The Impact of SOD3 on Prostatic Diseases: Elevated SOD3 Serves as a Novel Biomarker for the Diagnosis of Chronic Nonbacterial Prostatitis

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Research Article

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Abstract

BACKGROUND: Prostate is the most common gland for the three major diseases in male, such as chronic nonbacterial prostatitis (CNP), benign prostatic hyperplasia (BPH) and prostate cancer (PCa). However, there is lack of ideal biomarker for diagnosis with prostatic diseases, especially CNP.

METHODS AND RESULTS: Extracellular superoxide dismutase (SOD3) levels in serum or expressed prostatic secretion (EPS) with CNP, and in the prostate tissues of rat CNP modules, BPH, and PCa was quantified, which showed that SOD3 was significantly increased in CNP and BPH, but decreased in PCa compared to controls. Receiver operating characteristic curve (ROC) analysis suggested that SOD3 was an efficient diagnosis biomarker discriminating CNP versus normal controls (accuracy= 0.831, 95%CI: 0.726-0.937, P□0.001), CNP III versus CNP IV (accuracy=0.868, 95%CI: 0.716-0.940, P□0.001). SOD3 were associated with the clinical characteristics of patients with CNP including pelvic pain, blood pressure, and lecithin/leukocyte in EPS. Multiple bioinformatic analysis showed that SOD3 mainly participated in superoxide radicals degradation, apoptotic execution phase, and mesenchymal-to-epithelial transition, *etc*. Furthermore, the structural features of SOD3 and the interacting proteins were evaluated by molecular docking, and hotspot analysis indicated that better affinities between SOD3 and its interacting molecules were associated with the presence of Arginine (Arg) in the binding site.

CONCLUSIONS: According to the results, it can be concluded that SOD3 plays an important role in prostatic diseases, and it may potentially serve as an ideal diagnostic biomarker for CNP.

Introduction

Benign diseases of prostatitis, BPH, and the malignant disease of PCa are common pathologies of the prostate gland. Prostatitis, a common urological disease, is usually accompanied by urinary tract irritation and chronic pelvic pain[1, 2]. According to the National Institutes of Health (NIH), CNP is divided into Category III: chronic pelvic pain syndrome (CPPS), and Category IV: asymptomatic inflammatory prostatitis (AIP)[3]. CNP is one of the most common urological disorders with an incidence of 64%, making it the most common disease of the urinary system in men <50 years[4, 5]. BPH is the most common urinary disease in the elder male population, with a prevalence of 26.2% in a lifetime despite ethnic background[6]. The BPH is characterized with increased amount of epithelial and stromal cells in the periurethral area of the prostate. PCa is the second most frequently diagnosed cancer among men worldwide which accounts for 7.1% of total cancer cases[7]. Although the direct relationship between these diseases remains aguable, histological evidence of inflammatory infiltrates has almost been detected in BPH cases and the inflammation in prostate indicate a higher prevalence of PCa[8, 9]. However, there is lack of reliable and predictive surrogate biomarker for the diagnosis and therapeutic target for prostatic diseases. Therefore, it is necessary to investigate the potential etiologic mechanism of prostate diseases and search an ideal biomarker for administrating the diseases.

SOD3 is a member of the superoxide dismutase protein family that plays an extremely important role in antioxidant and modulating inflammation by catalyzing dismutation of $\bullet O^{2-}$ to H_2O_2[10]. Moreover, SOD3 also scavenges other reactive oxygen species (ROS) produced in cells and tissues affected by inflammation[11, 12]. Lack of SOD3 is associated with cellular ROS accumulation, activating danger signals, tissue remodeling, and even tumorigenesis[13]. Lower SOD3 in the extracellular space may enhance early cytokine responses and increase the expression of inflammatory factors leading to the generation of a hyperimmune response[14, 15]. In our previous study, SOD3 was filtered out in patients with prostatitis via 4-plex-iTRAQ combined with 2DLC-MS/MS[16].

Furthermore, multiple cancer cell types have been shown to downregulate the expression of SOD3, resulting in ROS accumulation, which supports tumor cell survival, metastasis, and tumor recurrence[17]. Loss of SOD3 is associated with increased cancer incidence—an aggressive phenotype and poor prognosis[18, 19]. Overexpressed SOD3 in PC-3 cells could suppress cells' proliferation, migration, and invasion which was concordant with the inhibition of MMP2 and MMP9 by the accumulated H_2O_2[20]. Bostwick[21] suggested that the oxidative stress(OS) was an early event in carcinogenesis, and the SOD3 could protect DNA against ROS-induced damage in benign epithelium.

In this study, the impact of SOD3 on prostatic diseases was further investigated at gene and protein level. The association between SOD3 concentration in serum and clinical characteristics in patients with CNP, as well as the diagnostic ability of SOD3 with CNP was assessed. The differential concentration of SOD3 in EPS of patients with CNP and healthy controls was estimated using western blot. Prostate cancer cell lines including 22RV1, VCaP, DU145, PC-3 and benign prostatic hyperplasia human cell lines BPH-1 were further used to investigate SOD3 expression in different prostate diseases. As our previous study described, ceruloplasmin (CP), desmoglein 2 (DSG2), retinol binding protein 4 (RBP4) and properdin (CFP) were also significantly upregulated in the pooled samples of sera from patients with CNP, and protein-protein interaction (PPI) was regarded as an important factor in the development of disease[16]. Therefore, it is necessary to explore the relationship between these proteins in prostate diseases.

Materials And Methods

Specimen collection

A total of 30 BPH, 16 PCa and adjacent normal control tissues were collected from the First Affiliated Hospital of Guangxi Medical University. BPH tissues were obtained after the Transurethral Resection of Plasma in severe prostatic hyperplasia patients. The PCa tissues were obtained by surgical resection. 94 cases of serum from patients with CNP were included in this study dividing into three groups: Category III, Category IV, and normal controls. 16 cases of EPS fluids were collected in 1.5 ml autoclaved tubes directly from the urethra after the patients' prostates were massaged via rectum. All patients had not received any antimicrobial treatment before this evaluation. Informed consent was obtained from individual participants included in this study. All specimens in this study were anonymously handled according to ethical and legal standards.

