Investigation of the Mechanism of Shengmai Injection on Sepsis by Network Pharmacology Approaches

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Shengmai injection (SMI) contains Ginsen Radix et Rhizoma Rubra, Ophiopogon japonicus, and Schisandrae Chinensis Fructus. It invigorates Qi, nourishes Yin, and promotes blood circulation. SMI is used as add-on for supportive treatment in managing patients with sepsis, systemic inflammatory syndrome, and septic or hemorrhagic shock. It is a compound with multiple components targeting multiple molecular networks; exploring its complex antisepsis mechanism in a suitable model is of great importance for sepsis treatment [12]. Network pharmacology is a useful tool for systemic investigation of the mechanisms of multiple component drugs [13, 14]. Its approach has been used to study “compound-protein/gene-disease” pathways which reveal complexities among drugs, biological systems, and diseases from

1. Introduction

Sepsis is a deregulated body response to infection, triggering inflammatory reactions that can cause systemic symptoms and damage multiple organs. Release of cytokines mediates uncontrolled inflammatory cascades that result in dysfunction and failure of multiple major organs and septic shock [1, 2]. Managing infection is the most critical strategy for sepsis therapy. However, these treatments could cause various side effects [3]. Clinically, sepsis is managed with early use of antibiotics and glucocorticoids. Traditional Chinese medicine, including Xuebijing injection [2, 4], Shenfu injection [5], and SMI [6], provides supportive effects in sepsis treatment.

Shengmai injection (SMI) origins from the ancient prescription of Chinese medicine Shengmaiyin; it contains Ginsen Radix et Rhizoma Rubra, Ophiopogon japonicus, and Schisandrae Chinensis Fructus. It invigorates Qi, nourishes Yin, and promotes blood circulation. SMI is used as add-on for supportive treatment in managing patients with sepsis, systemic inflammatory syndrome, and septic or hemorrhagic shock [7–9]. The frequency of adverse events associated with SMI is low [10, 11]. There have been few reports on the evaluation of SMI effective components and their underlying mechanisms despite they are used as supportive interventions for sepsis treatment. It is a compound with multiple components targeting multiple molecular networks; exploring its complex antisepsis mechanism in a suitable model is of great importance for sepsis treatment [12]. Network pharmacology is a useful tool for systemic investigation of the mechanisms of multiple component drugs [13, 14]. Its approach has been used to study “compound-protein/gene-disease” pathways which reveal complexities among drugs, biological systems, and diseases from
2. Materials and Methods

2.1. Equipment and Reagents. Water Acquity H-class UPLC, equipped with quaternary high-pressure pump, automatic sampler, and PDA detector (Milford, USA); METTLER AB265-S electronic analytical balance (Zurich, Switzerland); and SB25-12DT ultrasonic analytical balance (Ningbo, China) were used. Standard ginsenoside Rb1 (no. 171018), ginsenoside Rb2 (no. 171009), ginsenoside Rd (no. 170530), ginsenoside Re (no. 170924), ginsenoside RF (no. 171126), ginsenoside Rg1 (no. 180105), Ophiopogon D (no. 171126), schlisanol A (no. 180109), and schlisanin A (no. 171231) were all purchased from Shanghai Ronghe Medical Pharmaceutical Technology Co., Ltd (Shanghai, China). Ginko biloba leaf extract was purchased from Merck (Buckram, Germany). Distilled water was purchased from Watson (Hong Kong, China). There were 9 batches of SMI: S1, 16120101005; S2, 160502; S3, 17071014; S4, 17040423; S5, 1704252; S6, 17091302; S7, 17092903; S8, 17061103; and S9, 17053005.

2.2. Standards and Sample Solution Preparation. Standard stock solutions of ginsenoside Rb1 (9.49 mg·mL⁻¹), ginsenoside Rb2 (10.22 mg·mL⁻¹), ginsenoside Rb3 (6.69 mg·mL⁻¹), ginsenoside Rd (6.24 mg·mL⁻¹), ginsenoside Re (6.24 mg·mL⁻¹), ginsenoside Rf (7.35 mg·mL⁻¹), ginsenoside Rg1 (13.24 mg·mL⁻¹), ginsenoside Rg2 (6.58 mg·mL⁻¹), Ophiopogon D (5.12 mg·mL⁻¹), schlisanol A (5.60 mg·mL⁻¹), and schlisanin A (3.27 mg·mL⁻¹) were all purchased from Shanghai Ronghe Medical Pharmaceutical Technology Co., Ltd (Shanghai, China). Distilled water was purchased from Watson (Hong Kong, China). There were 9 batches of SMI: S1, 16120401005; S2, 160502; S3, 17071014; S4, 17040423; S5, 1704252; S6, 17091302; S7, 17092903; S8, 17061103; and S9, 17053005.

