
1 **Bioinformatics-based Prediction of the mechanism and**
2 **targets for BushenjieduDecoction in preventing relapse**
3 **of acute leukemia**

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13 **1. Abstract**

14 **1.1 Background**

15 Leukemia is a lethal myeloproliferative disorder, its' relapse following
16 chemotherapy is the major concern in clinical practice. For a long time, we found
17 that traditional Chinese medicines such as Bushenjiedudecoction (BSJD) have
18 significant effects on delaying relapse. However, the underlying mechanisms are
19 not clear, which limits the clinical application of BSJD decoction.

20 **1.2 Methods**

21 Therefore, we tried to make some explorations in this study. We isolated
22 mesenchymal stem cells (MSC) after treated them with BSJD for proteomic
23 analysis. And then 109 targets were screened out through analysis of the shared
24 proteins of that affected by BSJD and those related to leukemia. Subsequently, the
25 data were analyzed by GO functions, KEGG pathways, PPI network and
26 topological analysis, and then some nodes were selected for animal experiment.

27 **1.3 Results**

28 As a result, we demonstrated the effective targets of BSJD on MSC through
29 bioinformatics analysis and explored the potential mechanism of BSJD from its
30 influence on niches. These targets contains Hspb1、Dnmt1、Mmp2、Thbs1、Crebbp、
31 Hmgb1、Acta2、Cdkn1b、Atg7、Tsc2 and Icam1. Afterwards, we confirmed BSJD
32 reduced the gene expression of ICAM-1 through cultured MSC in vitro.

33 **1.4 Conclusions**

34 We screened the potential targets of BSJD on MSC through proteomics and

35 bioinformatics analysis, and selected some genes for experimental verification.
36 These studies demonstrated the effect of BSJD on MSC. We hope that this research
37 method could provide a new way of systematically studying the effects of
38 traditional Chinese medicine on diseases.

39 **Key words:**

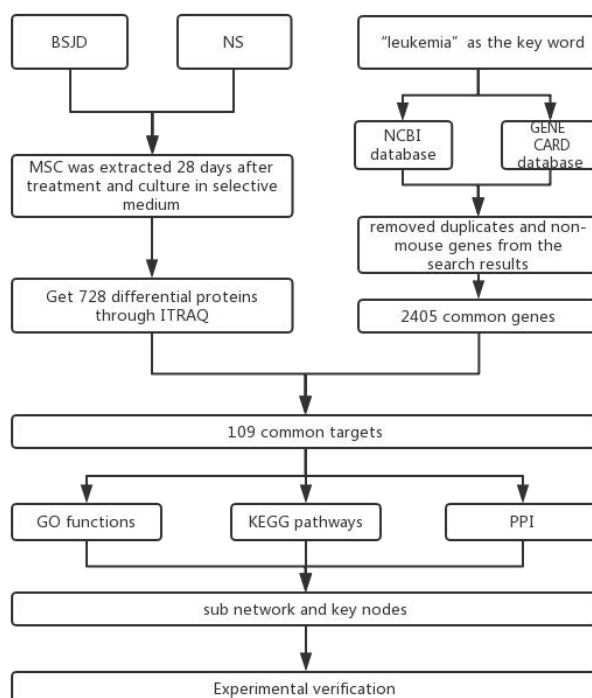
40 Drug target prediction, gene interaction network, acute leukemia, relapse,
41 predictors.

42 **2. Background**

43 Chemotherapy is the primary choice of adult acute leukemia in clinic, but
44 usually the prognosis is not satisfied (1, 2). Relapse after chemotherapy is t
45 he major concern for acute leukemia treatment. Both acute myeloid leukemia
46 (AML) and acute lymphoblastic leukemia (ALL) have low rates of long time
47 remission, especially the response rate is only 30%-40% in ALL (3, 4).AL
48 is a myeloproliferative disease that characterized by malignant clone of hema
49 topoietic stem cells in transformed niches. Bone marrow microenvironment(ni
50 che)supports the homing to bone marrow, self-renewal, proliferation and diffe
51 rentiation of hematopoietic stem cells in bone marrows(5). It is consisted of
52 osteocytes, perivascular cells, endodontic cells, adipocytes, macrophages, and
53 mesenchymal stem cells (MSCs)(6). And MSC plays an important role in the
54 se components. It is more than a material basis for niche, because it also m
55 ediates the homing of HSC through Nest in.(7). Recent studies suggested tha
56 t leukemia stem cells (LSCs) can transform niche into a LSC growth-permiss
57 ive and HSC growth-restrictive microenvironment through remodeling activity
58 in the course of leukemia (8). Different cytokines achieves this transformati
59 on through variety pathways and thereby mediates LSC mobilization, homing
60 and drug resistance, which eventually become an important cause of clinical
61 recurrence (9). Treatment to hijacked or transformed niches may provide no
62 vel therapies to delay the leukemia relapse(10).