Rat model of nonbacterial prostatitis

A total of 30 4-month-old male Sprague–Dawley rats, weighing 250–300g, were obtained from Guilin Medical University Animal Experiment Center. The experimental animals were randomly divided into 2 groups: saline group and xiaozhiling group. Following abdominal surgery, the prostate was exposed and injected with 0.1 ml of normal saline or an equal volume of xiaozhiling respectively as described[22]. One month later, rats were anesthetized with intraperitoneal injection of pentobarbital sodium, and the prostate tissue was excised by abdominal surgery.

Multiple Reaction Monitoring (MRM)

Serum samples were prepared for mass spectrometry as described[23]. Samples were lyophilized and redissolved in 2% ACN containing 0.1% formic acid, and peaked with 50 fmol of peptide mixture of β-galactosidase, as a relative internal standard peptide for LC-MS/MS analysis as described[24].

MRM experiments were performed on 4000 QTRAP mass spectrometer (Applied Biosystems) interfaced with a 2-D nanoLC (Eksigent) was used to perform LC-MS/MS analysis. MRM data on the 4000 QTRAP mass spectrometer were acquired with NanoSpray II source. The optimal acquisition parameters were as follows: ion spray voltage (2300 V), curtain gas (30 p.s.i.), nebulizer gas (16 p.s.i.), interface heater temperature (150 ℃), declustering potential (100). The resolution parameters of the first and the third quadrupoles were set as “unit”. In the MRM runs, the scan time was maintained at 50ms for each transition, and the pause between transition scans was set to 5ms. Result files (wiff and wiff.scan) were imported into peak area integration software, MultiQuant (Applied Biosystems, version 1.1) to extract the peak areas of transitions and to normalize using the peak area of internal standard peptide for the β-galactosidase peptide (VDEDQPFPAVPK, IDPNAWVER, GDFQFNISR) to adjust for variations between runs, as described.

Cell lines and cell cultures

The human BPH cell line BPH-1, and PCa cell lines 22RV1, VCaP, DU145, PC-3 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were grown in high-glucose Dulbecco’s Modified Eagle Medium /F12 (DMEM/F12; Gibco Company, USA), or Roswell Park Memorial Institute (RPMI-1640, Gibco Company, USA), supplemented with 10% fetal bovine serum (FBS; Gibco Company, USA) and 1% penicillin/streptomycin, respectively. All cells were maintained in a humidified incubator at 37°C with 5% CO_2.

Real time quantitative polymerase chain reaction analysis (RT-qPCR)

Total tissue RNA was isolated using the Trizol reagent (Invitrogen, USA) according to the protocol described by the manufacturer. The cDNA was obtained following reverse transcription with PrimeScript^{TM} RT reagent Kit (Takara, Shiga, Japan). The RT-qPCR cycling conditions were as following: predenaturation at 95°C for 10min, followed by 40 cycles of denaturation at 95°C for 15s, annealing at 58–60°C for 60s, and extension at 72°C for 10s. The primer sequences used were listed in Supplementary Table S1. Following the QR = $2^{-\Delta Ct}$, the relative expression levels of mRNA in each sample were calculated[25].

Immunohistochemistry and Hematoxylin-eosin staining

After fixed, the prostate tissues of rat or human were embedded in paraffin, respectively. The sections were dewaxed with xylene and the antigen was repaired with citrate buffer in the condition of hyperbaric for 15min. After blocked with 0.3% hydrogen peroxide and goat serum, the sections were incubated with the primary antibody: anti-SOD3 (1:1000, Proteintech) at 4°C for 12 hours. After washing with PBS solution, the sections were incubated with secondary antibody (1:2000, Proteintech) at room temperature for 30min. The Hematoxylin-eosin staining (HE) was done directly after dewaxed with xylene.

Western blot analysis

Total proteins of tissues were extracted using radio immunoprecipitation assay (RIPA) buffer. After measuring the concentrations, a total protein of 20μg were separated by electrophoresis on 10% Tris-HCl gels, transferred to polyvinylidene difluoride membranes, and blocked in 5% nonfat milk powder. Following the incubation with primary antibody: anti-SOD3 (1:1000, Proteintech) at 4°C for 12 hours, a horseradish peroxidase–conjugated secondary antibody (1:6000, Proteintech) were performed at RT for 60min. The bands were scanned and analyzed on the ChemiDoc XRS+ System (BioRad) at the end. Grayscale analysis were performed on Quantity One software.

Bioinformatics analysis

As described above，the potential interacting proteins of SOD3 were obtained from our previous study, that were also observed overexpression in the serum from patients with CNP. The online Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/)[26] was used to verify the PPI relationship of SOD3, CP, DSG2, RBP4, and CFP, and search for other potential interacting proteins of SOD3. For further detecting the function of SOD3 and its potential interactors, gene ontology (GO) analysis was done. FunRich3.1.3 was used to detect which biological process or component that SOD3 and its interacting proteins mainly involved in based on GO analysis.

Docking calculations

All protein files required were downloaded from the RCSB PDB website (https://www.rcsb.org/). The protein processing prior to docking was done using SYBYL-X 2.0 software. Protein-protein docking and image processing of the complexes were performed on HEX 8.0.0 software (http://hex.loria.fr). The docking condition was according to the correlation type of 'Shape + Electro' and the final search value of 30. Furthermore, we predicted the hotspots in the binding area using the KFC Server (Knowledge-based FADE and Contacts)[27].

Statistical analysis

Quantitative variables were expressed as mean ± SD, and were analyzed by ANOVA. Statistical significance was assumed when P< 0.05. Receiver operating characteristic (ROC) curves were conducted to estimate the diagnostic value of the markers. The best cut-off value for SOD3 was defined as the point with maximum Youden index (sensitivity□specificity□1) on the ROC curve.

Results

Higher SOD3 expression in the rat tissues of nonbacterial prostatitis

SOD3 expression was increased in prostatitis tissues at gene and protein in rat module detected via RT-qPCR and western blot, respectively (Figure 1A, 1B). Moreover, the results of HE showed that regular shaped acini and intact basement membrane in prostate tissue of the saline group under the microscope. On the contrary, a large count of irregular shaped acini was observed in xiaozhiling group with stromal infiltration of mast cells and lymphocytes (Figure 1C). And the IHC results showed that the SOD3 positive cells in prostatitis tissues possessing approximately 70% was more than that in normal tissues with about 20% totally at numbers and deepness (Figure 1C).