2.3. UPLC Conditions. The analyte was separated by a Waters Acquity UPLC BEH C18 (2.1 mm × 50 mm, 1.7 µm) column. The mobile phases used were solvent A (acetoneitrile) and solvent B (water) with gradient elution (Table 1). The analysis was carried out at a flow rate of 0.3 mL/min. The column temperature was set to 40°C. UV detection wavelength was over the range of 190 to 400 nm. 5 µL of the sample was injected. 210 nm was selected as the extraction wavelength of the fingerprints.

2.4. Precision of the Method. Method precision was determined by analyzing the same sample SMI (S1, 16120101005) five consecutive times in a day. The peak of schlisanin A was used as the reference peak. Relative standard deviation (RSD) was calculated from the relative peak area (RPA) or relative retention time (RRT) of each characteristic peak.

2.5. Sample Stability. Sample stability was evaluated using the same SMI (S1, 16120401005) after 0, 2, 4, 6, 8, 12, and 24 hours. The peak of schlisanin A was used as the reference peak. RSD was calculated from RPA or RRT of each peak to the reference peak from the chromatographic profiles of samples.

2.6. Repeatability. Repeatability was evaluated by analyzing six independently prepared SMI samples. The peak of schlisanin A was used as the reference peak. RSD was calculated from RPA or RRT of each peak to the reference peak from the chromatographic profiles of samples.

2.7. Information about Databases and Software of Network Pharmacology. TCMS database (https://tcmspw.com/tcmsp.php), PubChem CID (https://pubchem.ncbi.nlm.nih.gov/search/), STITCH (http://stitch.embl.de/), Human Phenotype Ontology (HPO, https://hpo.jax.org/app/), STRING database (https://string-db.org), OMIM database (https://omim.org/), and DAVID database (https://david.ncifcrf.gov/summary.jsp) were used. Cytoscape software v3.5.1 was used.

2.8. Network Construction. Data acquisition and processing were done in databases which include SciFinder and TCMS. Additionally, PubChem CID for each active ingredient of SMI was obtained from PubChem. We used SMILES format in STITCH chemical association networks and obtained the interaction complex between SMI bioactive components and the potential target protein in humans. Using HPO as a tool, we annotated and analyzed the core protein targets that participate in sepsis. The primary as well as predicted interactions between SMI target proteins and proteins involved in sepsis were analyzed in the STRING database.
Figure 1: UPLC fingerprints of 9 batches of SMI.

Figure 2: Contrast fingerprint of SMI.

Figure 3: UPLC chromatogram of SMI and mixed reference at 210 nm: (a) samples of SMI (S1); (b) mixed reference (10, ginsenoside Rg1; 11, ginsenoside Re; 15, ginsenoside Rf; 19, ginsenoside Rb1; 20, ginsenoside Rg2; 22, ginsenoside Rb2; 23, ginsenoside Rb3; 24, schisandrol A; 25, ginsenoside Rd; 37, Ophiopogon D; and 39, schisandrin A).
database. We collected core proteins that are highly associated with sepsis, while proteins with low correlation were filtered out [16]. The molecular interplays between SMI key targets and sepsis proteins were visualized in the Cytoscape platform. We calculated the degree, betweenness, and closeness of the targets; proteins with topological parameters greater than the corresponding median values were considered as major hits. The selected proteins were validated in

Table 2: Comparison of similarity of Shengmai injection in different batches.

|     | S1   | S2   | S3   | S4   | S5   | S6   | S7   | S8   | S9   | Control fingerprints |
|-----|------|------|------|------|------|------|------|------|------|---------------------|
| S1  | 1    | 0.954| 0.983| 0.976| 0.916| 0.938| 0.944| 0.942| 0.953| 0.968               |
| S2  | 0.954| 1    | 0.943| 0.945| 0.821| 0.869| 0.89  | 0.885| 0.922| 0.914               |
| S3  | 0.983| 0.943| 1    | 0.992| 0.931| 0.962| 0.967| 0.965| 0.973| 0.986               |
| S4  | 0.976| 0.945| 0.992| 1    | 0.927| 0.945| 0.952| 0.95  | 0.967| 0.978               |
| S5  | 0.916| 0.821| 0.931| 0.927| 1    | 0.944| 0.944| 0.939| 0.931| 0.963               |
| S6  | 0.938| 0.869| 0.962| 0.945| 1    | 0.996| 0.996| 0.983| 0.98  | 0.98                |
| S7  | 0.944| 0.89  | 0.967| 0.952| 0.944| 0.996| 1    | 0.998| 0.989| 0.984               |
| S8  | 0.942| 0.885| 0.965| 0.95  | 0.939| 0.996| 0.998| 1    | 0.989| 0.98                |
| S9  | 0.953| 0.922| 0.973| 0.967| 0.931| 0.983| 0.989| 0.989| 1    | 0.98                |
| Control fingerprints | 0.968| 0.914| 0.986| 0.978| 0.963| 0.98 | 0.984| 0.98 | 0.98 | 1                  |