63 As an important part of Chinese health system, traditional Chinese medicine
64 (TCM) has been used extensively. In disease free interval, we use a variety of
65 TCMs as adjuvant treatments to delay the recurrence. In these Chinese patent
66 medicines we found that Bushenjiedudecoction (BSJD) has a more obvious efficacy,
67 but the specific mechanism is still unclear. Although the research of TCM is facing
68 challenges, the developments of new technologies provide new methods to study
69 the complex mechanism of it. Especially, with the emerging of high-throughput
70 sequencing technology, it makes it possible to obtain large-scale protein data and
71 make comparative analysis of proteomic in a single experiment (11, 12).As a

72 next-generation of high-throughput technology through relative and absolute
 73 quantitative proteomics analysis with high sensitivity and repeatability, iTRAQ has
 74 been widely used in cancer research (13, 14). Meanwhile, various bioinformatics
 75 databases based on the proteomics also provide powerful support for functional
 76 annotation of proteins, classifying protein sequences into families, and comparative
 77 protein structure modeling (15). They all promote the research on the mechanism
 78 and target location of drugs (16). At the same time, it also provides a new objective
 79 way of scientific research and evaluation of TCM compounds (17). Inspired by
 80 these methods, we designed our experiment as shown in the figure, including: in
 81 vivo administration, cells culture, high-throughput sequencing, etc. Through these
 82 processes, possible targets of BSJD were obtained and bio-informatics analysis was
 83 carried out on these targets. (Fig.1)



84
 85 Fig.1|In the flowchart of target prediction and verification, the potential targets of BSJD were
 86 screened by proteomics and enrichment analysis, and the functional and net-work of these
 87 potential targets were analyzed by GO, KEGG, PPI and topological analysis. Finally, the effect of
 88 BSJD on the selected potential targets was demonstrated by experiments.

89 3. Materials and methods

90 3.1 Chinese medicine ingredients of BSJD

91 Bushenjiedudecoction is composed of Quanxie (*Buthusmartensii*Karsch),
 92 Jiangcan (*Bombyx Batryticatus*), Baifuzi (*TyphoniiRhizoma*), Lujiaopian (*Cornu*
 93 *Cervi*), Guiban (*Tortoise Shell Caraoax et PlastrumTestudinis*), Renshen (*Panax*
 94 *Ginseng C. A. Mey*), Gouqizi(*Lycii Fructus*),Gancao(licorice),Qingdai (*Indigo*

95 Naturalis), Huanshi (Talc Powder PulvisTalci).

96 **3.2 Proteomics**

97 The high-throughput sequencing was identified by Tianjin Seweisi
98 Biotechnology Co. Ltd. through ITRAQ technology developed by ABSCIEX
99 (Pro.No.QLSWS003-20190614001) .

100 **3.3 Prediction of common targets for BSJD and leukemia**

101 Leukemia as the key word was searched in two data bases: NCBI ([https://](https://www.ncbi.nlm.nih.gov/gene/)
102 www.ncbi.nlm.nih.gov/gene/)and GENE CARD(<https://www.genecards.org/>).We
103 removed duplicates and non-mouse genes from the search results and found
104 2045 common targets in two databases. Subsequently, these differential protei
105 ns were compared with 728 differential proteins after BSJD treatment.

106 **3.4 Analysis of GO, KEGG and key sub net.**

107 GO function and KEGG pathway common targets were analyzed with
108 Uniprot(<https://www.uniprot.org/>).Co-expression network of common targets was
109 established by Gene Mania (<http://pages.genemania.org/>). 82 common targets
110 which were greater than medium-confidence (> 0.6) were selected for further
111 analysis through STRING (<https://string-db.org/>) .In addition, topology analysis
112 was conducted by MCODE to calculate the degree centrality and extract
113 sub-network, in order to identify key nodes. Ultimately, targets of the sub-network
114 were selected to perform further animal experiment.

115 **3.5 Animal experiment**

116 All animal experiments were conducted in the animal center of the Institute of
117 Radiation Medicine Chinese Academy of Medical Sciences (IRM) and they all
118 have been approved. The BALB/c mice were purchased from Beijing Huafukang
119 Biotechnology Co. Ltd. (animal license no. SCXK -20190008) .

120 7 to 8 week-old male BALB/c mice were used for proteome analysis. Mice were
121 intragastric administrated of 0.2ml BSJD once a day for 4weeks and then got
122 euthanized.