SOD3 level in sera as well as the relationship with clinical characteristics

Furthermore, the expression of SOD3 was also increased in EPS from patients with CNP via western-blot (Figure 2A). Signature peptides were selected according to the combination of information from the Skyline 2.6 software (MacCoss Lab) and prediscovery experimental data. Unique peptides detected with the highest frequencies, which were SODE (LDAFFALEGFPTEPNSSSR) and β-galactosidase (IDPNAWVER, GDFQFNISR), were selected (Figure 2B). And the optimization of transitions and collision energies were shown in Table 1.

Table 1

Optimization of Transitions and Collision Energies

Peptide name	sequence	charge	Q1	Q3	CE
SODE	LDAFFALEGFPTEPNSSSR	2	1043	710.839	48.9
		2	1043	654.297	51.9
		2	1043	229.118	69.9
		2	1043	300.155	69.9
BGAL	IDPNAWVER	2	550.28	871.442	30.2
		2	550.28	774.389	30.2
		2	550.28	660.346	30.2
	GDFQFNISR	2	542.265	764.405	29.9
		2	542.265	636.346	29.9
		2	542.265	489.278	29.9

Serum SOD3 level was found to significantly elevated in patients with CNP III (6.491643 ±1.592292, P 0.001), and CNP IV (8.617879±1.535176, P0.001) compared to normal controls (4.705892± 1.484917) (Figure 2C, Table3). The AUC for SOD3 to predict CNP was 0.831 (95% CI 0.726-0.937) with a sensitivity of 92.8% and specificity of 57.9% (Figure 2D). And the AUC for SOD3 distinguishing prostatitis between Category III and Category IV was 0.868 (95% CI 0.716-0.940) with a sensitivity of 71.4% and specificity of 91.7% (Figure 2E, Table 2). Moreover, increased SOD3 was significantly related to high blood pressure (P=0.014), lower lecithin in EPS (P=0.012), severe pain of patients (P=0.042), and absence of leukocyte in EPS (P=0.037) (Table 3, 4). However, there was no significant association between SOD3 level and BMI (Body Mass Index), waist, nor CPSI (Table 3, 4).

Table 2

Sensitivity and specificity to diagnose CNP with SOD3

Project	AUC (95%CI)	Cut point (relative concentration)	Sensitivity (%)	Spesificity (%)
SOD3 for predicting CNP	0.831 (0.726-0.937)	4.523566	92.8	57.9
SOD3 for distinguish Category III and IV CNP	0.868 (0.716-0.940)	8.641081	71.4	91.7

SOD3 Extracellular superoxide dismutase, *CNP* chronic nonbacterial prostatitis, *AUC* area under the curve, *CI* confidence interval

Table 3

Association between SOD3 and clinical characteristics in chronic nonbacterial prostatitis (n=72)

Clinical features	Case	SOD3 level (mean± SD)	P value
Sample			
CNP III	48	6.491643±1.592292	0.000<0.05*
CNP IV	24	8.617879±1.535176	0.000<0.05*
Normal	22	4.705892±1.484917	
Age at diagnosis(years)			
≥30	32	7.472619±1.800889	0.096>0.05
<30	34	6.732808±1.753474	
unknown	6		
BMI(kg/m^2)			
≤24.9	44	7.268759±1.907236	0.523>0.05
>24.9	11	7.669010±1.520876	
unknown	17		
Waist(cm)			
≤90	51	7.261959±1.858539	0.491>0.05
>90	15	6.892413±1.647676	
unknown	6		
Blood pressure(mmHg)			
≤120	40	6.728730±1.542821	0.014>0.05*
>120	24	7.871034±2.041183	
unknown	8		
Lecithin in EPS(+++) [a]			
Normal	32	6.520641±1.467063	0.012>0.05*
Decreased	33	7.578082±1.819162	
unknown	7		
Leukocyte in EPS			
Positive	44	6.837507±1.984187	0.037>0.05*
Negative	24	7.678890±1.257645	
unknown	4		

SOD3 Extracellular superoxide dismutase, *CNP III/IV* chronic nonbacterial prostatitis III/IV, *BMI* body mass index, *EPS* expressed prostatic secretion, *SD* standard deviasion, a: Normal: +++, Decreased: lower than +++, *P□0.05

Table 4

Association between SOD3 and clinical characteristics in chronic nonbacterial prostatitis of Category III (n=48)

Clinical features	Case	SOD3 level (mean±SD)	P value
CPSI			
≤29	42	6.416413± 1.532597	0.801□0.05
□29	5	6.238512± 0.824442	
Unknown	1		
Pain score			
≤7	41	6.231694± 1.305283	0.042□0.05*
□7	6	7.530409± 2.106400	
Unknown	1		
LUTS score			
≤18	39	6.229589± 1.416299	0.083□0.05
□18	8	7.215990± 1.534366	
Unknown	1		

SOD3 Extracellular superoxide dismutase, *CPSI* Chronic Prostatitis Symptom Index, *LUTS* Lower urinary tract symptoms, *SD* standard deviasion, *P□0.05

Different expression of SOD3 in BPH, PCa and prostate cell lines

SOD3 was upregulated significantly in BPH tissues compared to normal control tissues at mRNA and protein levels (Figure 3A, 3C and 3E). In addition, SOD3 was decreased in PCa tissues compared to normal control tissues (Figure 3A, 3C and 3E). What's more, SOD3 was suppressed in malignant cell lines when compared to benign cell lines (Figure 3B, 3D).

Bioinformatic analyses of SOD3 and its interactors

After PPI analysis on STRING, the interaction between SOD3 and CP, DSG2, RBP4, and CFP was found, and PPI network was constructed (Figure 4A). In molecular function, SOD3 and its interactors were participated in superoxide dismutase activity and cell adhesion molecule activity (Figure 4B). For cellular component enrichment, they existed mostly in extracellular, plasma membrane, desmosome, cell junction, and catenin complex (Figure 4C). For biological process, they were associated with cell growth and/or maintenance (Figure 4D). The analysis of biological pathway showed that SOD3 and its interactors were mainly involved in superoxide radicals degradation, apoptotic execution phase, mesenchymal-to-epithelial transition (MET), *etc.* (Figure 4E).

