The impact of SOD3 on prostatic diseases: elevated SOD3 is a novel biomarker for the diagnosis of chronic nonbacterial prostatiti

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Research Article

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Abstract

Prostate is the most common gland for the three major diseases in men, 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. This prospective study quantified extracellular superoxide dismutase (SOD3) levels in serum or expressed prostatic secretion (EPS) with CNP, and in the prostatic tissues with BPH or PCa, to evaluate SOD3 as possible surrogate markers for diagnosis and treatment efficacy. The diagnostic ability of SOD3 with CNP was estimated by ROC analysis. The potential function of SOD3 in prostatic disoders was further investigated *via* bioinformatic analysis. As a result, SOD3 was significantly increased in CNP and BPH, but dereased in PCa compared to controls. SOD3 was significantly elevated in patients with CNP III (6.491643 ± 1.592292, P□0.001), CNP IV (8.617879 ± 1.535176, P□0.001) compared to normal controls (4.705892 ± 1.484917), 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). Furthermore, SOD3 in serum were associated with clinical characteristics of patients with CNP including plevic pain, blood pressure and lecithin/leukocyte in EPS. Functionally, SOD3 mainly interacted with CP, DSG2, RBP4, and CFP *via* spercific amino acid residue, and participated in the signal pathway of superoxide radicals degradation and apoptotic execution phase. Our findings suggested that SOD3 and its interactors might play an important role in prostatic diseases, and SOD3 could be an ideal diagnostic marker and therapeutic target for CNP.

Introduction

Benign diseases of the prostate including prostatitis, BPH and 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 NIH, CNP is divided into Category III: chronic pelvic pain syndrome (CPPS), and Category IV: asymptomatic inflammatory prostatitis(3). CNP is one of the most common urological disorders with an incidence of about 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 dtected 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 marker for the diagnosis and theraputic target for prostatic diseases. Therefore, it is necessary to investigate the potential etiologic mechanism of prostatic diseases and research 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 prostatitis patients 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 features in patients with CNP, as well as the diagnostic ability of SOD3 with CNP was assessed. The secretion 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 different SOD3 expression in different prostate diseases. To predict the potential function of SOD3 during the acurrence of prostatic disease, correlation analysis, protein docking, hot spot analysis and bioinformatics enrichment analysis were performed.

Materials And Methods

Samples collection

The tissues of 30 BPH, 16 PCa and adjacent normal control 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 were included in this study by dividing into three groups: Category III, Category IV, and healthy control. 16 cases of EPS fluidswere collected in 1.5 ml autoclaved tubes directly from the urethra after the patients' prostates were massaged via rectum. All patients had not receivedany antimicrobial treatment before this evaluation. 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 250–300 g, were obtained from Guilin Medical University Animal Experiment Center. The experimental animals were 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 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 50 ms for each transition, and the pause between transition scans was set to 5 ms. 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, respectly. All cells were cultured at 37°C with 5% CO_2.

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

Total tissues 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 PrimeScriptTM RT reagent Kit (Takara, Shiga, Japan). The annealing temperature of RT-qPCR and PCR cycle number were 58-60˚C and 40 cycles for SOD3, CP, DSG2, RBP4, and CFP. The primer sequences used were listed in Supplementary **Table S1**. Following the $QR = 2^{-\Delta Ct}$, we got the relative expression levels of mRNA in each sample(25).

Immunohistochemistry and Hematoxylin-eosin staining

After fixed, the rat and human prostate tissues 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 15 minutes. 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 30 minutes. 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 detecting the concentrations, a total protein of 20 μg 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 1 hour. The bands were scanned and analyzed on the ChemiDoc XRS+ System (BioRad) at the end. Grayscale analysis were performed on Quantity One software.

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 is that the correlation type is 'Shape + Electro' and the final search value is 30. Furthermore, we predicted the hot spots of between SOD3 and its interactomes using the KFC Server (Knowledge-based FADE and Contacts) online(26) .

Data collection of SOD3 and its interactors

Further the potential interacting proteins of SOD3 were obtained from the our previous study, that were also observed overexpression in the serum of CNP. The online Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/)(27) was also used to search for potential interacting proteins of SOD3. For further detecting the function of SOD3 and its potential interactors, gene ontology (GO) analyses 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 analyses.

