Network Pharmacology Based Prediction of Active Ingredients and Targets of Chinese Herb She Xiang in Treating Facial Paralysis

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Research

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Abstract

The traditional Chinese herb, She Xiang, has been found to accelerate the recovery of facial paralysis including Bell's palsy by acupoint application in China. However, the underlining mechanism was not well known which has become an obstacle on the way to further development. In this study, we attempted to explore the pharmacology mechanism of She Xiang on facial paralysis treatment preliminarily by bioinformatics analysis. As a result, 59 active ingredients were identified in She Xiang by Traditional Chinese Medicine Integrated Database, such as 17-Beta-Estradiol, testosterone, and 2,6-Decamethylene Pyridine. Totally 837 genes were identified to be differently expressed in the blood sample of facial paralysis patients by RNA sequencing. Finally, 33 overlapped proteins were obtained overlapped with the prediction of comparative toxicogenomics database (CTD) and BATMAN. Proteins of interest were closely related with 406 GO BP and 4 pathways. The hub protein in PPI network contained FOS, JUN, POMC, and GPER1. Pharmacology network was constructed with 15 active components of She Xiang, 33 protein targets and 4 pathways. The docking model of Androst-4-Ene-3, 17-Dione (ASD) and FUS-JUN complexes (1FOS) was predicted and constructed. In conclusion, this work indicated testosterone as the effective component of She Xiang may advance the recovery of facial paralysis by targeting FUN, MAPK and cAMP signaling pathway; docking of ASD and 1FOS might play a critical role in facial paralysis treatment by She Xiang. Further work will be carried out in human or experimental animals to test and verify the predicted results.

Introduction

Facial paralysis, also known as facial nerve paralysis, is caused by function loss of facial muscle innervated by the seventh cranial nerves in human [1] or even in animals [2]. This facial problem is generally characterized by muscle dysfunction on one side of the face. The common symptoms induced by facial paralysis include taste loss, hearing disorder and dry mouth. The most common cause of facial paralysis is suggested to be nonspecific or idiopathic which is also identified as Bell's palsy [3] and others factors contributed to facial paralysis contain traumatic injury, metabolic disorders and leukemic infiltration [4–6]. The underlying cause of facial paralysis is determined with the facilitation of history and physical examination. However, the etiologies of same cases remain to be unclear, which may add trouble in treatment.

The typical therapies for facial paralysis including oral administration of corticosteroids, antiviral drugs, vitamins and physiotherapy [7–10]. Severe patients may need surgery for facial nerve decompression [11]. Despite the advances in the treatment for facial paralysis, the recovery of function remains incomplete in many cases. Traditional medicine served as the traditional, complementary and alternative medicine has been applied world-widely in under-developing countries, especially in Asia. In China, Traditional Chinese Medicine (TCM) has widely accepted for the advantages of fewer side effect, easy accessibility, low cost and unexpected effect [12]. She-xiang (Moschus), a Chinese herb, has shown therapeutic effect on cardiovascular disorders [13–15]. Compendium of Materia Medica an ancient Chinese Literature written by the well-known pharmacist Shizhen Li, pointed out for the first time that She-
xiang is expert in dredging the channel and meridians. Recent evidence also supports the clinical application of She-xiang for facial paralysis [16–19]. However, the underlying mechanism of She-xiang in treating facial paralysis has not been clarified. In this study, the pharmacology network of She-xiang was constructed based on the RNA sequencing data and bioinformatics methods in order to explore the potential pharmacological mechanism of She-xiang on facial paralysis treatment.

Methods

Components of the traditional Chinese medicine

Traditional Chinese Medicine Integrated Database (TCMID) is a collection of the information of TCM, which bridges the gap between TCM, common drug and diseases [20]. In our study, the chemical components of TCM She-xiang were mined from TCMID (http://www.megabionet.org/tcmid/).

Prediction of the targets for active ingredients of She-xiang

Bioinformatics analysis tool for molecular mechanism of traditional Chinese medicine (BATMAN-TCM) tool is the first used bioinformatic analysis tool for studying the molecular mechanism of TCM. The proteins that targeted by the effective components of She-xiang were predicted by BATMAN-TCM online tool [21] (http://bionet.ncpsb.org/batman-tcm/). The protein-component interaction pairs with score > 20 were collected.

The RNA sequencing and differentially expressed gene analysis

The whole blood samples were collected from 5 facial paralysis patients and 5 healthy controls. RNA samples were extracted and subjected to RNA sequencing. The raw reads were mapped to human reference genome to generate a raw count. Then, the raw counts were normalized by TMM (trimmed mean of M-values) algorithm with the application of edgeR package [22] and then transformed to logCPM (logarithm of counts per million reads) to estimate the expression value of genes. Subsequently, the genes with differential expression between patients and controls were analyzed. The p values were controlled by BH (Benjamini–Hochberg) method. The adj.P.Value < 0.05 and |logFC(fold change)| > 2 were set as the cutoff value for screening differentially expressed genes.