Docking between SOD3 and its interactors

In the results, the PDB ID 4ENZ of CP with SOD3 (PDB ID 2jlp) has the least E-total value of -1430.74 and it is the most closely functioning state between CP and SOD3. Another structure (PDB ID 5J5J) of DSG2 has the least E-total value of -1196.62 when docking with SOD3.The E-total value of SOD3-RBP4 and SOD3-CFP complex is -987.41 and -946.58, respectively (Table 5). The 3D modules of the interaction position were shown in Figure 5. It is worth to notice that a residue of Arg in SOD3 has a higher frequency presented in the binding site (Table 5). Totally, the expression of DSG2 and CFP were decreased in PCa tissues comparing to the normal tissues, however, the CP and RBP4 were elevated in PCa and BPH when compared to the normal control tissues (Figure 5A-D).

Table 5

The hotspot analysis of SOD3 and its interacting proteins

Target protein	The name of PDB file	Position	Etotal Kcal/mol	Hot spots of target protein	Hot spots of SOD3
CP	4ENZ	1-1065	-1430.74	ASP(122) TYR(136)	ARG(134) TRP(139) ARG(140)
DSG2	5J5J	157-380	-1196.62	ILE(335) HIS(336) LYS(338) SER(339) SER(340) VAL(341) ILE(342) SER(343) ILE(344) TYR(345) GLU(348) SER(349) ARG(352) SER(353) SER(354) LYS(355) GLY(356) GLN(357) ILE(358) PHE(365) ASP(366) PRO(372) ALA(373) HIS(374) ARG(376) LEU(380) GLU(381) ARG(383) ASP(384) ASN(385) ASP(390) SER(391) VAL(392) THR(393) GLU(395) ALA(399) LYS(400) LEU(401) ASP(403) PHE(404) SER(406) TYR(408) VAL(409) GLN(410) GLY(412) THR(413) LYS(417) VAL(419) ILE(421) SER(422) GLU(423) LYS(428) THR(429) ILE(430) THR(431) ASN(437)	THR(40) HIS(42) GLN(46) THR(61) LEU(69) ALA(70) PRO(71) ARG(72) ALA(73) LYS(74) GLU(82) GLY(83) PRO(85) THR(86) ASN(89) SER(90) SER(91) SER(92) ARG(93) TYR(114) PRO(125) GLY(126) ASN(130) ALA(132) VAL(133) ARG(134) ASP(135) GLY(136) SER(137) TRP(139) ARG(140) ARG(142) ALA(143) GLY(144)

					ALA(146) GLY(151) PRO(152) HIS(153) SER(154) ARG(158) CYS(190) VAL(191) VAL(194) CYS(195) PRO(197) LEU(199) TRP(200) GLN(203)
RBP4	5NU9	19-201	-987.41	ASN(66) ASP(68) TYR(173)	TRP(139) ARG(140) TYR(141)
CFP	6RUS	28-469	-946.58	ASP(463) GLU(466) LEU(469) TYR(473)	SER(90) SER(92) ASN(130) ALA(132) ARG(134) ARG(140)

SOD3 Extracellular superoxide dismutase, *CP* ceruloplasmin, *DSG2* desmoglein 2, *RBP4* retinol binding protein 4, *CFP* properdin, *PDB* Protein Data Bank

Discussion

This study examined SOD3 levels in three common prostate diseases compared to normal controls. Among these disorders, SOD3 was increased in CNP and BPH, however, was decreased in PCa. Previous studies had reported that the imbalance of ROS was a critical factor in the development and the progression of prostate diseases[28]. Usually, antioxidant enzyme functioned to prevent cell damage from ROS. However, cell defense systems were destroyed because of the irregulation of antioxidant enzyme or others. For CNP, Ilter Alkan[29] found that superoxide anion ($O_2^{\bullet -}$) and total ROS production in semen of male with CNP III was significantly higher than in normal controls. Scientific evidence suggested that high ROS might reduce the clinical outcomes for patients with BPH and PCa *via* protein, lipid, and DNA damage[30, 31]. Reports suggested that SOD3 suppressed the development of PCa and improved the cancer response to chemotherapy by modulating ROS in cells[32]. While the mechanism of ROS in prostate diseases remains largely unclear, it is approved that the imbalance of ROS production interconnects CNP with BPH, and PCa[33]. Synthesis of the results of previous studies and our research, we presumed that the infiltration of leukocyte and the release of inflammatory factors in prostate stimulated the production of ROS which subsequently stimulated the expression of SOD3.

At present, there was no optimal diagnostic marker to confirm CNP and distinguish different categories in patients. In this study, the AUC of SOD3 was 0.831, and the cut-off point of the relative concentration was 4.523566 with a sensitivity of 92.8% and specificity of 57.9% indicating that elevated SOD3 was an important biomarker for filtering out CNP. Further, once SOD3 level was higher than 8.641081, that might indicate a higher risk of Category IV but not Category III prostatitis. With respect to the clinical characteristics, high serum SOD3 might indicate more severe pelvic pain and higher blood pressure in patients, although the pathological mechanisms of pelvic pain or lower urinary tract symptoms in patients with CNP remain largely unclear. Many patients who for the first time to diagnosis with CNP because of the pelvic pain which causes mental trauma in male. However, pelvic pain has no correlation with severity of inflammation, which can confuse doctor in management the CNP standardly. High levels of SOD3 also suggested to decrease lecithin levels in EPS, indicative of prostatitis. Yet, the concentration of SOD3 does not affect the total chronic prostatitis symptom index (CPSI) in this study (P=0.801), indicating that SOD3 might not reflect other lower urinary tract symptoms such as frequent urination, urgency, and dysuria. Continuous exposure of prostate tissue to the inflammation can induce the vehement release of ROS, causing changes in lipids, protein structure and function, and DNA modifications[34]. Migration of activated granulocytes in prostate and the accumulation of TNF-α and interleukin-1β pro-inflammatory cytokines were the vital factor in the production of ROS[35]. However, our findings suggested that SOD3 in the group of leukocyte negative in EPS was higher than the positive one. This discrepancy may be due to multiple regulation of the leukocyte migration and the secretion of SOD3 to serum during the CNP.