Figure 4: Prediction of component-target of Shengmai injection by STITCH.
the OMIM database to establish protein-disease association and construct “SMI Targets-Sepsis Targets” network.

2.9. Prediction of the SMI-Antisepsis Mechanism. A list of the selected top 20 key proteins was uploaded to the DAVID database for functional annotation and enrichment analysis to obtain the main pathways and network distribution that confer potential mechanisms for SMI treatment. Only pathways with \( p < 0.05 \) were considered for mechanism prediction.

3. Results and Discussion

3.1. Establishment of SMI Fingerprints and the Results of Methodological Evaluation. The RSDs of RPA and RRT for precision, repeatability, and sample stability were lower than 3%, respectively. The results showed that the fingerprint method developed for analysis of SMI is reliable and applicable. Figures 1 and 2 show the UPLC chromatogram fingerprints of 9 batches of SMI.

3.2. Reference Peak and Common Peak. The peak of schisantinerin A was used as the reference peak; it showed as an intense peak with preferable chromatographic peak resolution and RRT. Peaks that existed in all SMI samples were appointed as “common peaks.” 44 common peaks were detected in SMI samples, in which 11 peaks were identified (Figure 3): ginsenoside Rg1 (peak #10), ginsenoside Re (peak #11), ginsenoside Rf (peak #15), ginsenoside Rb1 (peak #19), ginsenoside Rb2 (peak #20), ginsenoside Rb2 (peak #22), ginsenoside Rb3 (peak #23), schisandrol A (peak #24), ginsenoside Rd (peak #25), Ophiopogonin D (peak #37), and schisantherin A (peak #39).

3.3. Similarity of Fingerprints of 9 Batches of SMI. The similarities of all chromatographic patterns among the samples (Table 2) were calculated using software “Chromatographic Fingerprints of Traditional Chinese Medicine, version: 2004A.” The similarities of 9 SMI batches were greater than 0.91. Therefore, our method was precise, stable, reproducible, and reliable.

3.4. Putative Targets of SMI Ingredients. The SMI components were screened by TCMSP, and the criteria are OB (oral bioavailability) \( \geq 30\% \) and, meanwhile, DL (drug-like) \( \geq 0.18 \) [17–19]. All the components were confirmed in the PubChem database [19] (total 9 bioactive SMI components: ginsenoside Rb1, ginsenoside Rb2, ginsenoside Re, ginsenoside Rg1, ginsenoside Re, ginsenoside Rf, ginsenoside Rb1, ginsenoside Rb2, ginsenoside Rb3, schisandrol A, ginsenoside Rd, Ophiopogonin D, schisantherin A, and schisantherin B). The putative targets of SMI were predicted and listed in Table 3.