Cross-validation of target proteins

The comparative toxicogenomics database (CTD; http://ctdbase.org) is a publicly available resource that records the association between chemical, gene products, and disease. The facial paralysis related gene products were retrieved from CTD 2019 update. Then, the results were compared with the target proteins of effective components of She-xiang and differentially expressed genes products. The overlapped proteins were obtained for further analysis.

GO function and pathway enrichment analysis
Gene ontology (GO) resource is widely used for providing function annotation for genes and gene products in three categories of molecular function (MF), cellular component (CC) and biological process (BP) [23]. The proteins of interest were subjected to GO enrichment analysis by clusterprofiler [24]. The significant GO terms with p value $\leq 0.05$ in BP category were screened out. The Kyoto encyclopedia of genes and genomes (KEGG) is a knowledge resource containing the pathway terms for genes or gene products [25]. In our study, the significant pathways for proteins with $p \leq 0.05$ were also analyzed with clusterProfiler in R.

**Target protein interaction analysis**

The protein-protein interactions (PPIs) were analyzed by STRING database (version: 10.0, http://www.string-db.org/) [26–27]. The protein interaction pairs were selected by Required Confidence (combined score) $> 0.4$ and the PPI network was constructed by Cytoscape software [28].

**Construction for pharmacology network**

Network pharmacology can illuminate the systematic understanding of drug action. In order to explore the therapeutic action of She-xiang, the integrated network with active components of herb She-xiang, their protein targets, and related pathways was constructed by Cytoscape software interactive docking prediction of key target proteins and effective component.

Protein Data Bank (PDB) is a worldwide repository that facilitates macromolecular structure studies by providing the three-dimensional (3D) structure of a given protein [29]. PubChem is a public resource of chemical structures and corresponding biological activities, which contains three inter-linked databases, such as Substance, Compound and BioAssay [30]. In this study, the 3D structure of key target protein was retrieved from PDB database and the molecule structure of effective component was downloaded from PubChem Compound database. The raw SDF format file was transformed to mol2 format by pymol (Version 2.0 Schrödinger, LLC.). The docking possibility of key protein and effective component was predicted by Lamarckian Genetic Algorithm with the application of AutoDock software [31].

**Results**

**Components of herb She-xiang**

The chemical components of herb She-xiang were retrieved from TCMID with the key words of “She-xiang”. As shown in Table S1, there are 59 active ingredients in She-xiang, such as 17-Beta-Estradiol, testosterone, 2,6-Decamethylene Pyridine, 3,5-Dihydroxybenzoic Acid and 3-Methylcyclotridecan-1-One.

**The differentially expressed genes related with facial paralysis**

With the cutoff value, total 837 genes were identified to be differentially expressed in the blood sample of facial paralysis patients, comparable to healthy controls, of which 457 genes were overexpressed and
380 were down-expressed. The differential expression genes were visualized in the volcano plot by combining significant p values and fold change (Fig. 1A). The heatmap for the differentially expressed genes illustrates that the samples in case and control groups were clearly distinguished based the expression profile (Fig. 1B).

**Protein targets and cross-validation**

Based on the information in BATMAN, there were 1081 protein targets for 26 active components. Total 11862 records of gene products related with facial paralysis were deposited in CTD. Then, the proteins obtained above were compared with the differentially expressed genes. Finally, 33 overlapped proteins were obtained (Fig. 2).

**Significant GO function and pathways enriched by proteins of interest**

In order to understand the target protein involved biological function and pathways, the overlapped proteins were subjected to GO and pathway analysis. Results showed that the proteins were significantly enriched in 406 GO BP and 4 pathways. The top 20 GO BP terms were displayed in Fig. 3, such as response to metal ion, response to cAMP, and cellular response to calcium ion. The significantly enriched pathways included Endocrine resistance, MAPK signaling pathway, Sulfur metabolism and cAMP signaling pathway (Fig. 4).

**PPI network**

The protein interactions of overlapped proteins were predicted by STRING database. Total 32 protein interaction pairs were obtained. The PPI network was constructed with 32 edges connecting with 24 proteins (Fig. 5). The significant nodes with high degrees in PPI network included FOS (degree = 9), JUN (degree = 8), POMC (degree = 6), GPER1 (degree = 4), and CALB1 (degree = 3).