As described previously, SOD3 acts as a superoxide dismutase protein that eliminates ROS preventing cells from oxidative damage. Superoxide is approved to be a signal molecular in a distinct manner to regulate the physiological or pathological process, although the mechanism remains largely unknow. However, several studies have suggested that superoxide function to mediate the activation of protein kinases, such as protein kinase C/D (PKC/D), and mitogen-activated protein kinase (MAPK)[36, 37]. In this study, we further investigated the potential mechanism of SOD3 in regulating the process of prostate diseases *via* bioinformatics analysis. SOD3 and its interactors were found involving in processes associated with removal of superoxide radicals and response to stimulus stress. Biological pathway analysis suggested that they mainly participated in the superoxide radical degradation, as well as in MET. So, it's interesting to further investigate whether SOD3 regulates the similar signal pathway *via* modulating the level of ROS.

Epithelial-to-mesenchymal transition (EMT), characterized by downregulation of epithelial markers including ALK2 and E-cadherin, and the upregulation of mesenchymal markers like Zeb-1, Twist-1 and Vimentin *etc*, has been wildly acknowledged that inhibits cell apoptosis and differentiation, and promotes carcinoma metastasis in clinic. MET considered to be the reverse process of EMT, which prevents the progression and metastasis of cancer cells[38]. Although contrary to EMT, the essential mechanism of MET remains poorly understood, the re-expression of epithelial markers is a key hallmark of MET, especially E-cadherin[39]. Sarah K. Martin and colleagues[40]implied that MET alleviated drug resistance and reduced tumorigenesis of PCa cells. Studies also suggested that SOD3 deficiency promoted liver

fibrosis by activating hepatic stellate cell *via* EMT[41]. Our findings suggested that SOD3 and its interactors participated in the process of MET, although rare functional mechanism about it was performed.

For interactors, CP was a mammalian blood plasma ferroxidase which carried more than 95% of the copper found in plasma[42]. Cooper was a cofactor in many enzymes responsible for important processes, however, the free cooper in plasma could product amount of oxidant causing the cell damage. So, whether the CP affects SOD3 levels by controlling the cooper? DSG2, one of the four isoforms of desmosomes, which controlled cell adhesion and proliferation via directly interacting with epidermal growth factor receptor (EGFR)[43]. Literatures reported that EGFR inhibited the expression of DSG2 *via* the accumulation of ROS[44]. The RBP4 gene was located on chromosome 10 (10q23–q24) which encoded the Retinol-binding protein 4 inducing the production of ROS[45, 46]. It was worth to further investigate whether the RBP4 mediated the progression of BPH and PCa *via* accumulating ROS. CFP gene encoded the protein of complement factor properdin that mediated the inflammatory processes[47]. In a word, different levels of the four interacting proteins in prostate diseases might act as different roles in the development and progression of the diseases. Further analysis of our study focused on the hotspots in the binding site between SOD3 and CP, DSG2, RBP4, as well as CFP. According to work by Svetlana V. Antonyuk[48], the SOD3 protein is a tetramer consisting of 8 antiparallel β-strands. Interestingly, all of the identified SOD3 hotspots included Arg, which might indicate that Arg was vital for the binding between SOD3 and its interacting proteins. Previous studies have suggested that Arg deprivation induces atypical cell death of PCa via modulating histone acetylation, metabolic, and DNA-repair genes[49, 50]. Thus, it's necessary to further investigate the clear function of Arg in the binding site of SOD3 in prostate diseases.

Conclusion

In summary, SOD3 was upregulated in benign prostatic diseases, while down- regulated in malignant disease. ROC analysis revealed that high level of SOD3 in serum was a potent diagnostic biomarker for CNP. The interaction between SOD3 and its potential interactors was revealed *via* protein docking. And hotspot analysis suggested that Arg residue was the key point for SOD3 interacting with the proteins, indicating that it might play a vital role in the binding site. Above all, SOD3 might play an important role during the development and progression of prostate diseases, and interacted with CP, DSG2, RBP4, and CFP, which mainly participated in specific pathway such as superoxide radicals degradation, apoptotic execution phase, and MET. However, more experimental research should be conducted to explore the clear molecular mechanism of SOD3 in prostate diseases.

Declarations

The authors declare that they have no conflicts of interest.

Availability of data and material

Data will be made available on reasonable request.

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Authors' contributions

Shengfeng Zheng, Hongtao Li: Samples collection, experiment conducting, data analysis and manuscript editing. Xuandong Lin and Xiang Gan: Participated in the animal experiments. Shengjun Xiao, Jiayi Liu, and Zhibin Xie: Manuscript writig and Samples collection. Zhidi Lin and Weiru Song: Helped in statistical analysis. Xiao Wang, Meiqing Li, Yue Lan, Mingjing Zhang: Data collection and analysis. Weijin Fu and Xiaoli Yang: Revised the manuscript.

Research involving human participants and/or animals

This study was approved by the Ethics and Human Subject Committee of the First Affiliated Hospital of Guangxi Medical University and the Animal Experiment Committee of Guilin Medical University.

Informed consent

Informed consent was obtained from individual participants included in this study.

