWORDS | Transcription regulation | 5 | 0.2338 | EDRF1, TEAD1, BAZ2B, ZNF3, FOXP2 | 0.8282 | 94.3394 |
| UP_KEYWORDS | Transcription | 5 | 0.2496 | EDRF1, TEAD1, BAZ2B, ZNF3, FOXP2 | 0.8287 | 95.4801 |
| GOTERM_BP_DIRECT | GO:0006351~ transcription, DNA-templated | 4 | 0.4491 | EDRF1, BAZ2B, ZNF3, FOXP2 | 1.0000 | 99.9156 |

D, Annotation cluster 4 (enrichment score: 0.01)

| Category | Term | Count | P-value | Genes | Benjamini | FDR |
|---|---|---|---|---|---|---|
| GOTERM_CC_DIRECT | GO:0016021~ integral component of membrane | 4 | 0.9615 | FOLH1, BACE2, HECTD4, ADAMTS1 | 1.0000 | 100 |
| UP_KEYWORDS | Transmembrane helix | 4 | 0.9631 | FOLH1, BACE2, HECTD4, ADAMTS1 | 1.0000 | 100 |
| UP_KEYWORDS | Transmembrane | 4 | 0.9637 | FOLH1, BACE2, HECTD4, ADAMTS1 | 1.0000 | 100 |
| UP_KEYWORDS | Membrane | 4 | 0.9958 | FOLH1, BACE2, HECTD4, ADAMTS1 | 1.0000 | 100 |

FDR, false discovery rate; GO, gene ontology; BP, biological process; CC, cellular component.

*RNA tissue specificity analysis*. Genes have unique expression patterns that are broadly classified as tissue-specific or housekeeping. In a multicellular organism, knowledge of the tissue-specificity of a gene contributes to a better