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 and used 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 expressed in the rat tissues of nonbacterial prostatitis

The results of HE showed that the 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). Moreover, SOD3 expression was increased in prostatitis tissues at gene and protein level detected via RT-qPCR and western blot, respectively(Figure 1A, 1B).

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

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 2C). And the optimization of transitions and collision energies were shown in **Table 1**.

Serum SOD3 level was found to increase in CNP (Figure 2B, Table3). The AUC for SOD3 is 0.831 (95% CI 0.726-0.937) (Figure 2D) and its sensitivity and specificity for predicting CNP is 92.8% and 57.9% respectively. And the AUC for SOD3 is 0.868 (95% CI 0.716-0.940) distinguishing Category III and Category IV prostatitis with 71.4% sensitivity and 91.7% specificity (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). But, there were no significant association between SOD3 level and BMI (Body Mass Index), waist, nor CPSI (Table 3, 4). Furthermore, the expression of SOD3 also increased in EPS from nonbacterial prostatitis patients via western-blot (Figure 2A).

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

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

Bioinformatic analyses of SOD3 and its interactors

After PPI analysis, the interaction between SOD3 and CP, DSG2, RBP4, and CFP was found, and PPI network was constructed(Figure 5A). The module analysis was also visualized in Cytoscape (Figure 5B). For cellular component enrichment, they existed mostly in extracellular, plasma membrane, desmosome, cell junction, and catenin complex (Figure 5C). In molecular function, SOD3 and its interactors were participated in superoxide dismutase activity and cell adhesion molecule activity (Figure 5D). For biological process, they were associated with cell growth and/or maintenance (Figure 5E). The analysis

of biological pathway showed that SOD3 and its interactors were mainly involved in superoxide radicals degradation, apoptotic execution phase (Figure 5F).

Docking between SOD3 and its interactors

In the results, the PDB ID 4ENZ with SOD3 (PDB ID 2jlp) has the least E-total energy -1430.74 and it is the most closely functioning state of CP and SOD3. PDB ID 5J5J is another structure of DSG2 docking with SOD3 has the biggest E-total energy -1196.62. And the Etotal energy for SOD3-RBP4 and SOD3-CFP complex was -987.41 and -946.58 respectively (Table 5). The 3D modules of the interaction position were shown in **Figure 4**. It is worth to notice that a residue of ARG (140) in SOD3 was the common hot spot interacting with the four proteins (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 tissues (Figure 4A-D).

Discussion

This study examined SOD3 levels in healthy controls compared to three common prostatic diseases. Among these disorders, SOD3 was increased in CNP and BPH, however, was decreased in PCa. Previous studies had reported that OS accumulated by the inbalance of ROS was a critical factor in the development and the progression of prostatic diseases(28). Usually, antioxidant enzyme functioned to prevent cell damage from ROS. However, cell defense systems were destroyed because of the iiregulation of antioxidant enzyme or others. For CNP, Ilter Alkan(29) found that superoxide anion ($O_2^{\bullet -}$) and total ROS production in semen of men with CNP III was significantly higher than in healthy control. Scientific evidence suggested that high OS might reduce the clinical outcomes for patients with BPH and PCa *via* protein, lipid and DNA damage(30,31). Reports suggested that SOD3 supressed the development of PCa and improved the cancer response to chemotherapy by modulating OS in cells(32). In this investigation, 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 ROC of SOD3 was 0.831, and the cut-off point of the relative concentration was 4.523566 with a sensitivity of 92.8% and a specificity of 57.9% indicating that elevated SOD3 was an important marker for filtering out CNP. Further, once SOD3 level was higher than 8.641081, that might indicate a higher risk of Category IV prostatitis. With respect to the clinical characteristics, high serum SOD3 might indicate more severe pelvic cavity pain, which might correlate with higher blood pressure in patients, although the pathological mechanisms of pelvic cavity pain or lower urinary tract symptoms in prostatitis patients remain largely unclear. High levels of SOD3 also suggested to decrease lecithin levels in EPS, indicative of prostatitis. Therefore, SOD3 might perform well as a biomarker for the diagnosis of CNP and discrimination of clinical subtypes.