123 The animal model of ALL was generated using 6-week-old male BALB/c
124 mice. 5×10^5 L1210 cells were transplanted into half lethally (5Gy, ^{137}Cs γ ra
125 y source, Gammacell-40, Canada) irradiated mice by tail vein injection. And
126 on the 7th day after transplantation, one mouse was randomly selected from
127 each group to evaluate the proliferation of B-ALL cells in the bone marrow
128 by bone marrow smear.

129 **3.6 Treatment protocol**

130 Their chemotherapy regimen was cytoxan (3mg/m², for onetime) , vindesine
131 (600mg/m² for one time) and prednisone (9mg/kg/day, for 3d) at 10th day after
132 transplanted.

133 BSJD was prepared as decoction at a concentration of 10g/50ml, oral gavage

134 treated once a day at 0.2ml.

135 **3.7 Cell Line and Cell Culture**

136 L1210 cells were purchased from the cell compartment of the Chinese Academy
137 of Medical Sciences (Shanghai, China) and cultured in DMEM (GIBCO,
138 #12800017) with 10% FBS (GIBCO, 10270106) and 1% penicillin-streptomycin at
139 37°C and 5% CO₂.

140 MSC were obtained from mouse femurs and cultured in MesenCult
141 (STEMCELL, CAT#05513), the selective medium for mouse MSC. The adherent
142 culture method was used to culture the MSC, and three subcultures were performed
143 before the experiment.

144 **3.8 RNA Isolation and Real-Time PCR Analysis**

145 Total RNA was extracted using TRIzol. Reverse transcription and real-time
146 quantitative PCR was conducted by Real-time PCR Fluorescent Quantitative
147 Kit (ZSTC,ZS-M-1009) on PCR machine (Eppendorf). Target gene primer seq
148 uences: ICAM-1: Forward primer-CTGAGCCTGCTGGATGAGAC, Reverse prim
149 er-GCCACCATCCTGTTCTGTGA; SHP2: Forward primer-ACTGTGCAGACC
150 CTACCTCT, Reverse primer-GCACGGAGAGAACGAAGTCT. The relative e
151 xpression quantity $2^{-\Delta\Delta Ct}$ value was calculated to compare the differences am
152 ong two groups (three technical replicates were used for each gene).

153

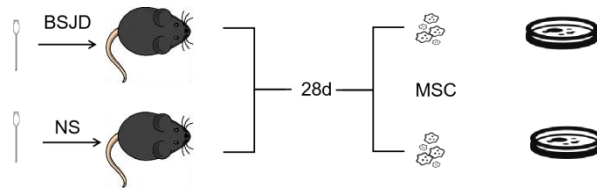
154 **3.9 Statistical Analysis**

155 All data were represented in mean \pm SD. Statistical analysis was performed using
156 GraphPad Prism 8 software with student's t test ($P < 0.05$ was considered with
157 statistical significance). Histogram was made using the Graphpad software.

158 **4. Results**

159 **4.1 Potential Targets of BSJD.**

160 In order to obtain the BSJD target genes, we performed high-throughput
161 proteomics analysis by iTRAQ. First, the 6-week old mice were divided
162 randomly into two groups (n=6). BSJD and Control groups were treated with
163 decoction of BSJD (0.2ml every day) and normal saline (0.2ml every day)
164 respectively. After 28 days treatment, mice were killed and the BM cells were
165 collected. Then MSC were cultured in selective medium for mouse MSC. (Fig.2)
166 A comparative proteomic analysis was carried out between the two groups after
167 three generations. We finally got 728 differential proteins,
168 including 303 down-regulations and 425 up-regulations.

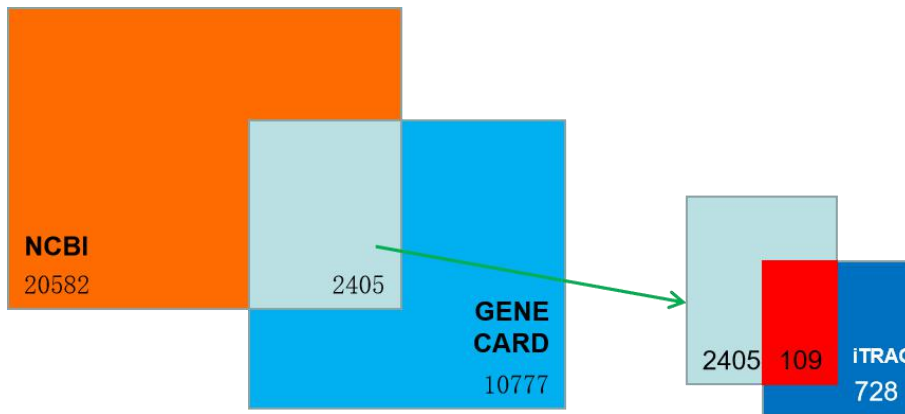


169

170 Fig.2| the 6-week-old mice were divided randomly into two groups (n=6). BSJD and Control
 171 groups were treated with decoction of BSJD (0.2ml every day) and normal saline (0.2ml every
 172 day) respectively. After 28 days treatment, mice were killed and the BM cells were collected.