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Figures

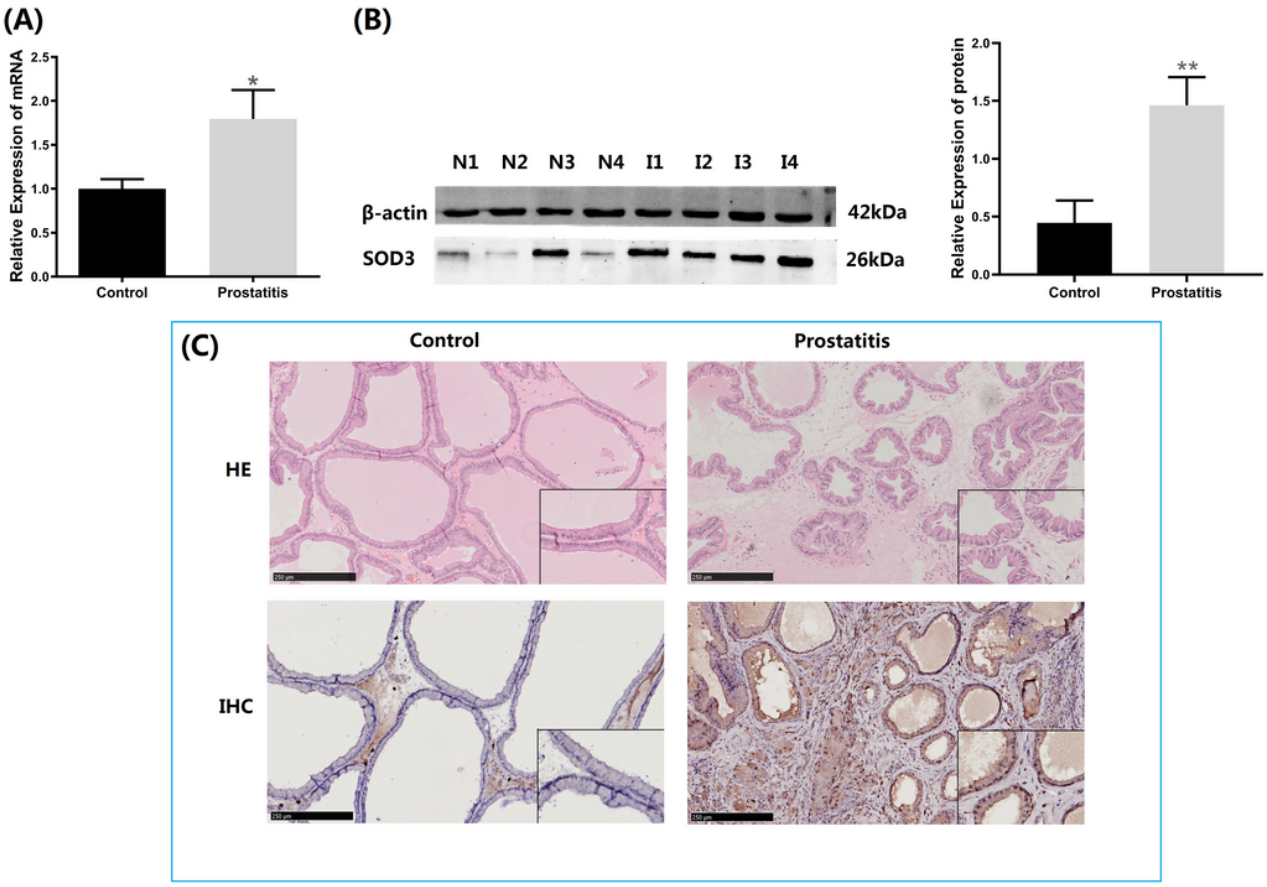

Figure 1

The expression of SOD3 in rats. A. The mRNA expression of SOD3 in the tissues of prostatitis and control. B. Protein levels of SOD3 detected by Western blot in prostate tissues of prostatitis and control. (Control: N1~4, Prostatitis: I1~4). protein was quantitatied by Quantity one software, *p<0.05. C. The HE and IHC of SOD3 image.