| No. | Targets               | Evidence-Based Complementary and Alternative Medicine |
|-----|----------------------|-------------------------------------------------------|
| 1   | E2F5                 |                                                       |
| 2   | HDAC2                |                                                       |
| 3   | VPS33A               |                                                       |
| 4   | TFDP2                |                                                       |
| 5   | MSN                  |                                                       |
| 6   | KCNE1                |                                                       |
| 7   | RB1                  |                                                       |
| 8   | CDK1                 |                                                       |
| 9   | TFDP1                |                                                       |
| 10  | MDM2                 |                                                       |
| 11  | VPS16                |                                                       |
| 12  | VPS39                |                                                       |
| 13  | E2F4                 |                                                       |
| 14  | E2F2                 |                                                       |
| 15  | CDK2                 |                                                       |
| 16  | VPS41                |                                                       |
| 17  | KCNA4                |                                                       |
| 18  | HSP90AA1             |                                                       |
| 19  | CDK4                 |                                                       |
| 20  | CDK6                 |                                                       |
| 21  | HDAC1                |                                                       |
| 22  | CCNA2                |                                                       |
| 23  | E2F1                 |                                                       |
| 24  | RBBP4                |                                                       |
| 25  | ABL1                 |                                                       |
| 26  | SPI1                 |                                                       |
| 27  | CCND2                |                                                       |
| 28  | E2F3                 |                                                       |
| 29  | RBL2                 |                                                       |
| 30  | CCNE1                |                                                       |
| 31  | KCNA3                |                                                       |
| 32  | KCNA1                |                                                       |
| 33  | RAF1                 |                                                       |
| 34  | GNAS                 |                                                       |
| 35  | LRRK1                |                                                       |
| 36  | STNN4                |                                                       |
| 37  | HMOX1                |                                                       |
| 38  | PPI1R3A              |                                                       |
| 39  | PHAG                 |                                                       |
| 40  | PTFG5E3              |                                                       |
| 41  | GNAL                 |                                                       |
| 42  | CCND1                |                                                       |
| 43  | CYP3A4               |                                                       |
| 44  | PPIB                 |                                                       |
| 45  | LRRK1                |                                                       |
| 46  | GALE                 |                                                       |
| 47  | PPIC                 |                                                       |
| 48  | RHCE                 |                                                       |
| 49  | VPS18                |                                                       |
| 50  | ANGPT1L4             |                                                       |
| 51  | IC1T                 |                                                       |
| 52  | DLG4                 |                                                       |
| 53  | CKS2                 |                                                       |
| 54  | TMPS511D             |                                                       |
| 55  | KCNQ1                |                                                       |
| 56  | CCNB1                |                                                       |
| 57  | CCNB2                |                                                       |
| 58  | CCNB3                |                                                       |
| 59  | CDC20                |                                                       |

Table 3: Continued.

| No. | Targets               | Evidence-Based Complementary and Alternative Medicine |
|-----|----------------------|-------------------------------------------------------|
| 60  | CDC37                |                                                       |
| 61  | CDC6                 |                                                       |
| 62  | CDKN1B               |                                                       |
ginsenoside Rf, ginsenoside Rg1, ginsenoside Rg2, schisandrol A, schisantherin A, and Ophiopogon D. The chemical structures and molecular properties were analyzed and uploaded to the STITCH database for predicting targets that interact with SMI ingredients [20]. A total of 62 targets (Figure 4 and Table 3) showed potential interaction with 9 SMI ingredients.

3.5. Acquisition of Known Therapeutic Sepsis Targets. Sepsis targets were collected from the HPO database. The keyword "sepsis" was used to search known therapeutic targets for sepsis in humans [21]. A total of 58 sepsis targets (Table 4) were acquired from the HPO database, and targets were further verified in the NCBI database.

3.6. Results of Network Construction. The putative targets of SMI active ingredients and sepsis disease targets were determined based on the protein–protein interactions [22]. The interplays amongst SMI targets, known therapeutic targets for sepsis, and interactional human targets were combined to construct the network. The network illustrates the relationship between SMI targets and sepsis targets. The overall interaction network (Figure 5) was visualized using Cytoscape (sepsis targets in red circles and SMI targets in blue squares); the larger a node, the more targets it contains and more important in sepsis management. Targets with higher values of “degree,” “betweenness,” “closeness,” and “coreness” (above the median value of all the network nodes) were identified [23, 24]. Targets which might play unimportant roles in the network according to the topological features were discarded [25]. Median value of “degree,” “betweenness,” and “closeness” was 19, 0.014, and 0.449, respectively. Top twenty proteins were selected as key sepsis therapeutic targets (Figure 6 and Table 5), including ABL1, CCND1, CDK family (CDK1, CDK2, CDK6, and CDKN1B), RB1, HSP90AA1, SMARCA4, RBL2, CTNNB1, MDM2, SP1, LRRK1, BTK, PIK3R1, TMPRSS11D, ACTG2, CD79A, and RET.

Sepsis causes life-threatening organ dysfunction due to a host’s complex systemic inflammatory response to infection [26]. In the present study, we identified core proteins that may play important roles in SMI-supportive treatment in sepsis. ABL1 is a tyrosine-protein kinase which is important for cell growth and survival, cytoskeleton remodeling in response to extracellular stimuli, autophagy, and apoptosis [27–29]. It also regulates multiple pathological signaling cascades during infection that alter vascular permeability and the endothelium barrier in inflammation [30].