173 **4.2 Common Targets of BSJD and Leukemia**

174 Being the key word, “Leukemia” was searched in two databases: NCBI and
 175 GENE CARD. 20582 relate genes were screened out from NCBI database
 176 and 10777 were screened out from GENE CARD. When we processed these results,
 177 we deleted duplicates and non-mouse genes. To increase the confidence of finally
 178 result, we selected 2405 shared genes of both databases for further analysis. At last,
 179 we compared these 2405 genes with the 728 differentially expressed genes
 180 previously identified by proteomics, and then obtained 109 co-expressed genes.
 181 These 109 co-expressed genes may be the potential targets to reveal the mechanism
 182 of BSJD. (Fig.3)

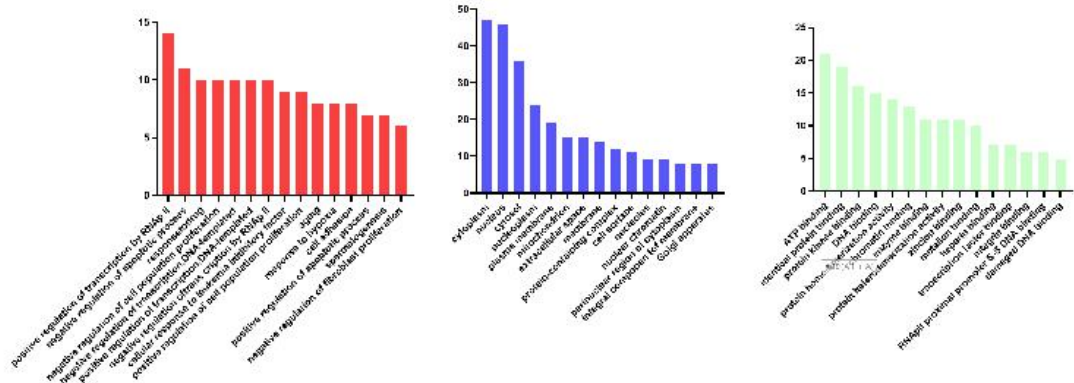


183

184 Fig.3| Common targets of BSJD and leukemia

185 **4.3 GO Function and KEGG Pathway Analysis of 109 Potential Targets**

186 In order to further analyze these 109 genes, we used the MOU species annotation
 187 in Uniprot to annotate their GO functions and KEGG pathways. Uniprot contains
 188 millions of real and available protein data, which can be used for protein
 189 information analysis of different species (18, 19). This Enrichment analysis can
 190 identify valuable functional classes or pathways from identified proteomic results
 191 or differentiated protein data (20). The functions were categorized according to
 192 their cellular components (CC), molecular functions (MF) and biological
 193 processes (BP). (Fig.4, table 1) In the higher ranking results, the words related to
 194 the “binding” appeared more frequently.



195

196 Fig.4| GO enrichment of 109 common genes. Different categories of biological process, cellular
 197 component, and molecular function were represented by a red, blue, and green bar.

