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

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1. Abstract

1.1 Background

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

1.2 Methods

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

1.3 Results

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

1.4 Conclusions

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

Key words:

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

2. Background

Chemotherapy is the primary choice of adult acute leukemia in clinic, but usually the prognosis is not satisfied (1, 2). Relapse after chemotherapy is the major concern for acute leukemia treatment. Both acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) have low rates of long time remission, especially the response rate is only 30%-40% in ALL (3, 4). AL is a myeloproliferative disease that characterized by malignant clone of hematopoietic stem cells in transformed niches. Bone marrow microenvironment(niche) supports the homing to bone marrow, self-renewal, proliferation and differentiation of hematopoietic stem cells in bone marrows(5). It is consisted of osteocytes, perivascular cells, endodontic cells, adipocytes, macrophages, and mesenchymal stem cells (MSCs)(6). And MSC plays an important role in these components. It is more than a material basis for niche, because it also mediates the homing of HSC through Nest in.(7). Recent studies suggested that leukemia stem cells (LSCs) can transform niche into a LSC growth-permissive and HSC growth-restrictive microenvironment through remodeling activity in the course of leukemia (8). Different cytokines achieves this transformati on through variety pathways and thereby mediates LSC mobilization, homing and drug resistance, which eventually become an important cause of clinical recurrence (9). Treatment to hijacked or transformed niches may provide novel therapies to delay the leukemia relapse(10).

As an important part of Chinese health system, traditional Chinese medicine (TCM) has been used extensively. In disease free interval, we use a variety of TCMs as adjuvant treatments to delay the recurrence. In these Chinese patent medicines we found that Bushenjiedudecoction (BSJD) has a more obvious efficacy, but the specific mechanism is still unclear. Although the research of TCM is facing challenges, the developments of new technologies provide new methods to study the complex mechanism of it. Especially, with the emerging of high-throughput sequencing technology, it makes it possible to obtain large-scale protein data and make comparative analysis of proteomic in a single experiment (11, 12). As a
next-generation of high-throughput technology through relative and absolute quantitative proteomics analysis with high sensitivity and repeatability, iTRAQ has been widely used in cancer research (13, 14). Meanwhile, various bioinformatics databases based on the proteomics also provide powerful support for functional annotation of proteins, classifying protein sequences into families, and comparative protein structure modeling (15). They all promote the research on the mechanism and target location of drugs (16). At the same time, it also provides a new objective way of scientific research and evaluation of TCM compounds (17). Inspired by these methods, we designed our experiment as shown in the figure, including: in vivo administration, cells culture, high-throughput sequencing, etc. Through these processes, possible targets of BSJD were obtained and bio-informatics analysis was carried out on these targets. (Fig.1)

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

3. Materials and methods

3.1 Chinese medicine ingredients of BSJD

Bushenjiedudecoction is composed of Quanxie (ButhusmartensiiKarsch), Jiangcan (Bombyx Batryticatus), Baifuzi (TyphoniiRhizoma), Lujiaopian (Cornu Cervi), Guiban (Tortoise Shell Caraoax et PlastrumTestudinis), Renshen (Panax Ginseng C. A. Mey), Gouqizi(Lycii Fructus), Gancao(licorice), Qingdai (Indigo
3.2 Proteomics

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

3.3 Prediction of common targets for BSJD and leukemia

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

3.4 Analysis of GO, KEGG and key sub net.

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

3.5 Animal experiment

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

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

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

3.6 Treatment protocol

Their chemotherapy regimen was cytoxan (3mg/m^2, for onetime), vindesine (600mg/m^2 for one time) and prednisone (9mg/kg/day, for 3d) at 10^th day after transplanted.

BSJD was prepared as decoction at a concentration of 10g/50ml, oral gavage.
treated once a day at 0.2 ml.

3.7 Cell Line and Cell Culture

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

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

3.8 RNA Isolation and Real-Time PCR Analysis

Total RNA was extracted using TRIzol. Reverse transcription and real-time 
quantitative PCR was conducted by Real-time PCR Fluorescent Quantitative 
Kit (ZSTC, ZS-M-1009) on PCR machine (Eppendorf). Target gene primer seq 
quences: ICAM-1: Forward primer-CTGAGCCTGCTGGATGAGAC, Reverse prim 
er-GCCACCATCCTGGTCTGTA; SHP2: Forward primer-ACTGTGCAGACC 
CTACCTCT, Reverse primer-GCACGGAGAGAACGATCT. The relative e 
xpression quantity 2^-ΔΔCt value was calculated to compare the differences am 
ong two groups (three technical replicates were used for each gene).