We further investigated the potential mechanism of SOD3 in regulating the process of prostatic diseases. 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 mesenchymal-to-epithelial transition(EMT), as well as in superoxide radical degradation and so on, that might play an important role in the processes of prostatic diseases.

For interactors, CP was a mammalian blood plasma ferroxidase which carried more than 95% of the copper found in plasma(33). Cooper was a cofactor in many enzymes responsible for important processes, however, the free cooper in plasma could product amount of oxidant cuasing the cell damage. So, would that CP affected the SOD3 levels by controlling the cooper? DSG2, one of the four isoforms of desmosomes, which controlled cell adhension and proliferation via directly interacting with epidermal growth factor receptor (EGFR)(34). Literature reported that EGFR inhibited the expression of DSG2 *via* the accumulation of ROS(35). The RBP4 gene was located on chromosome 10 (10q23–q24) which encoded the Retinol-binding protein 4 inducing the production of ROS(36,37). 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(38). In a word, different levels of the four interacting proteins in prostatic diseases might act as different roles in the development and progression of the diseases. Further analysis of our study focused on the hot spots on SOD3 and CP, DSG2, RBP4, and CFP. According to work by Svetlana V. Antonyuk(39), the SOD3 protein is a tetramer consisting of 8 antiparallel β-strands. In this study, SOD3 interacted with other proteins through its special structure and shared a common amino acid residue of ARG (140).

Conclusion

In conclusion, 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 and might be an effective theraputic marker for prostatic diseases. The interaction between SOD3 and its potential interactors was revealed *via* protein docking. And hot spot analysis suggested that ARG (140) residue was the key point for SOD3 interacting with the proteins. Above all, SOD3 might play an important role during the development and progression of prostatic diseases, and interacted with CP, DSG2, RBP4, and CFP and participated in spercific pathway such as superoxide radicals degradation and apoptotic execution phase. Further, this study provides insightful data regarding a potential diagnostic biomarker and an effective therapeutic target for CNP.

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

Z.F. L.T. samples collection, experiment conducting, data analysis and manuscript editing. L.D. and G.X. participated in the animal experiments. X.J. L.Y. and S.W. manuscript writig and Samples collection. X.B. L.D. helped in statistical analysis. W.X. L.Q. L.Y. and Z.J. Data collection and analysis. F.J. and Y.L. 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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Tables