Cyclin-dependent kinase 1 (CDK1) is a member of the Ser/Thr protein kinase family. Its kinase activity is controlled by cyclin [31, 32]. CCND1/CDK4 and CDK2 are critical for G1/S phase transition. It has been shown that a kidney injury is associated with G1 phase arrest in cecal ligation and puncture- (CLP-) induced sepsis, while upregulation of CCND1/CDK4 and CCNE/CDK2 activates Rb leading to revival of cell cycle progress and recovery of kidney function.

| No. | Targets |
|-----|---------|
| 1   | CYBB    |
| 2   | RMRP    |
| 3   | RAG1    |
| 4   | RAG2    |
| 5   | TGM1    |
| 6   | LIG4    |
| 7   | SEMA3D  |
| 8   | NIPAL4  |
| 9   | SEMA3C  |
| 10  | TCF3    |
| 11  | MYH11   |
| 12  | ATP7A   |
| 13  | WAS     |
| 14  | WIPF1   |
| 15  | GALT    |
| 16  | HLA-B   |
| 17  | G6PC3   |
| 18  | DCLRE1C |
| 19  | NRTN    |
| 20  | ABCA12  |
| 21  | PIK3R1  |
| 22  | IGHM    |
| 23  | NFKB2   |
| 24  | BTK     |
| 25  | NCF1    |
| 26  | BLNK    |
| 27  | APC     |
| 28  | LRRK1A  |
| 29  | ELANE   |
| 30  | ACTG2   |
| 31  | CYP4F22 |
| 32  | AK2     |
| 33  | CD79A   |
| 34  | CD79B   |
| 35  | NCF2    |
| 36  | IKZF1   |
| 37  | ALOX3E3 |
| 38  | NCF4    |
| 39  | SERAC1  |
| 40  | LIPN    |
| 41  | CHD7    |
| 42  | IGLL1   |
| 43  | RET     |
| 44  | PLEC    |
| 45  | CTNNB1  |
| 46  | ECE1    |
| 47  | ADA     |
| 48  | IL2RG   |
| 49  | ITGB4   |
| 50  | GDNF    |
| 51  | MUT     |
| 52  | ALOX12B |
| 53  | EDN3    |
| 54  | EDNRB   |
| 55  | IL7R    |
| 56  | FERMT3  |
| 57  | TFC     |
| 58  | CYBA    |
Figure 5: Prediction of protein interaction between SMI and sepsis by STRING (blue-square nodes are targets of SMI; red circular nodes are targets of sepsis).

Figure 6: Key targets of the SMI-sepsis protein interaction network (yellow is the key target; blue-square nodes are SMI targets; and red circular nodes are sepsis targets).
48 hours after CLP [33, 34]. The findings demonstrate that cell cycle arrest occurs in sepsis, and drugs that regulate cell cycle proteins may be a means to rescue organ injury [35]. In addition, the targets of SMI are more involved in DNA replication and transcription; for example, MDM2, E3 ubiquitin-ligase, mediates ubiquitination and degradation of p53. It mediates apoptosis in organ injury and malignant transformation [36]. SMI may inhibit MDM2 and keep p53 active; therefore, it promotes cells staying in the G1/G2 phase and alleviates cell injury in sepsis.

Molecular chaperone heat-shock protein (HSP 90) is extensively expressed by cells, and its expression increases upon stimulation [37]. HSPs are associated with multiple organ failure in sepsis [38]. In the vast immune response in sepsis, stressed cells release HSPs that are regarded as “danger signal” to neighboring and immune cells [39]. HSP90-α has been shown to interact with about 200 client proteins, including signal proteins in the inflammatory pathway such as NF-κB, Akt, and IKK, to interfere inflammation [40–42]. Moreover, HSP90-α, as abundant “chaperone,” is one of the main mediators that activates bacterial lipopolysaccharide. It interacts with proteins in the PI3K/Akt pathway and is essential in promoting the immune response and improving host defense to pathogens. Inhibition of HSP90-α prevents sepsis-associated acute lung injury; therefore, block HSP90-α offers a novel treatment for lung injury in sepsis [43–45].

BTK (tyrosine-protein kinase) is a component of the toll-like receptor (TLR) pathway and plays important roles in innate and adaptive immunity. Key target CD79A is required for efficient differentiation of pre- and pre-B-cells. It cooperates with CD79B and binds to the B-cell antigen receptor complex (BCR) for initiation of the signal transduction cascade. It is pivotal in regulating immunity and inflammation [46]. Network pharmacology revealed SMI represses BTK expression/activation, blocks signals through multiple pathways (TLR, B-cell antigen receptor signal, and apoptosis), and consequently ameliorates cell apoptosis and organ injury. SMI acts as a whole, and each formula has its corresponding targets/syndromes; thus, SMI prescription acts on multiple key targets, and the network pharmacology study of SMI provides insights into understanding its fundamental mechanisms.