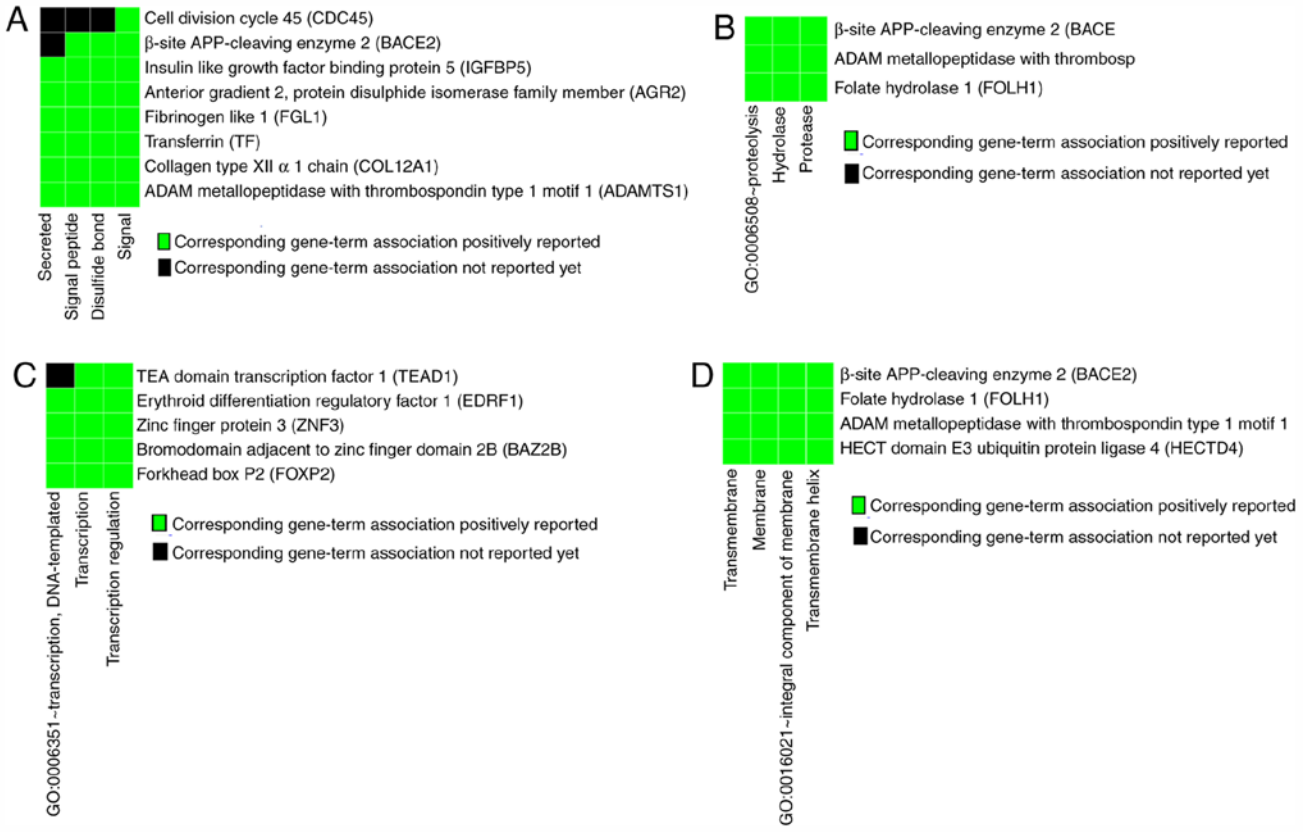


Figure 5. Functional annotation clustering analysis performed using the DAVID tool. (A) Annotation cluster 1; (B) annotation cluster 2; (C) annotation cluster 3; (D) annotation cluster 4. GO, gene ontology.

understanding of its function (68). In the present study, tissue specificity was measured by counting the number of tissues each gene was expressed in.

Both disease and trait phenotypes are under dynamic tissue-specific regulation. The major purpose of performing RNA tissue specificity analyses was to better understand how the expression of genes and its regulatory processes may be affected by disease or other biological factors. The ADAMTS1 gene is expressed in numerous tissues, including the ovary, adipose tissue, gallbladder and placenta. Fig. 7 presents the results of the tissue specificity analysis of certain DEGs in BPH identified in the present study. It was observed that the prostate tissue specificity score of all of the DEGs identified was low (normalized expression, <25), and the differential expression of these genes in BPH samples may be due to genetic variations leading to BPH.

*Molecular docking and interaction analysis*

*Extraction of phytochemical compounds*. In the present study, only six compounds (namely cinnamonitride, astragaloside, cornuside, polyporenic acid C, berberine and alisol A monoacetate) were considered, since they have been used for the treatment of BPH and its phytochemical compound structures are available.

*Docking*. By docking ligands (phytochemical compounds) to receptor proteins, it was indicated that polyporenic acid C and alisol A were notable to bind to the proteins under any of the tested conditions. Dockings were possible only between the prostatic receptors and cinnamonitride, astragaloside, berberine and cornuside. Fig. 8 presents the best binding positions for each receptor-ligand complex docking.

*Interaction analysis*. After comparing the dockings, it was revealed that berberine had the higher binding affinity for BPH target receptors. Therefore, the best-bound complexes with berberine were selected and subjected to a PLIP interaction analysis. Fig. 9 demonstrates interactions formed between berberine and the selected target receptors, including hydrophobic interactions, hydrogen bonds and salt bridges. Out of the four complexes subjected to the interaction analysis, the interactions formed between AGR2 (protein databank ID, 2LNT) and berberine were the most stable due to a balance between the number of hydrogen and hydrophobic bonds. The complex between berberine and AGR2 had 5 hydrogen bonds stabilizing the complex (the more hydrogen bonds in a complex, the more stable the complex).

*Conclusions*. In the present study, genes with differential expression between BPH and normal prostatic tissues were discovered and interaction analyses associated with BPH phenotypes were performed. A general framework for mapping complex interactions from genome-wide genotype data was established and interactions with Chinese herbal drugs were identified.