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

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

Table 5. The hot spots 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

Figures

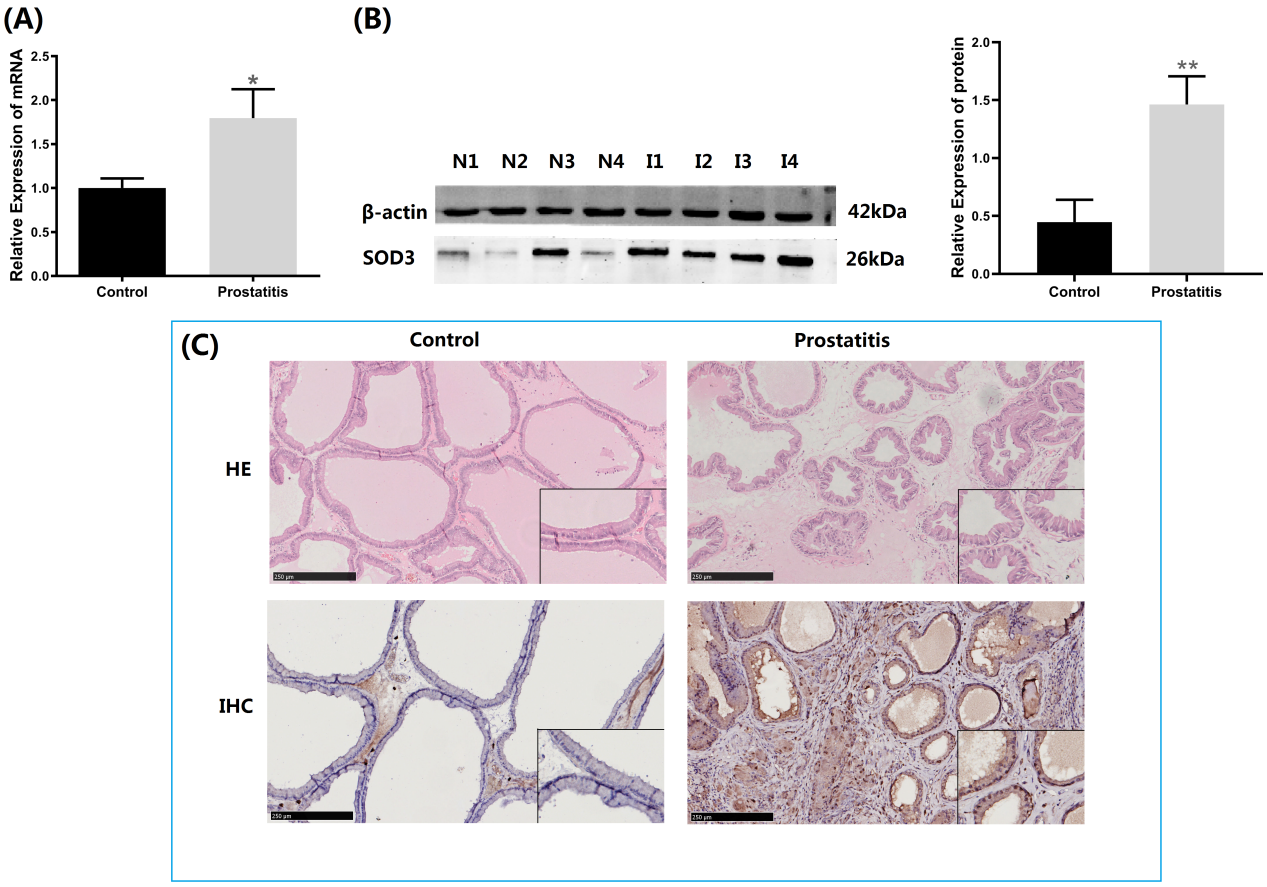

Figure 1

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.