3.9 Statistical Analysis

All data were represented in mean ± SD. Statistical analysis was performed using 
GraphPad Prism 8 software with student’s t test (P < 0.05 was considered with 
statistical significance). Histogram was made using the Graphpad software.

4. Results

4.1 Potential Targets of BSJD.

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

4.2 Common Targets of BSJD and Leukemia

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

4.3 GO Function and KEGG Pathway Analysis of 109 Potential Targets

In order to further analyze these 109 genes, we used the MOU species annotation in Uniprot to annotate their GO functions and KEGG pathways. Uniprot contains millions of real and available protein data, which can be used for protein information analysis of different species (18, 19). This Enrichment analysis can identify valuable functional classes or pathways from identified proteomic results or differentiated protein data (20). The functions were categorized according to their cellular components (CC), molecular functions (MF) and biological processes (BP). (Fig. 4, table 1) In the higher ranking results, the words related to the “binding” appeared more frequently.
**Fig.4** GO enrichment of 109 common genes. Different categories of biological process, cellular component, and molecular function were represented by a red, blue, and green bar.

**Tab.1**

| TermID     | Term                                         | gene number | Enrichment | FDR   |
|------------|----------------------------------------------|-------------|------------|-------|
| GO:0019901 | protein kinase binding                       | 16          | 9.9071     | 0.0000|
|            |                                               | 24          | 0.00       |       |
| GO:0005737 | cytoplasm                                    | 47          | 9.5442     | 0.0000|
|            |                                               | 87          | 0.00       |       |
| GO:0005829 | cytosol                                      | 36          | 8.1799     | 0.0000|
|            |                                               | 51          | 0.01       |       |
| GO:1990830 | cellular response to leukemia inhibitory factor | 9           | 8.9202     | 0.0000|
|            | negative regulation of fibroblast proliferation | 93          | 0.02       |       |
| GO:0048147 | chromatin binding                            | 13          | 7.5602     | 0.0000|
|            |                                               | 93          | 0.04       |       |
| GO:0003682 | identical protein binding                     | 19          | 7.0561     | 0.0000|
|            |                                               | 40          | 0.09       |       |
| GO:0005634 | nucleus                                      | 46          | 6.6869     | 0.0000|
|            |                                               | 09          | 0.12       |       |
| GO:0000790 | nuclear chromatin                            | 9           | 6.8114     | 0.0000|
|            |                                               | 58          | 0.12       |       |
| GO:0042493 | response to drug                             | 10          | 7.4432     | 0.0000|
|            |                                               | 88          | 0.15       |       |
| GO:0019899 | enzyme binding                               | 11          | 6.5488     | 0.0000|
|            |                                               | 56          | 0.23       |       |
| GO:0043200 | response to amino acid                       | 5           | 6.8952     | 0.0000|
|            |                                               | 95          | 0.41       |       |
| GO:0007568 | aging                                        | 8           | 6.4092     | 0.0001|
|            |                                               | 38          | 0.00       |       |
In addition, the pathway analysis of KEGG is shown in figure 5. We listed the top 20 pathways, including mmu01524, mmu05203, mmu01523, mmu05215, mmu00670, mmu05212, mmu05220, mmu04110, mmu05200, and mmu05206. Platinum drug resistance, P53 pathway, CML pathway are closely related to the pathogenesis, treatment and prognosis of leukemia (21-23). Especially in relapsed or refractory leukemia (24), one research showed a significantly increase of platinum-based drugs resistance (24).

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

Tab.1] The top 20 pathways of KEGG analysis.

4.4 Protein-protein Interaction Network (PPI) Analysis

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

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

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.
4.6 Sub Network

We've done a literature review of genes’ function in this core network, some key genes in the study of leukemia were screened. The Tsc2 gene is involved in PI3K/AKT/mTOR signaling pathways that can be phosphorylated by Akt (26). Tsc2 phosphorylation mediated by different cytokines can promote leukemia cell growth and inhibit apoptotic pathways in both AML and ALL (27, 28). Icam-1 (CD54) is the receptor of lymphocytes cell surface molecule LFA-1, they are both involved in the intercellular adhesion of lymphocytes (29). In leukemia, icam-1 has also been shown to be associated with niches transformation process caused by leukemia stem cells: LSC secrete stimulating factors that enhance the level of Icam-1 in niches, thereby promoting the adhesion between themselves and niches (30).