198

199

Tab.1

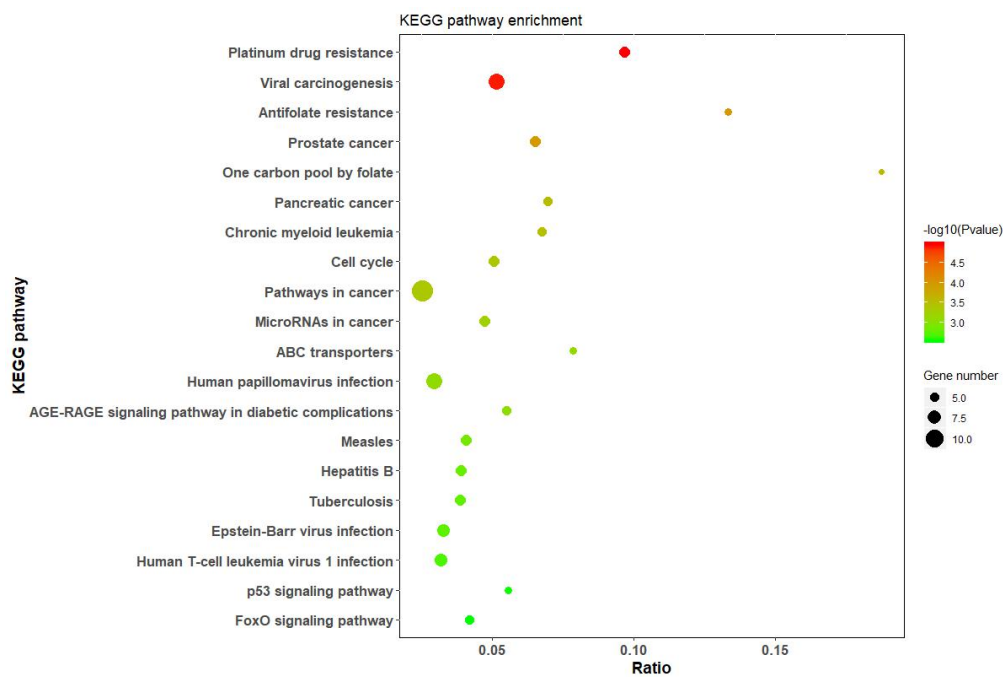
TermID	Term	gene number	Enrichment	FDR
GO:0019901	protein kinase binding	16	9.9071 24	0.0000 00
GO:0005737	cytoplasm	47	9.5442 87	0.0000 00
GO:0005829	cytosol	36	8.1799 51	0.0000 01
GO:1990830	cellular response to leukemia inhibitory factor	9	8.9202 93	0.0000 02
GO:0048147	negative regulation of fibroblast proliferation	6	8.5261 17	0.0000 02
GO:0003682	chromatin binding	13	7.5602 93	0.0000 04
GO:0042802	identical protein binding	19	7.0561 40	0.0000 09
GO:0005634	nucleus	46	6.6869 09	0.0000 12
GO:0000790	nuclear chromatin	9	6.8114 58	0.0000 12
GO:0042493	response to drug	10	7.4432 88	0.0000 15
GO:0019899	enzyme binding	11	6.5488 56	0.0000 23
GO:0043200	response to amino acid	5	6.8952 95	0.0000 41
GO:0007568	aging	8	6.4092 38	0.0001 00

GO:0005654	nucleoplasm	24	5.6318	0.0001
			81	12
GO:0001666	response to hypoxia	8	6.1116	0.0001
			89	65
GO:0008201	heparin binding	7	5.2605	0.0003
			85	53
GO:0042803	protein homodimerization activity	14	4.9513	0.0004
			69	50
GO:0005178	integrin binding	6	5.0757	0.0004
			47	51
GO:0003684	damaged DNA binding	5	4.9746	0.0004
			72	88
GO:0035767	endothelial cell chemotaxis	3	5.4521	0.0006
			04	44

200 Tab.1| The top 20 terms of GO analysis.

201

202 In addition, the pathway analysis of KEGG is shown in figure 5. We listed
 203 the top 20 pathways, including mmu01524、mmu05203、mmu01523、mmu
 204 u05215、mmu00670、mmu05212、mmu05220、mmu04110、mmu05200 and
 205 mmu05206. Platinum drug resistance, P53 pathway, CML pathway are closely
 206 related to the pathogenesis, treatment and prognosis of leukemia (21-23).
 207 Especially in relapsed or refractory leukemia (24), one research showed a
 208 significantly increase of platinum-based drugs resistance.(24)



209

210 Fig.5| KEGG enrichment of 109 common targets, the size of the bubbles represents the degree of
 211 gene enrichment, and the color represents the P value.