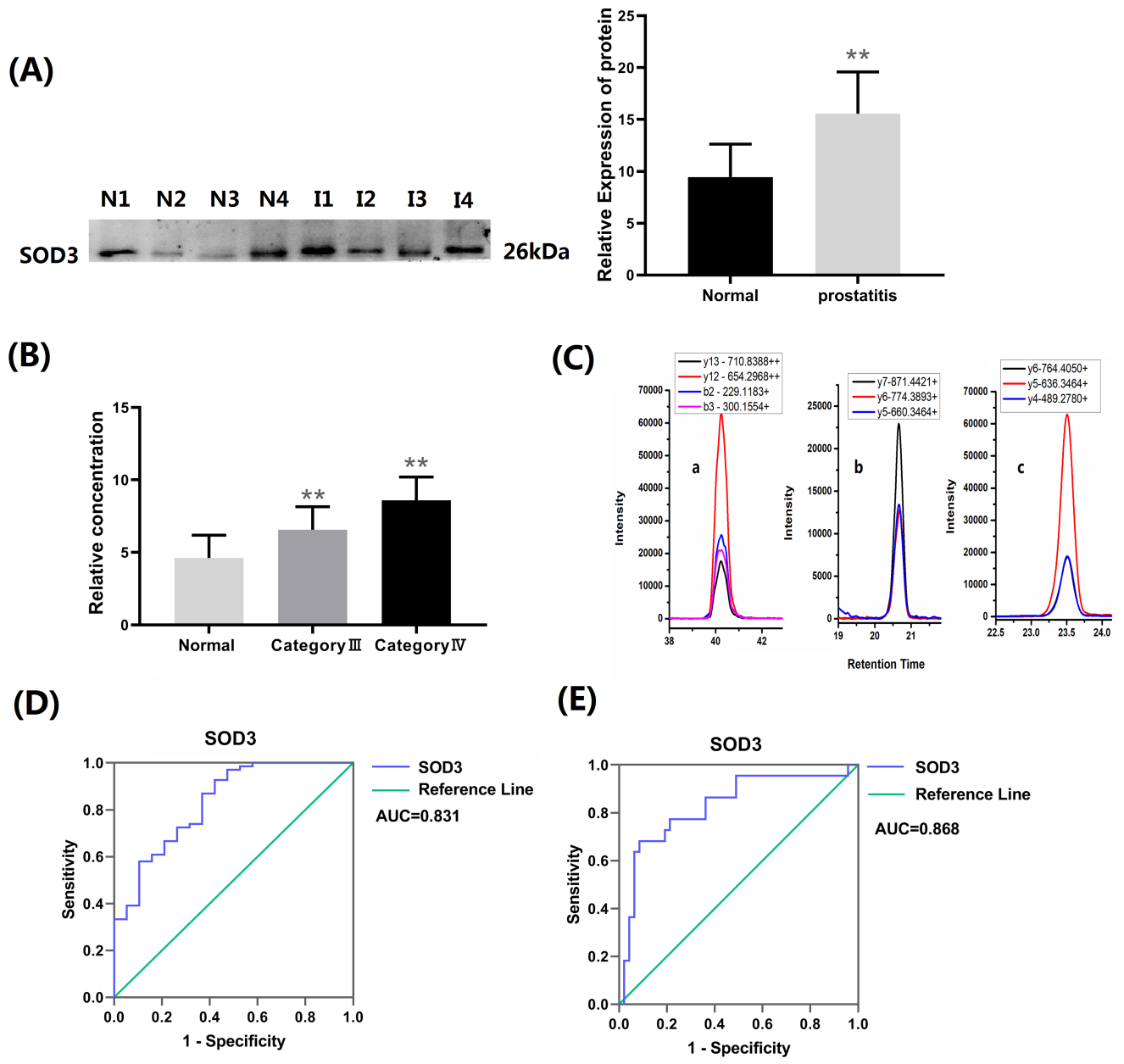

Figure 2

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.

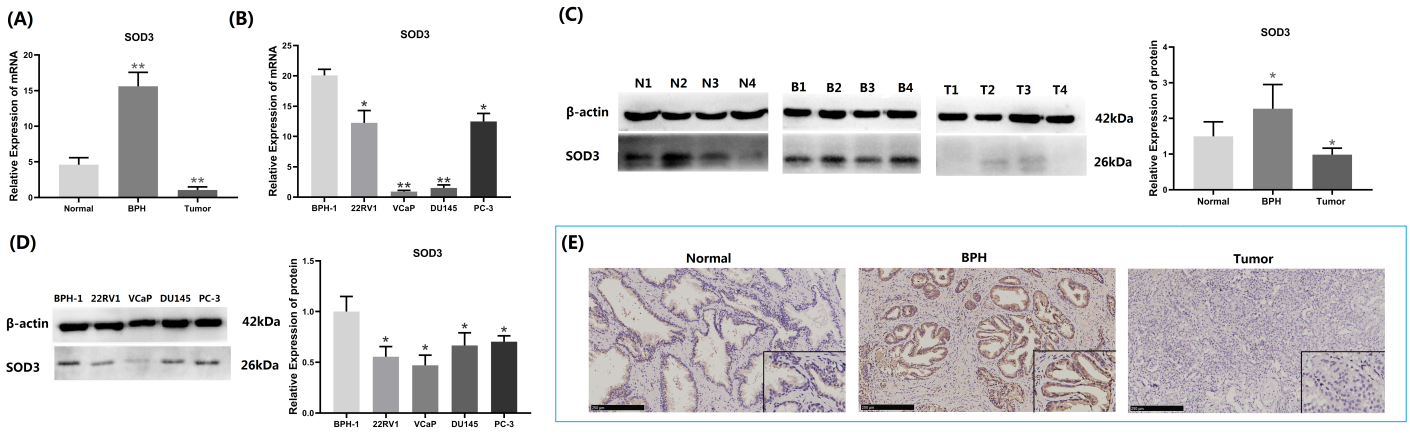

Figure 3

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: 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 nomal tissues.