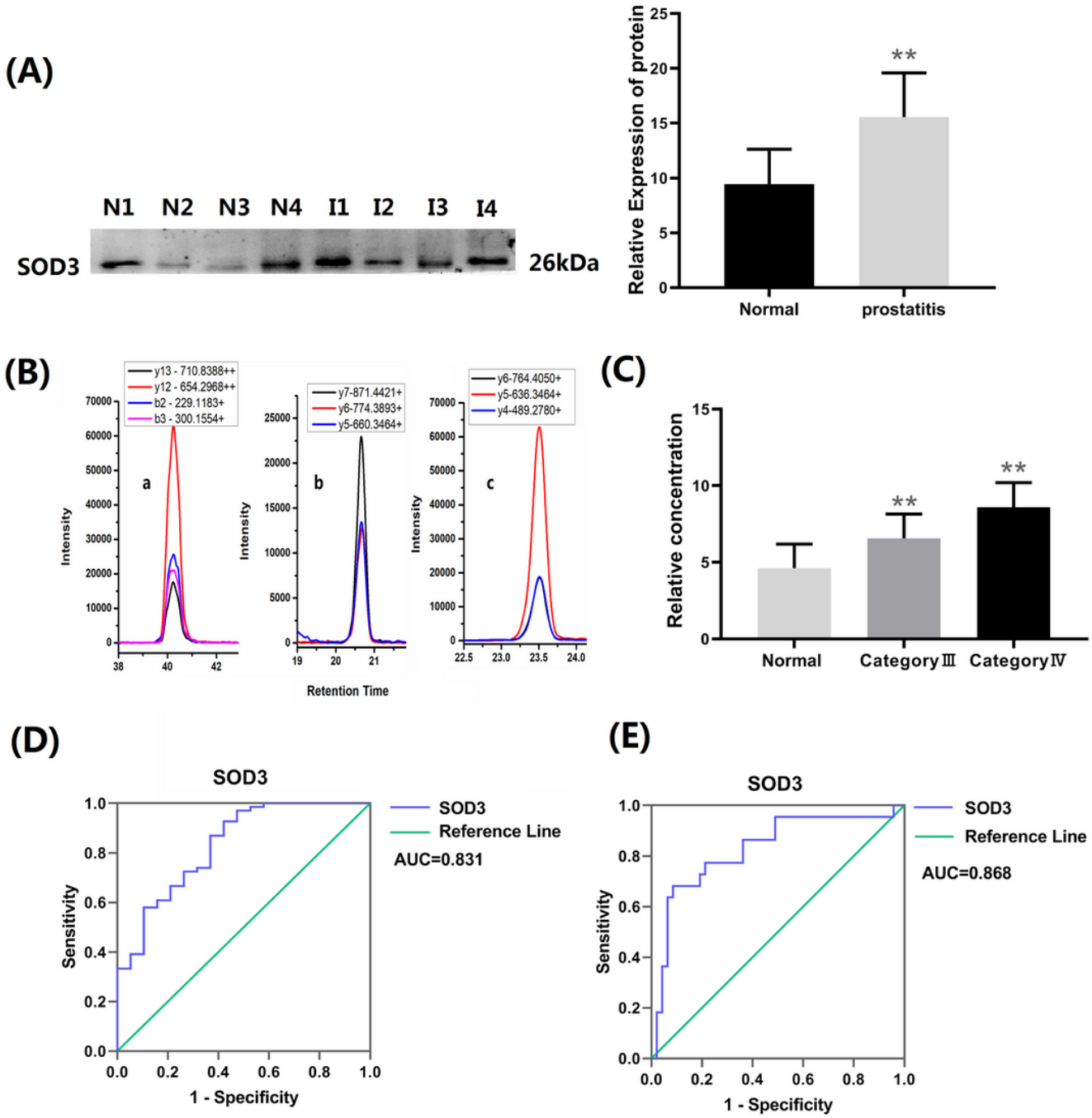

Figure 2

The results of SOD3 levels in human sera and EPS. A. SOD3 level in EPS (control: N1~4, prostatitis: I1~4); relative expression of protein quantitatied by Quantity one software, **p<0.01. B. SOD3 in sera obtained from control and prostatitis (Category III and IV) patients. Serum concentrations of SOD3 is presented as histogram, *p<0.05, ** p<0.01. C. Chromatogram views on Skyline. Chromatograms are displayed in a tabular format with one row per replicate in which the peptide was measured. The first column displays the selected fragment ion, produced by the precursor ion. The last column displays the retention time of the target peptide and standard peptide. (a: SODE (LDAFFALEGFPTEPNSSSR); b: BGAL (IDPNAWVER); c:

BGAL (GDFQFNISR). D. ROC Curve for SOD3 predicting nonbacterial prostatitis. E. ROC Curve for SOD3 predicting differential nonbacterial prostatitis between category III and category IV.

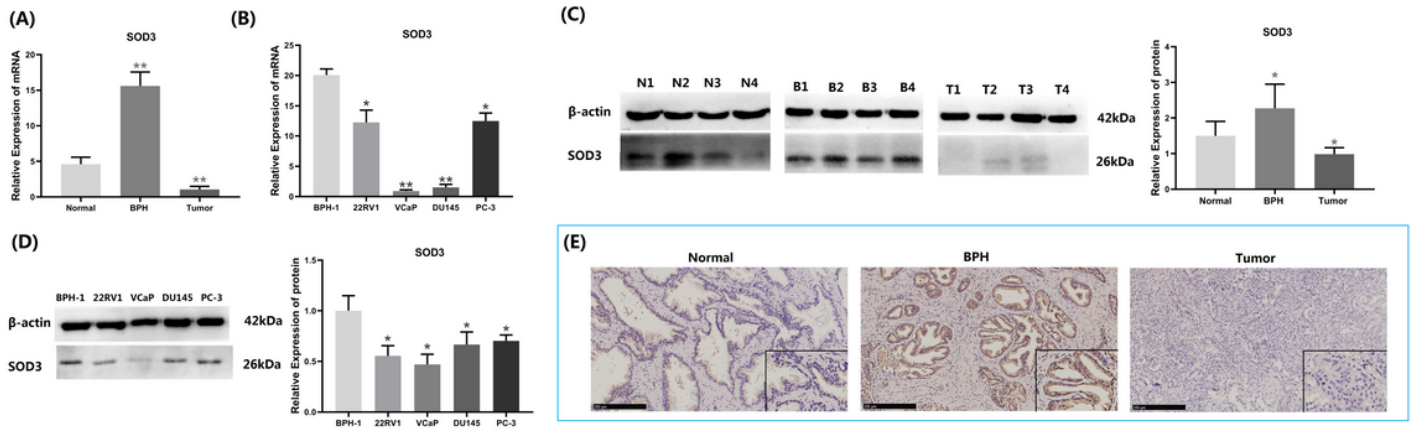

Figure 3

The expression of SOD3 in human prostate. A. The mRNA expression of SOD3 in the tissues of BPH, PCa and control. B. The mRNA expression of SOD3 in prostate cell lines. C. Protein levels of SOD3 detected by Western blot in prostate tissues (Normal adjacent: N1~4, BPH: B1~4, Tumor: T1~4). D. Protein levels of SOD3 detected by Western blot in prostate cell lines. E. IHC results of SOD3 in BPH, prostate cancer and normal adjacent tissues.