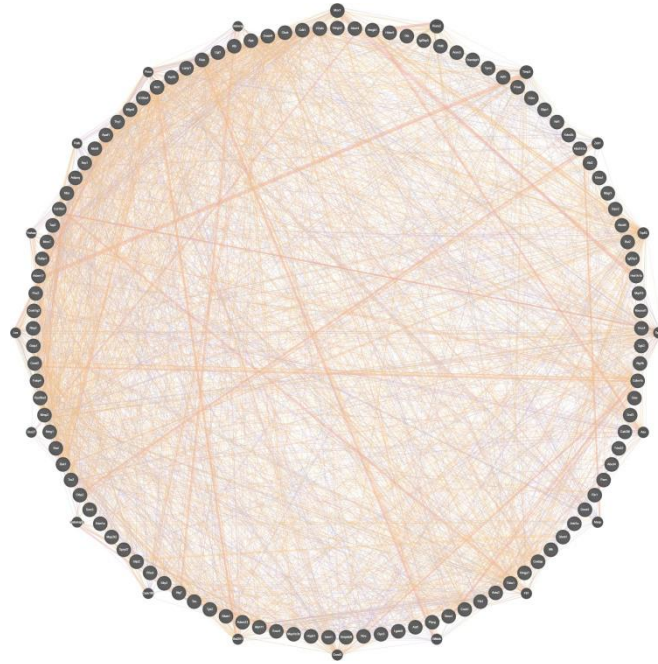
212

Term ID	Term	Term num	Enrichment	FDR
path:mmu01524	Platinum drug resistance	6	4.973571	0.002030
path:mmu05203	Viral carcinogenesis	9	4.935924	0.001107
path:mmu01523	Antifolate resistance	4	4.007099	0.006263
path:mmu05215	Prostate cancer	6	3.992614	0.004857
path:mmu00670	One carbon pool by folate	3	3.555667	0.010627
path:mmu05212	Pancreatic cancer	5	3.525363	0.009496
path:mmu05220	Chronic myeloid leukemia	5	3.469654	0.009253
path:mmu04110	Cell cycle	6	3.380453	0.009942
path:mmu05200	Pathways in cancer	12	3.365681	0.009143
path:mmu05206	MicroRNAs in cancer	6	3.229578	0.011258
path:mmu02010	ABC transporters	4	3.103411	0.013685
path:mmu05165	Human papillomavirus infection	9	3.089588	0.012950
path:mmu04933	AGE-RAGE signaling pathway in diabetic complications	5	3.055819	0.012920
path:mmu05162	Measles	6	2.881639	0.017917
path:mmu05161	Hepatitis B	6	2.792969	0.020510
path:mmu05152	Tuberculosis	6	2.764337	0.020539
path:mmu05169	Epstein-Barr virus infection	7	2.744732	0.020223
path:mmu05166	Human T-cell leukemia virus 1 infection	7	2.665063	0.022946
path:mmu04115	p53 signaling pathway	4	2.544895	0.028667
path:mmu04068	FoxO signaling pathway	5	2.538302	0.027650

214 Tab.1| The top 20 pathways of KEGG analysis.

215 4.4 Protein-protein Interaction Network (PPI) Analysis

216 To further reveal the function of these 109 targets during the interaction with
 217 other proteins, we constructed a PPI network through the web tool GENE MANIA.
 218 Those 109 targets established a co-expression network with other 20 targets in
 219 GENE MANIA by PPI analysis methods including Co-expression, physical
 220 interactions, pathways, and Co-localization. (Fig.6)

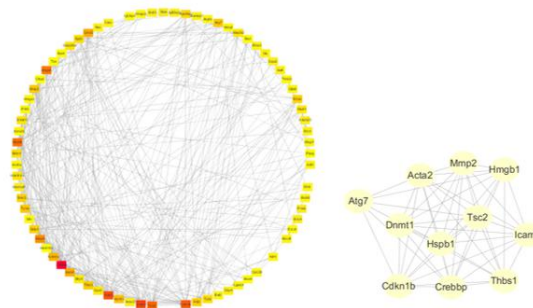


图表 6

221
222
223

4.5 Co-expression and Topology Analysis of over medium-confidence.

224
225 Although we obtained the PPI network of these 109 proteins, their underlying
226 connections cannot be directly reflected by this network structure. Therefore,
227 on the basis of GENE MANIA analysis, we selected 82 proteins with score
228 above 0.6 for k-core analysis in Cytoscape3.7.1 using MCODE.K-core. It
229 is a topological analysis that decomposes the network relation of interaction,
230 and find out the important nodes in the complex network relation structure
231 (25). In this study, we set the parameters of degree>15 and k-core=2 to identify
232 key nodes in the co-expression network (Fig.7). This sub network contains
233 sHspb1、Dnmt1、Mmp2、Thbs1、Crebbp、Hmgb1、Acta2、Cdkn1b、Atg7、Tsc2
234 and Icam1.



235
236
237

Fig.7| 82 proteins with score above 0.6 for k-core analysis. On the right is the Core sub-network obtained by k-core analysis.