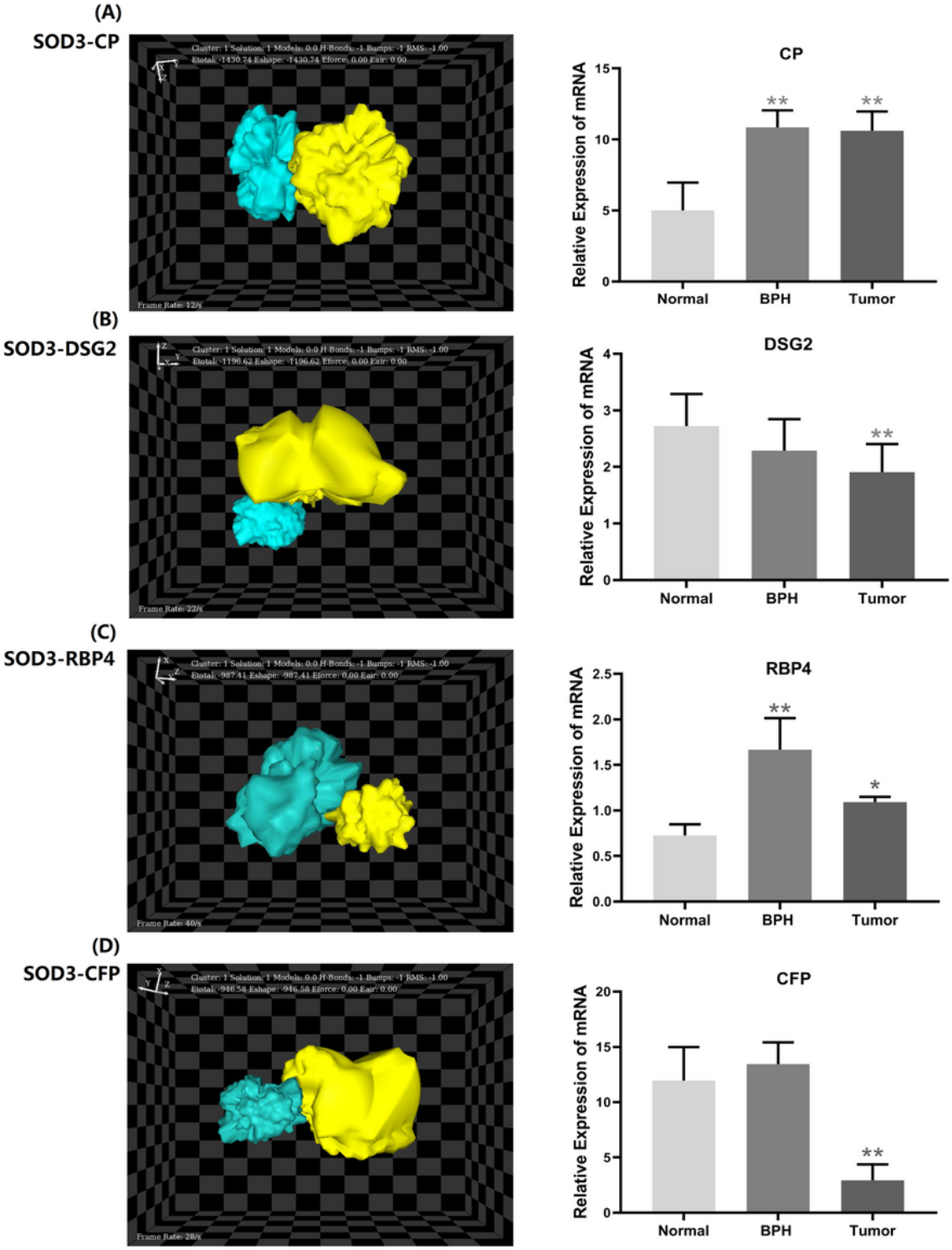

Figure 4

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.