### 3.7. KEGG and GO Analysis

To cluster the biological functions of SMI and its targets, data were uploaded to KEGG, and results revealed SMI active formulae target pathways including cancer, cell cycle, p53, B-cell receptor, and ErbB pathways (Table 6). SMI regulates the interplay and synergy among the pathways of immunity, inflammation, and apoptosis to protect cellular and organ injury in sepsis. p53 pathway regulates mitochondrial fission and mitochondrial biogenesis via AMPK, and it alters PKM2-dependent glycolysis. Global deletion of PKM2 results in systemic inflammation in mice [47]. Our GO analysis (Table 7) showed that SMI ingredients regulate multiple
biological processes including cell cycle, energy metabolism, cellular signal transduction, transcription regulation, and immunity development. Our data indicate the putative role of SMI in alleviating systemic inflammation and deregulating immunity in the host; moreover, it regulates energy utilization and promotes energy homeostasis and therefore ameliorates multiple organ failure associated with sepsis. It is also in agreement with the idea of SMI used in the early phase of sepsis.

4. Conclusion
An UPLC method was developed for analysis of SMI fingerprints. Forty-four peaks were selected as common peaks, of which 11 peaks were identified. The consistency in the chromatograms of 9-batch samples reflects the presence of similar chemical constituents (similarities greater than 0.91). The technique was proven to be useful in SMI quality control. A total of 9 active components of SMI target 20 key proteins including ABL1, CDK, HSP90, BTK, PIK3R1, and CD79A. These proteins are enriched in cell cycle, p53 signaling pathway, B-cell receptor signaling pathway, and ErbB pathway. It is likely that the pharmacological mechanisms of SMI in sepsis treatment are of multiple dimensions that are associated with regulation of cell cycle, energy metabolism, cellular signal transduction, transcription regulation, and immunity development. Further experiments are needed to validate our prediction.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare no conflicts of interest.

Authors’ Contributions
Juan Lu and Xinkai Lyu contributed equally to this work. XC, ZD, and JL conceived, designed, and supervised the project. RC, YY, XL, MD, and XZ performed the experiments. JL and XL integrated all the data and wrote the paper.

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Table 7: GO biological analysis.

| No. | Name                                           | Count | p value      |
|-----|------------------------------------------------|-------|-------------|
| 1   | Cell cycle                                      | 10    | 3.5×10^{-7} |
| 2   | Phosphorylation                                 | 9     | 6.1×10^{-6} |
| 3   | Phosphorus metabolic process                    | 9     | 2.6×10^{-5} |
| 4   | Intracellular signaling cascade                 | 9     | 1.6×10^{-4} |
| 5   | Regulation of transcription                     | 9     | 1.8×10^{-2} |
| 6   | Mitotic cell cycle                              | 8     | 4.1×10^{-7} |
| 7   | Protein amino acid phosphorylation              | 8     | 2.1×10^{-3} |
| 8   | Positive regulation of macromolecule metabolic process | 8 | 1.0×10^{-4} |
| 9   | Interphase                                      | 7     | 5.0×10^{-9} |
| 10  | Regulation of cell proliferation                | 7     | 5.4×10^{-3} |
| 11  | Regulation of transcription, DNA-dependent      | 7     | 3.0×10^{-2} |
| 12  | Regulation of RNA metabolic process             | 7     | 3.3×10^{-2} |
| 13  | Regulation of mitotic cell cycle                | 6     | 2.6×10^{-15}|
| 14  | Hemopoiesis                                     | 6     | 1.5×10^{-5} |
| 15  | Hemopoietic or lymphoid organ development       | 6     | 2.4×10^{-5} |
| 16  | Immune system development                       | 6     | 3.1×10^{-5} |
| 17  | Positive regulation of nitrogen compound metabolic process | 6 | 1.6×10^{-3} |
| 18  | Positive regulation of cellular biosynthetic process | 6 | 2.1×10^{-3} |
| 19  | Positive regulation of biosynthetic process     | 6     | 2.2×10^{-3} |
| 20  | Regulation of transcription from RNA polymerase II promoter | 6 | 2.7×10^{-3} |
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