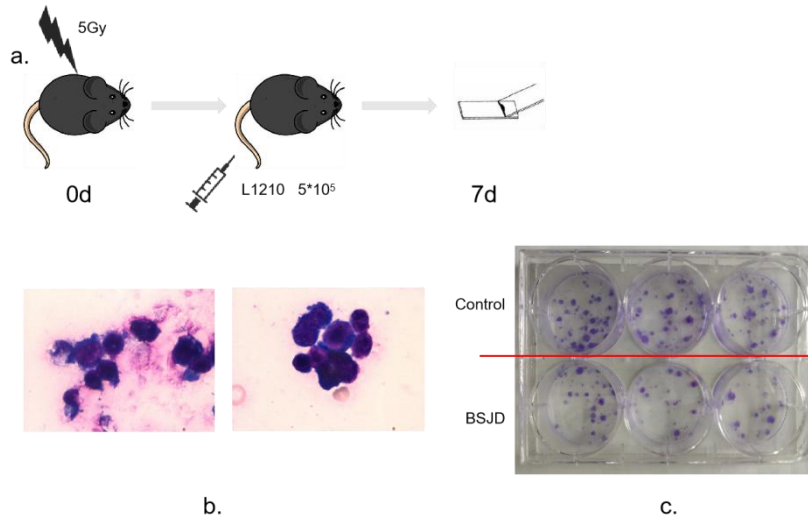
238

4.6 Sub Network

239 We've done a literature review of genes' function in this core network, some key
240 genes in the study of leukemia were screened. The Tsc2 gene is involved in
241 PI3K/AKT/mTOR signaling pathways that can be phosphorylated by Akt (26). Tsc2
242 phosphorylation mediated by different cytokines can promote leukemia cell growth
243 and inhibit apoptotic pathways in both AML and ALL (27, 28). Icam-1 (CD54) is
244 the receptor of lymphocytes cell surface molecule LFA-1, they are both involved in
245 the intercellular adhesion of lymphocytes (29). In leukemia, icam-1 has also been
246 shown to be associated with niches transformation process caused by leukemia
247 stem cells: LSC secrete stimulating factors that enhance the level of Icam-1 in
248 niches, thereby promoting the adhesion between themselves and niches (30).
249 Recent studies demonstrated that Atg7 increased chemo resistance in leukemic cells
250 By combining with EVI1 (31). Moreover, Atg7 plays a key role in the protection of
251 AL cells that was mediated by niche. Inhibiting the expression of Atg7 can enhance
252 the sensitivity to chemotherapy and prolong the survival time of leukemia mice
253 after chemotherapy (32). These evidences suggested that the therapeutic targets of
254 BSJD and leukemia are related at the level of PPI analysis.

255 4.7 BSJD Reduced the in Vitro Cloning of B-ALL Mouse MSC

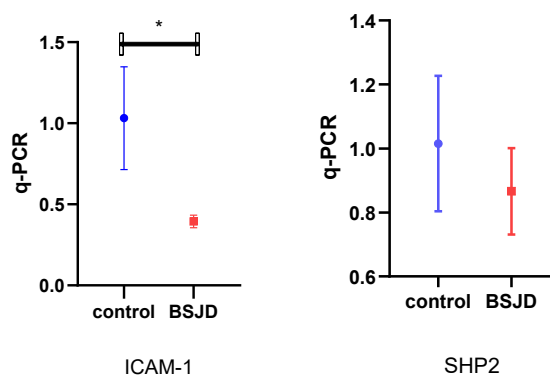
256 In order to verify the targets of BSJD, we constructed an ALL mouse mo
257 de. BALB/c mice were randomly divided into control group and BSJD group
258 (n=6), and L1210 cells were transplanted after 5Gy irradiation (Fig.8a). Bon
259 e marrow smears confirmed the proliferation of ALL cells in mice after 7 days
260 since transplantation (Fig.8b). On the 10th day since transplantation, both two
261 groups were treated with the same chemotherapy of cytoxan, vindesine and p
262 rednisone. In addition, the BSJD treatment group received intragastric admini
263 stration of BSJD decoction from the 7th day since transplantation (0.2ml/day
264 and lasted three weeks). After 28 days since transplantation, all mice wer
265 e killed and their MSCs were cultured in vitro. As shown in results, through
266 an in vitro colony formation assay, we found that the colony-forming ability
267 of BSJD treated MSC was significantly reduced (Fig.8c).



268
 269 Fig.8| a. The method of ALL mice model; b. At 7th days after transplantation, one randomly
 270 selected mouse from each group underwent bone marrow smear, which showed a large
 271 proliferation of ALL cells in the marrow; c. The in vitro clonogenic assay of BSJD and control
 272 group.

273 4.8 BSJD down-regulated the expression of ICAM-1 in MSC.

274 To verify the effect of BSJD on the potential targets, we measured ICAM-1 in
 275 BSJD group and control group by quantitative real-time PCR
 276 (qRT-PCR). Meanwhile, the downstream targets of icam-1, SHP2, was selected for
 277 qRT-PCR detection. Some studies demonstrated that the SHP2 is involved in Scr
 278 activation mediated by icam-1 and enhanced adhesion of leukocytes to niche. (33,
 279 34)。 In addition, SHP2 is also involved in the FLT3-ITD-mediated activation of
 280 Atg7 to enhance the proliferation and survival of leukemia cells (35). We calculated
 281 the mRNA expression values based on the $2^{-\Delta\Delta CT}$ method (36). The results showed
 282 BSJD significantly reduced the gene expression of ICAM-1, whereas did not
 283 change SHP2 ($P > 0.05$). (Fig.9)。



284
 285 Fig.9|qRT-PCR of BSJD and control group, BSJD significantly reduced the gene expression of
 286 ICAM-1. Significance is defined as $p < 0.05$ (* $p < 0.05$).