The recent discovery of novel genomic mutations and the availability of high-throughput screening and microarray technologies have facilitated the uncovering of gene

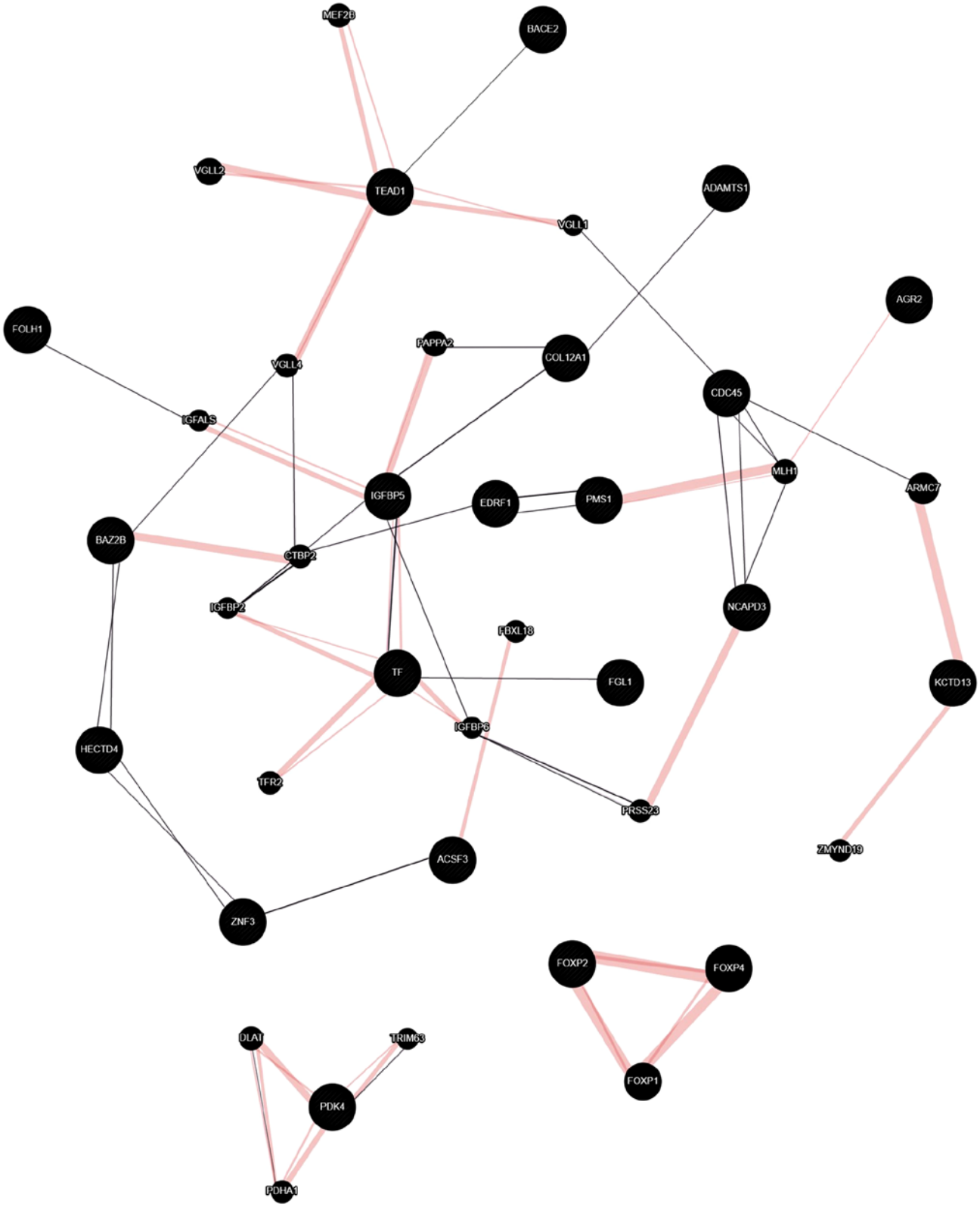


Figure 6. Map of interactions among genes. Gene-gene physical interactions (pink edges) and gene co-expression interactions only (black edges). The larger size nodes in the interaction network represent the differentially expressed genes identified in this study, whilst smaller nodes represent other interacting genes.