Recent studies demonstrated that Atg7 increased chemo resistance in leukemic cells by combining with EVI1 (31). Moreover, Atg7 plays a key role in the protection of AL cells that was mediated by niche. Inhibiting the expression of Atg7 can enhance the sensitivity to chemotherapy and prolong the survival time of leukemia mice after chemotherapy (32). These evidences suggested that the therapeutic targets of BSJD and leukemia are related at the level of PPI analysis.

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

In order to verify the targets of BSJD, we constructed an ALL mouse model. BALB/c mice were randomly divided into control group and BSJD group (n=6), and L1210 cells were transplanted after 5Gy irradiation (Fig.8a). Bone marrow smears confirmed the proliferation of ALL cells in mice after 7 days since transplantation (Fig.8b). On the 10th day since transplantation, both two groups were treated with the same chemotherapy of cytoxan, vindesine and prednisone. In addition, the BSJD treatment group received intragastric administration of BSJD decoction from the 7th day since transplantation (0.2ml/day and was lasted three weeks). After 28 days since transplantation, all mice were killed and their MSCs were cultured in vitro. As shown in results, through an in vitro colony formation assay, we found that the colony-forming ability of BSJD treated MSC was significantly reduced (Fig.8c).
4.8 BSJD down-regulated the expression of ICAM-1 in MSC.

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

![Fig.8](image_url)

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

![Fig.9](image_url)

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

LSC transforms niches into tumorous tissues by influencing the stromal cells through growth factors, chemokines, cytokines and anoxic environment in leukemia development process (37). The tumorous niches, in turn, promotes LSC survival, proliferation, and drug resistance through "screening" mechanism, while crowding out the survival space of normal HSC (38). For example, HIF-1α high-expression-mediated microenvironment hypoxia in MSC is one of the important triggers of the niches tumorous process (37). Hypoxia can not only enhance the adhesion and chemotaxis between LSC and MSC, but also reduce the sensitivity of LSC to chemotherapy drugs (39, 40). Down regulation of HIF-1α in MSC can improve hypoxia in niches and suppress the activity and invasion of LSC (41). In addition, reversing the hypoxic microenvironment could prolong the survival of leukemia-bearing mice (42).

These evidences from one side prove that niches represented by MSC have a vicious cycle with LSC in leukemia. On the one hand, LSC transforms niches into tumor environments through MSC to make them more suitable for survival; On the other hand, tumorous niches protect the LSC while crowding out the normal HSC from the bone marrow microenvironment. Targeting the tumor niches may be a potential therapeutic approach for relapsed/refractory leukemia.

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

Network pharmacology and biological information analysis provide a new way to study the mechanism of complex components in traditional Chinese medicine. They objectified the synergy of natural drugs or compounds on different targets and pathways (45). However, with no unified database and the differences in search logic between different databases, study of network pharmacology cannot fully reflect the targets of drugs (46). Therefore, we selected proteomics to comprehensively identify the differential proteins of MSC on BSJD treatment. And then we screened out the targets of BSJD from these differential proteins through bioinformatics analysis. Finally, the potential targets were preliminarily verified by...
qRT-PCR. These results demonstrated the effects of BSJD on MSC in leukemia. We will systematically validate other potential targets in the core sub-network in our following studies. We hope to systematically, comprehensively and objectively explain the regulatory effect of BSJD on MSC after chemotherapy in leukemia, by verifying the interaction between potential targets.

6. Conclusion

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

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 |

8. Declarations

8.1 Ethics approval and consent to participate

Not applicable.

8.2 Consent for publication

Not applicable.

8.3 Availability of data and materials

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

8.4 Competing interests

The authors declare no conflicts of interest.
8.5 Funding
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8.6 Authors' contributions
WS designed the experiment, performed the major experiments and data analysis; FY designed the experiment and provided technical support, both are major contributor in writing the manuscript. WS, XT, ZX, YL, XX participated in the experiment and organized the literature. All authors read and approved the final manuscript.

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10. Supplementary information
Additional file 1, targets of leukemia in GeneCards.
Additional file 2, targets of leukemia in NCBI.
Additional file 2, con-targets of NCBI and GeneCards.

References
1. Paul S, Kantarjian H, Jabbour EJ. Adult Acute Lymphoblastic Leukemia. Mayo Clin Proc. 2016;91(11):1645-66.
2. Meyers J, Yu Y, Kaye JA, Davis KL. Medicare fee-for-service enrollees with primary acute myeloid leukemia: an analysis of treatment patterns, survival, and healthcare resource utilization and costs. Appl Health Econ Health Policy. 2013;11(3):275-86.
3. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J. 2017;7(6):e577.