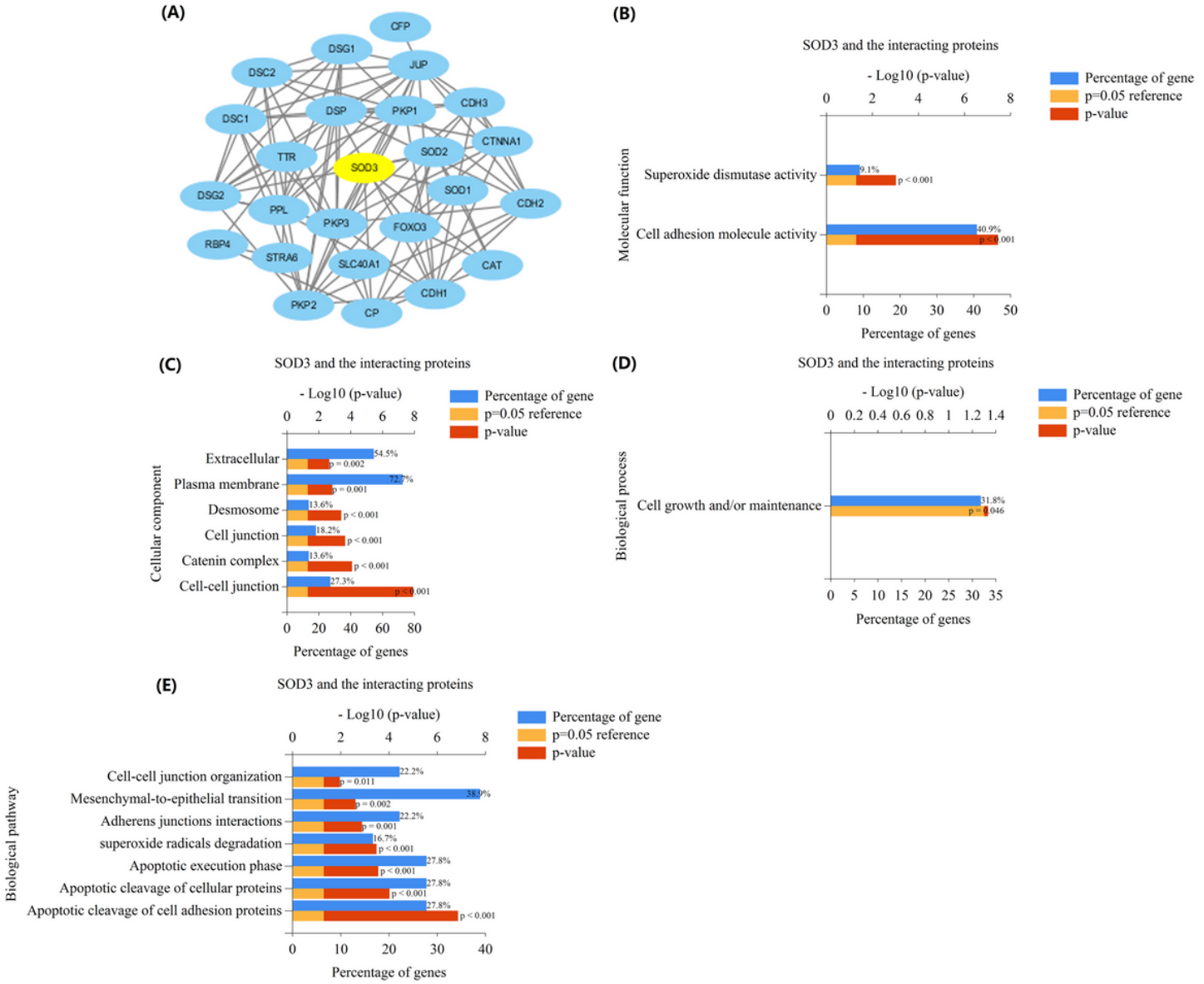

Figure 4

Results of bioinformatic enrichment analysis for SOD3 and its interactors: A. PPI network of SOD3 and its interacting proteins. B. molecular function; C. cellular component; D. biological process; E. biological pathway.

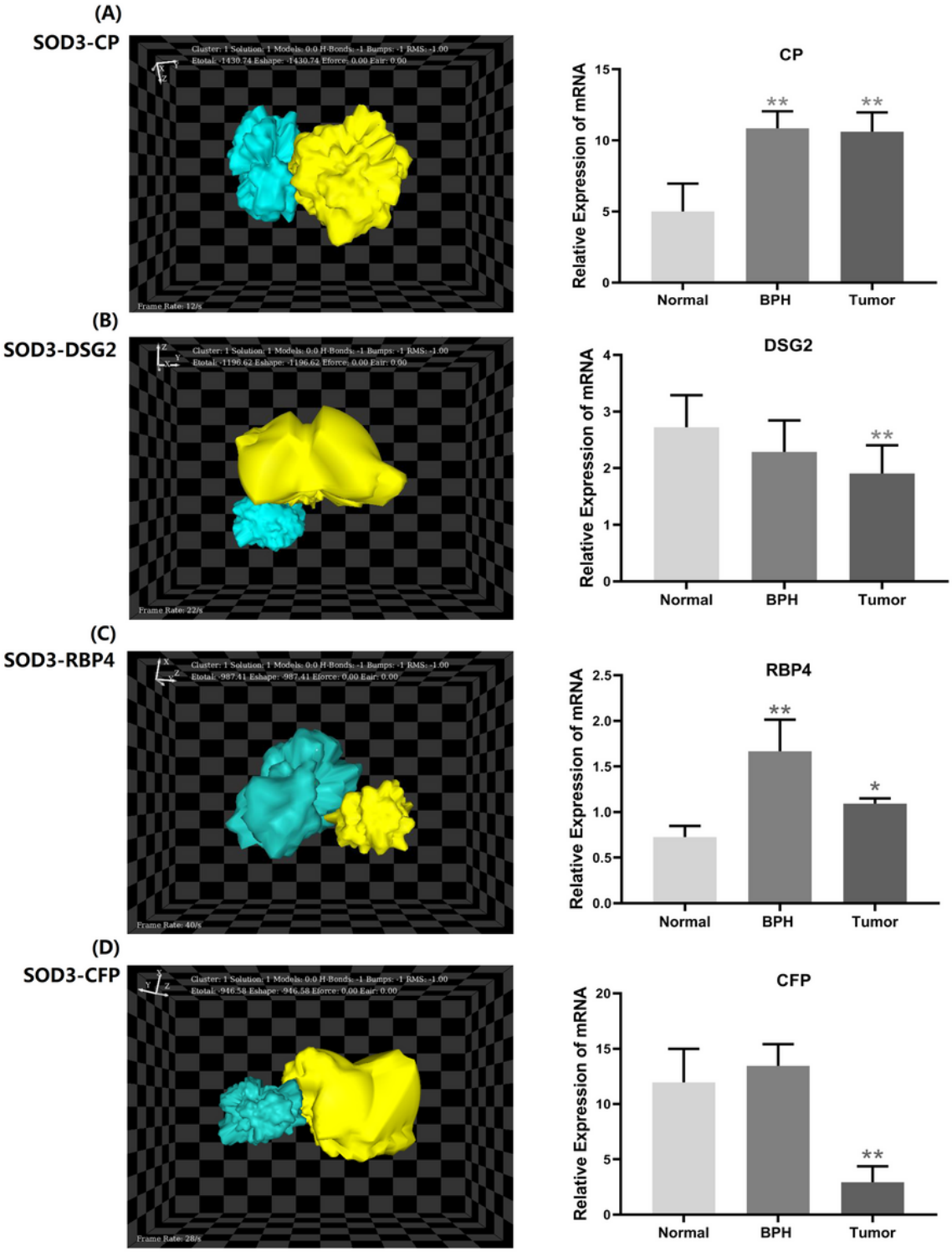

Figure 5

The interaction between SOD3 and its interactome. A. Predicted constructions of SOD3-CP, and the relative mRNA expression of CP. B. Predicted constructions of SOD3-DSG2, and the relative mRNA expression of DSG2. C. Predicted constructions of SOD3-RBP4, and the relative mRNA expression of RBP4. D. Predicted constructions of SOD3-CFP, and the relative mRNA expression of CFP. , *p<0.05, **p<0.01.

Supplementary Files

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- TableS1.doc