biomarkers for the diagnosis, prognosis and treatment of various diseases, such as BPH (31-33). With the help of *in silico* approaches, high-throughput microarray data were analyzed to identify DEGs as biomarkers for BPH; furthermore, PPI, gene clustering and tissue specificity analyses were performed to associate their expression to BPH phenotypes. In addition, molecular docking studies of certain short-listed gene biomarkers [AGR2 (2LNT), SRD5A2 (6OQX), ZNF3 (5T00) and COL12A1 (1U5M)] were performed to identify alternative Chinese herbal drugs for the treatment of BPH. The

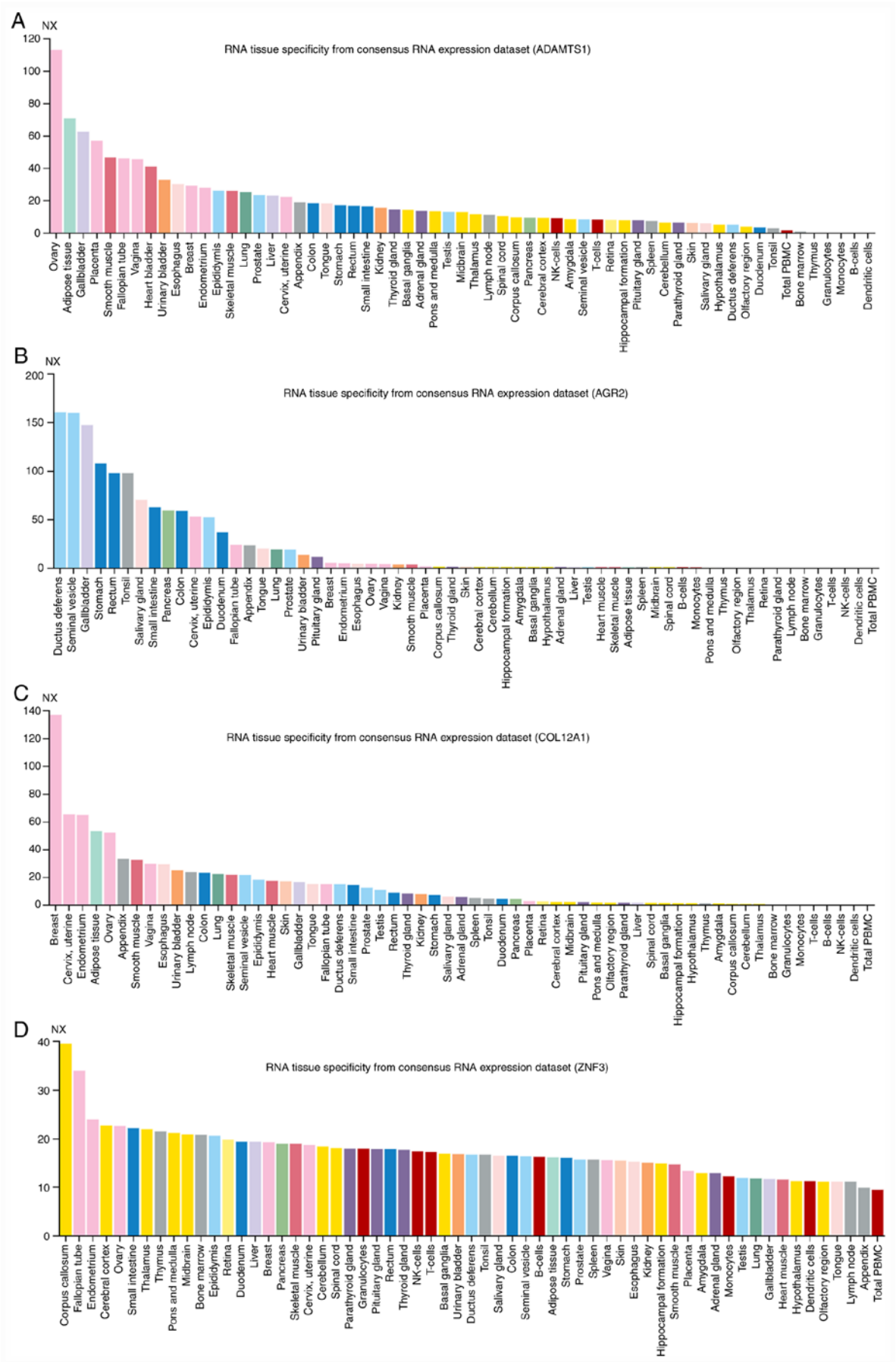