4. De Kouchkovsky I, Abdul-Hay M. 'Acute myeloid leukemia: a comprehensive review and 2016 update'. Blood Cancer J. 2016;6(7):e441.

5. Jones DL, Wagers AJ. No place like home: anatomy and function of the stem cell niche. Nat Rev Mol Cell Biol. 2008;9(1):11-21.

6. Yu VWC, Scadden DT. Hematopoietic Stem Cell and Its Bone Marrow Niche. Curr Top Dev Biol. 2016;118:21-44.

7. Ménendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 2010;466(7308):829-34.

8. Kumar B, García M, Weng L, Jung X, Murakami JL, Hu X, et al. Acute myeloid leukemia transforms the bone marrow niche into a leukemia-permissive microenvironment through exosome secretion. Leukemia. 2018;32(3):575-87.

9. Wang A, Zhong H. Roles of the bone marrow niche in hematopoiesis, leukemogenesis, and chemotherapy resistance in acute myeloid leukemia. Hematology. 2018;23(10):729-39.

10. Cheng H, Sun G, Cheng T. Hematopoiesis and microenvironment in hematological malignancies. Cell Regen (Lond). 2018;7(1):22-6.

11. Blackstock WP, Weir MP. Proteomics: quantitative and physical mapping of cellular proteins. Trends Biotechnol. 1999;17(3):121-7.

12. Pareek CS, Smoczynski R, Tretyn A. Sequencing technologies and genome sequencing. J Appl Genet. 2011;52(4):413-35.

13. Yan B, Chen B, Min S, Gao Y, Zhang Y, Xu P, et al. iTRAQ-based Comparative Serum Proteomic Analysis of Prostate Cancer Patients with or without Bone Metastasis. J Cancer. 2019;10(18):4165-77.

14. Li F, Zhao D, Yang S, Wang J, Liu Q, Jin X, et al. iTRAQ-Based Proteomics Analysis of Triptolide On Human A549 Lung Adenocarcinoma Cells. Cellular Physiology and Biochemistry. 2018;45(3):917-34.

15. Chen C, Huang H, Wu CH. Protein Bioinformatics Databases and Resources. Methods Mol Biol. 2017;1558:3-39.

16. Wooller SK, Benstead-Hume G, Chen X, Ali Y, Pearl FMG. Bioinformatics in translational drug discovery. Biosci Rep. 2017;37(4):BSR20160180.

17. Liu Z-H, Sun X-B. Network pharmacology: new opportunity for the modernization of traditional Chinese medicine. Yao Xue Xue Bao. 2012;47(6):696-703.

18. UniProt C. UniProt: a hub for protein information. Nucleic Acids Res. 2015;43(Database issue):D204-D12.

19. The UniProt Consortium. UniProt: the universal protein knowledgebase. Nucleic Acids Res. 2017;45(D1):D158-D69.

20. Sun D, Liu Y, Zhang X-S, Wu L-Y. CEA: Combination-based gene set functional enrichment analysis. Sci Rep. 2018;8(1):13085-.

21. Tonino SH, Mulkens CE, van Laar J, Derks IAM, Suo G, Croon-de Boer F, et al. Induction of TAp73 by platinum-based compounds to overcome drug resistance in p53 dysfunctional chronic lymphocytic leukemia. Leuk Lymphoma. 2015;56(8):2439-47.
22. Ball B, Abdel-Wahab O. Activating p53 and Inhibiting Superenhancers to Cure Leukemia. Trends Pharmacol Sci. 2018;39(12):1002-4.

23. Henklewska M, Pawlak A, Pruchnik H, Obminska-Mrukowicz B. Complex of Platinum(II) with Tris(2-carboxyethyl)phosphine Induces Apoptosis in Canine Lymphoma/Leukemia Cell Lines. Anticancer Res. 2017;37(2):539-46.

24. Styczynski J, Wysocki M, Debski R, Juraszewska E, Malinowska I, Stanczak E, et al. Ex vivo drug resistance profile in childhood acute myelogenous leukaemia: no drug is more effective in comparison to acute lymphoblastic leukemia. Leuk Lymphoma. 2002;43(9):1843-8.

25. García-Algarra J, Pastor JM, Iriondo JM, Galeano J. Ranking of critical species to preserve the functionality of mutualistic networks using the k-core decomposition. PeerJ. 2017;5:e3321-e.

26. Inoki K, Li Y, Zhu T, Wu J, Guan K-L. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol. 2002;4(9):648-57.

27. Feng Y, Wu L. mTOR up-regulation of PFKFB3 is essential for acute myeloid leukemia cell survival. Biochem Biophys Res Commun. 2017;483(2):897-903.