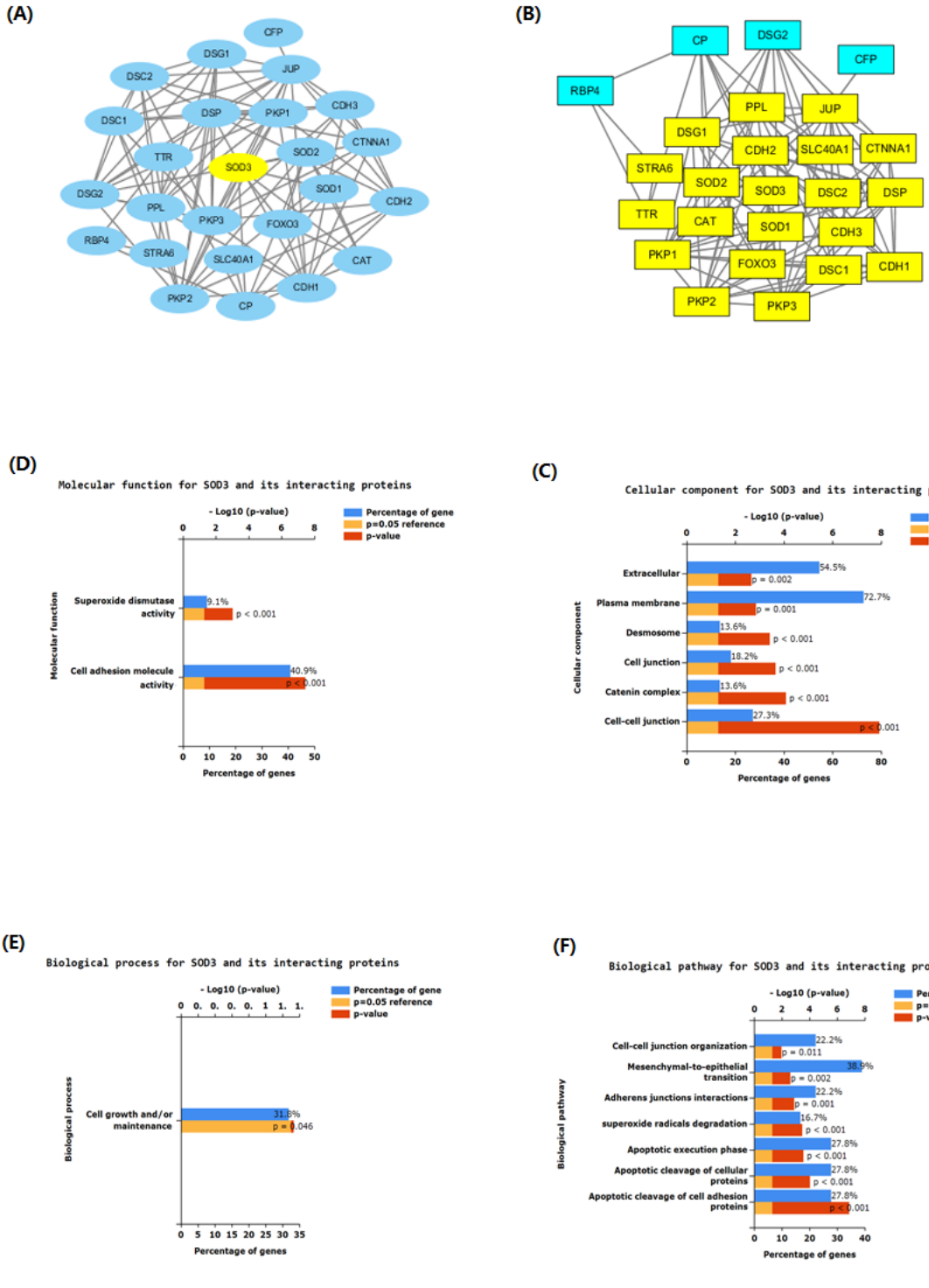

Figure 5

Results of bioinformatic enrichment analysis for SOD3 and its interactors: A. PPI network of SOD3 and its interacting proteins. B. Core module for SOD3 and its interaction partners. Yellow nodes represented module protein, while blue nodes represented potential protein that may interact with SOD3. C. cellular component; D. molecular function; E. biological process; F. biological pathway.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.doc