Figure 7. RNA tissue specificity analysis using the Tissue Atlas of the Human Protein Atlas. (A) ADAMTS1, (B) AGR2, (C) COL12A1 and (D) ZNF3. PBMC, peripheral blood mononuclear cells; NX, normalized expression which is computed using Trimmed Mean of M-values methods within the Human Protein Atlas; ADAMTS1, ADAM metallopeptidase with thrombospondin type 1 motif 1; AGR2, anterior gradient 2; COL12A1, collagen type XII α1 chain; ZNF3, Zinc finger protein 3.

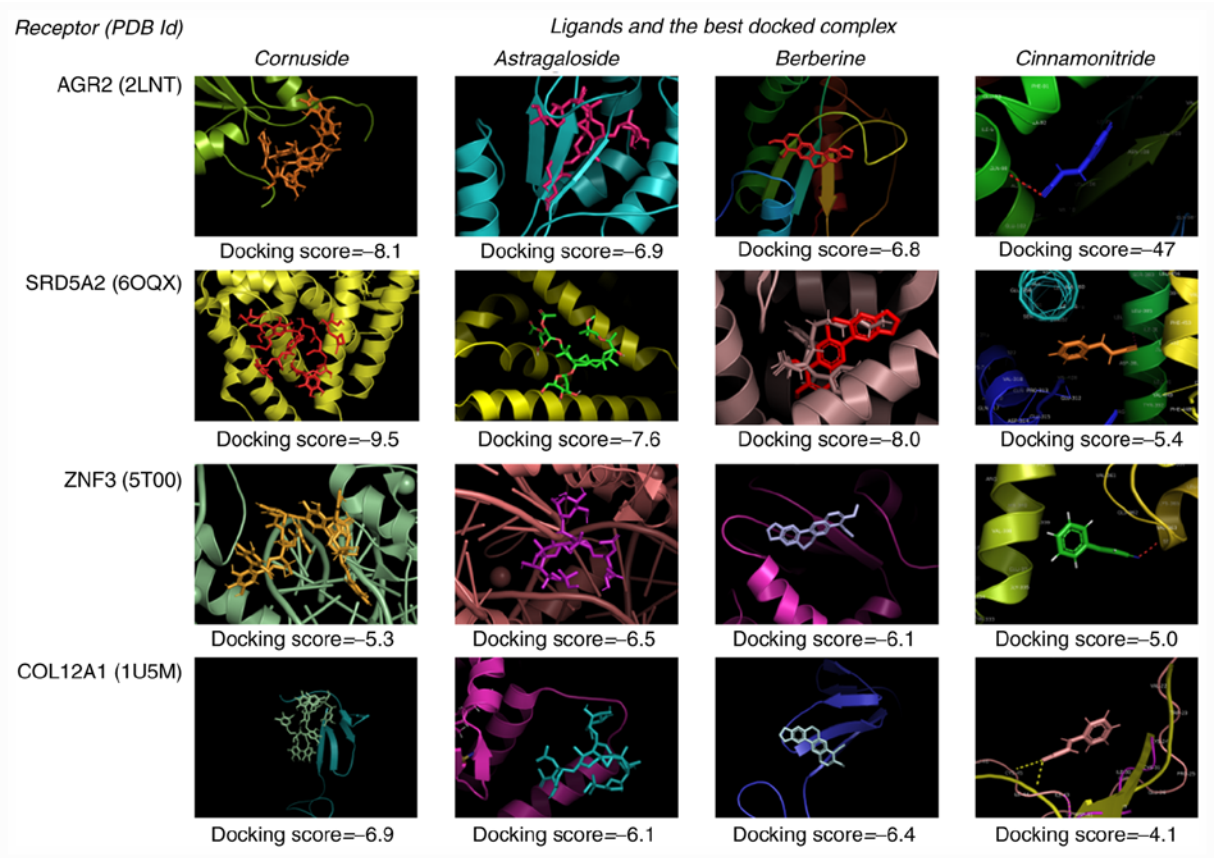


Figure 8. Best docked complexes with docking scores of receptors. AGR2 (2LNT), SRD5A2 (6OQX), ZNF3 (5T00), and COL12A1 (1U5M). PDB, protein databank; SRD5A, 5α reductase; AGR2, anterior gradient 2; COL12A1, collagen type XII α1 chain; ZNF3, Zinc finger protein 3.