28. Chiang YJ, Liao WT, Ho KC, Wang SH, Chen YG, Ho CL, et al. CBAP modulates Akt-dependent TSC2 phosphorylation to promote Rheb-mTORC1 signaling and growth of T-cell acute lymphoblastic leukemia. Oncogene. 2019;38(9):1432-47.

29. Long EO. ICAM-1: getting a grip on leukocyte adhesion. J Immunol. 2011;186(9):5021-3.

30. Zhang W-h, Qiao Z-h, Fan X-h, Zhang X-l, Li D-q. The role of intercellular adhesion molecule-1 in binding of acute myeloid leukemic blasts cells to human umbilical vein endothelial cells. Zhonghua Nei Ke Za Zhi. 2003;42(6):413-6.

31. Niu Y, Yang X, Chen Y, Jin X, Li L, Guo Y, et al. EVI1 induces autophagy to promote drug resistance via regulation of ATG7 expression in leukemia cells. Carcinogenesis. 2019:bgz167.

32. Piya S, Kornblau SM, Ruvolo VR, Mu H, Ruvolo PP, McQueen T, et al. Atg7 suppression enhances chemotherapeutic agent sensitivity and overcomes stroma-mediated chemoresistance in acute myeloid leukemia. Blood. 2016;128(9):1260-9.

33. Liu G, Place AT, Chen Z, Brovkovych VM, Vogel SM, Muller WA, et al. ICAM-1-activated Src and eNOS signaling increase endothelial cell surface PECAM-1 adhesivity and neutrophil transmigration. Blood. 2012;120(9):1942-52.

34. Yan M, Zhang X, Chen A, Gu W, Liu J, Ren X, et al. Endothelial cell SHP-2 negatively regulates neutrophil adhesion and promotes transmigration by enhancing ICAM-1-VE-cadherin interaction. FASEB J. 2017;31(11):4759-69.

35. Watanabe D, Nogami A, Okada K, Akiyama H, Umezawa Y, Miura O. FLT3-ITD Activates RSK1 to Enhance Proliferation and Survival of AML Cells by Activating mTORC1 and eIF4B Cooperatively with PIM or PI3K and by Inhibiting Bad and BIM. Cancers (Basel). 2019;11(12).

36. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008;3(6):1101-8.

37. Tabe Y, Konopleva M. Advances in understanding the leukaemia microenvironment. Br J Haematol. 2014;164(6):767-78.

38. Tabe Y, Konopleva M. Role of Microenvironment in Resistance to Therapy in AML. Curr Hematol Malig Rep. 2015;10(2):96-103.
39. Dong-Feng Z, Ting L, Cheng C, Xi Z, Xue L, Xing-Hua C, et al. Silencing HIF-1α reduces the adhesion and secretion functions of acute leukemia hBMSCs. Braz J Med Biol Res. 2012;45(10):906-12.

40. Zhu B, Pan S, Liu J, Wang S, Ni Y, Xiao L, et al. HIF-1α forms regulatory loop with YAP to coordinate hypoxia-induced adriamycin resistance in acute myeloid leukemia cells. Cell Biol Int. 2020;44(2):456-66.

41. Migliavacca J, Percio S, Valsecchi R, Ferrero E, Spinelli A, Ponzoni M, et al. Hypoxia inducible factor-1α regulates a pro-invasive phenotype in acute monocytic leukemia. Oncotarget. 2016;7(33):53540-57.

42. Valsecchi R, Coltella N, Belloni D, Ponente M, Ten Hacken E, Scielzo C, et al. HIF-1α regulates the interaction of chronic lymphocytic leukemia cells with the tumor microenvironment. Blood. 2016;127(16):1987-97.

43. Vairiy S, Garcia JL, Teira P, Bittencourt H. CTL019 (tisagenlecleucel): CAR-T therapy for relapsed and refractory B-cell acute lymphoblastic leukemia. Drug Des Devel Ther. 2018;12:3885-98.

44. Winer ES, Stone RM. Novel therapy in Acute myeloid leukemia (AML): moving toward targeted approaches. Ther Adv Hematol. 2019;10:2040620719860645-.

45. Yuan H, Ma Q, Cui H, Liu G, Zhao X, Li W, et al. How Can Synergism of Traditional Medicines Benefit from Network Pharmacology? Molecules. 2017;22(7):1135.

46. Zhang R, Zhu X, Bai H, Ning K. Network Pharmacology Databases for Traditional Chinese Medicine: Review and Assessment. Front Pharmacol. 2019;10:123-.