5. Discussion

288 LSC transforms niches into tumorous tissues by influencing the stromal cel
289 ls through growth factors, chemokines, cytokines and anoxic environment in
290 leukemia development process(37). The tumorous niches, in turn, promotes L
291 SC survival, proliferation, and drug resistance through "screening" mechanis
292 s, while crowding out the survival space of normal HSC (38). For example,
293 HIF-1 α high-expression-mediated microenvironment hypoxia in MSC is one of
294 the important triggers of the niches tumorous process(37). Hypoxia can not
295 only enhance the adhesion and chemotaxis between LSC and MSC, but also
296 reduce the sensitivity of LSC to chemotherapy drugs (39, 40). Down regula
297 ted expression of HIF-1 α in MSC can improve hypoxia in niches and suppres
298 s the activity and invasion of LSC (41). In addition, reversing the hypoxic
299 microenvironment could prolong the survival of leukemia-bearing mice (42).
300 These evidences from one side prove that niches represented by MSC have
301 a vicious cycle with LSC in leukemia. On the one hand, LSC transforms ni
302 ches into tumor environments through MSC to make them more suitable for
303 survival; On the other hand, timorous niches protect the LSC while crowdin
304 g out the normal HSC from the bone marrow microenvironment. Targeting t
305 he tumor niches may be a potential therapeutic approach for relapsed/refracto
306 ry leukemia.

307 Chemotherapeutics for single target is the primary treatment of AL in clinic.
308 Although new drugs keep achieving clinical efficacy, relapse is still the most
309 important obstacle for long-term remission (3, 4). In addition, immunotherapies
310 represented by CAR-T-cell have been proved effective in the treatment of
311 relapse/refractory acute leukemia. But serious and unforeseen side effects and huge
312 medical costs have limited its clinical application(43). Although researchers
313 recognized the interaction between leukemia and tumorous niches, drug
314 developments to reverse tumorous niches, especially tumorous MSC, is still in
315 infancy. Only a small part of the new drug development for AML, such as
316 Uproleselan (gmi-1271), targets the microenvironment (44). In recent years, with
317 the wide application of artemisinin, the research on the effective ingredients in
318 single or compound natural herbs has been paid more attention, which provides a
319 new option for drug research aimed at reversing tumorous niches.

320 Network pharmacology and biological information analysis provide a new way
321 to study the mechanism of complex components in traditional Chinese medicine.
322 They objectified the synergy of natural drugs or compounds on different targets and
323 pathways (45). However, with no unified database and the differences in search
324 logic between different databases, study of network pharmacology cannot fully
325 reflect the targets of drugs.(46). Therefore, we selected proteomics to
326 comprehensively identify the differential proteins of MSC on BSJD treatment. And
327 then we screened out the targets of BSJD from these differential proteins through
328 bioinformatics analysis. Finally, the potential targets were preliminarily verified by

329 qRT-PCR. These results demonstrated the effects of BSJD on MSC in leukemia.
330 We will systematically validate other potential targets in the core sub-network in
331 our following studies. We hope to systematically, comprehensively and objectively
332 explain the regulatory effect of BSJD on MSC after chemotherapy in leukemia, by
333 verifying the interaction between potential targets.

334 **6. Conclusion**

335 We screened the potential targets of BSJD on MSC through proteomics and
336 bioinformatics analysis, and selected some genes for experimental verification.
337 These studies demonstrated the effect of BSJD on MSC. We hope that this research
338 method could provide a new way of systematically studying the effects of
339 traditional Chinese medicine on diseases.

340 **7. List of abbreviations**

Abbreviations	Paraphrase
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BP	Biological processes
BSJD	Bushenjiedudecoction
CC	Cellular components
LSC/LSCs	Leukemia stem cells
MF	Molecular functions
MSC/MSCs	Mesenchymal stem cells
PPI	Protein-protein Interaction
TCM	Traditional Chinese medicine

341

342 **8. Declarations**

343 **8.1 Ethics approval and consent to participate**

344 Not applicable.

345 **8.2 Consent for publication**

346 Not applicable.

347 **8.3 Availability of data and materials**

348 The datasets used and/or analysed during the current study are available from
349 the corresponding author on reasonable request.

350 **8.4 Competing interests**

351 The authors declare no conflicts of interest.

352 **8.5 Funding**

353 This study was supported by the National Natural Science Foundation of
354 China (No.81774048).

355 **8.6 Authors' contributions**

356 WS designed the experiment, performed the major experiments and data
357 analysis; FY designed the experiment and provided technical support, both are
358 major contributor in writing the manuscript. WS, XT, ZX, YL, XX participated in
359 the experiment and organized the literature. All authors read and approved the
360 final manuscript.

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375 **10. Supplementary information**

376 Additional file 1, targets of leukemia in GeneCards.

377 Additional file 2, targets of leukemia in NCBI.

378 Additional file 2, con-targets of NCBI and GeneCards.

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