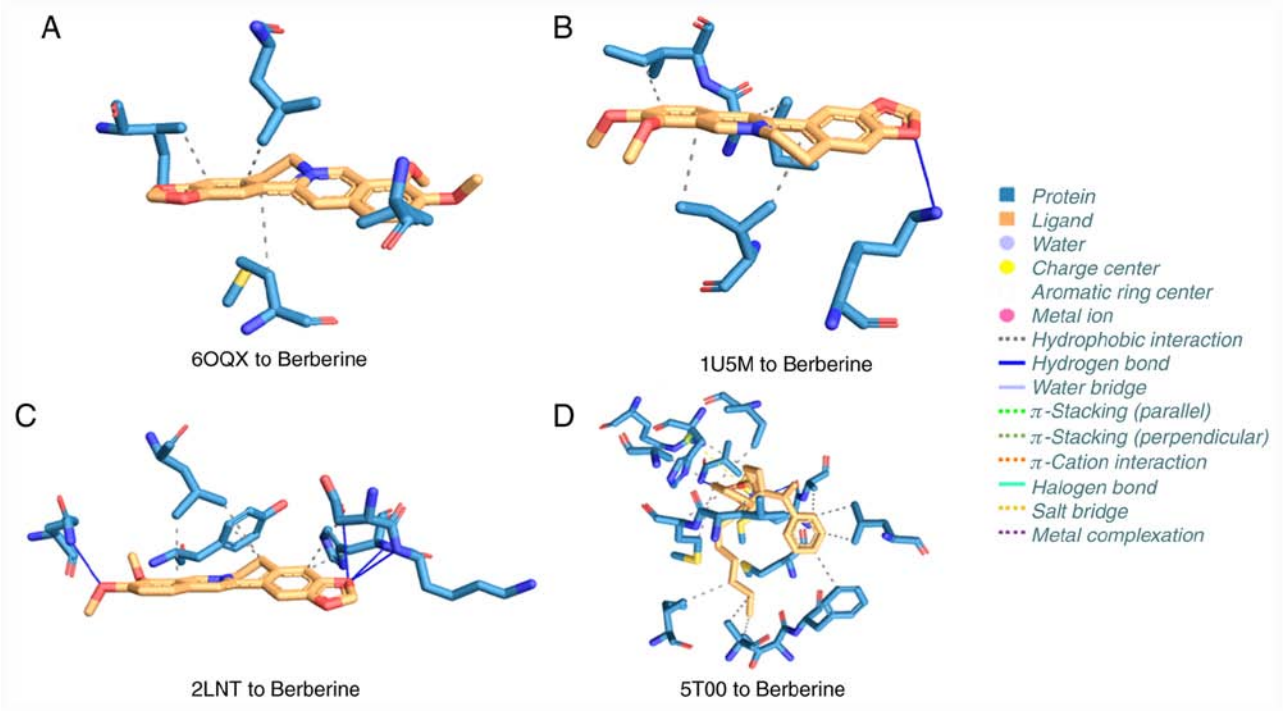


Figure 9. Interactions between berberine and the selected target receptor proteins. (A) 6OQX to Berberine, (B) 1U5M to Berberine, (C) 2LNT to Berberine, (D) 5T00 to Berberine.

results suggest that the AGR2 receptor (2LNT) and berberine (Huang Bo) form a stable complex that maybe examined in further pharmacological studies. Further experimental studies are required to confirm the present computational results.

**Acknowledgements**

Not applicable.

**Funding**

No funding was received.

**Availability of data and materials**

The datasets generated and/or analyzed during the current study are available in the NCBI-GEO repository (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6099).

**Authors' contributions**

MG conceived and designed the study; MG and ZC collected and analyzed the data for this study; ZC wrote the manuscript; and MG reviewed and edited the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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