**Pharmacology network for She-xiang**

Pharmacology network was constructed by integrating activated components, protein targets and pathways. As shown in Fig. 6, the network contains 52 nodes and 111 edges, of which there are 15 active components of She-xiang, 33 protein targets and 4 pathways. The drug active components of Androst-4-Ene-3,17-Dione (degree = 10), 5-Cis-Cyclopentadecen-1-One (degree = 10), Testosterone (degree = 8) and 17-Beta-Estradiol (degree = 8) were significant nodes in pharmacology network. The significant gene nodes included JUN (Jun proto-oncogene, AP-1 transcription factor subunit, degree = 13), FOS (Fos proto-oncogene, AP-1 transcription factor subunit, degree = 13) and GPER1 (G protein-coupled estrogen receptor 1, degree = 11).
Interactive docking of key proteins and effective components of herb She-xiang

Androst-4-Ene-3,17-Dione (ASD) as the key effective component, FOS and JUN as the key protein in pharmacology network were subjected to interactive docking prediction. The chemical structure of ASD was obtained from PubChem Compound database and shown in Fig. 7A. The 3D structure of FOS-JUN complexes (1FOS) was downloaded from the PDB database (Fig. 7B and C). Total 6 docking models were predicted for 1FOS and ASD (Table 1). The model 1 was the best docking model with the highest affinity and lowest root mean square deviation (RMSD). The docking global graph based on model 1 was visualized in Fig. 7D. The interaction sequences between 1FOS and ASD were LYS267, ASN271, DC26 and DT14 (Fig. 7E).

![Fig. 7A](image1)
![Fig. 7B](image2)
![Fig. 7C](image3)
![Fig. 7D](image4)
![Fig. 7E](image5)

Table 1
| Model | Affinity (kcal/mol) | Dist from best mode | rmsd l.b. | rmsd u.b. |
|-------|---------------------|---------------------|-----------|-----------|
| 1     | -7.9                | 0                   | 0         | 0         |
| 2     | -7.3                | 30.947              | 32.317    |           |
| 3     | -7.3                | 30.061              | 32.216    |           |
| 4     | -7.2                | 36.577              | 38.296    |           |
| 5     | -7                  | 3.231               | 5.32      |           |
| 6     | -7                  | 16.781              | 19.863    |           |
| 7     | -6.7                | 42.248              | 45.056    |           |
| 8     | -6.6                | 24.786              | 27.777    |           |
| 9     | -6.5                | 13.127              | 13.771    |           |

Rmsd: the root mean square deviation.

Discussion

Recently, TCM has been widely recognized worldwide. She-xiang as a traditional Chinese herb, has been found to facilitate the recovery of facial paralysis. However, the mechanism of the effect of She-xiang on facial paralysis has not been illuminated. In the present study, the active ingredients of herb She-xiang were predicted based on the records of TCMD. The protein targets of active ingredients were predicted by the BATMAN and differentially expressed genes analysis. Finally, pharmacology network was constructed with 33 protein targets, 15 chemical components and 4 signaling pathways.
Our results showed that testosterone was an active ingredient of She-xiang, which has been validated in the pharmacology network. Testosterone is a primary male hormone and plays an essential role in human health and well-being [32]. Evidence has shown that testosterone as an anabolic steroid is applied for male hypogonadism treatment and certain types of breast cancer [33–34]. In addition, testosterone has been documented to be implicated in muscle growth and development of humans [35]. It is reported that androgens show function in stimulating muscle generation, and facilitating reinnervation and angiogenesis [36]. Testosterone, a kind of androgens, has been found to stimulate the activation of androgen receptor of nerve, which promotes reinnervation in the process of muscle grafts [37–38]. Previous evidence has suggested that testosterone plays a differential regulatory role in the regeneration of facial motoneurons [39]. A study in male hamsters showed that testosterone mediated the accelerative recovery of facial paralysis [40], which was consistent with therapeutic effect of She-xiang on facial paralysis.

Besides, JUN was predicted to be a protein target for testosterone. Jun protein family plays a regulatory role in collagenase expression after stimulated by various extracellular signals [41]. Jun protein has been found to be selectively expressed in peripheral nerves of rats after axotomy and plays a role in nerve generation [42]. Besides, the expression of Jun protein is increased after the activation of mitogen-activated protein kinase (MAPK) pathway induced by UV radiation in skin [43]. Previous report has suggested that MAPK is activated in mice with facial pain induced by occlusal interference [44]. MAPK signaling pathway has been suggested to be involved in the evolution of facial palsy in the mice model [45]. A previous study suggested that MAPK signaling was one of the downstream pathways underlying the role of ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF) in improving facial nerve regeneration and functional recovery [46]. In the present study, the interaction was identified between JUN protein and MAPK signaling pathway. Taken together, we suggested that JUN protein and MAPK signaling pathway were implicated in the recovery of facial nerve disorder.

Furthermore, our data showed that cAMP signaling was another pathway involved with Jun protein. cAMP is the second messenger involved in the central nervous system (CNS) axonal regeneration. The level of cAMP is elevated under the process of advanced growth of CNS neurite induced by neurotrophies such as BDNF and glia-derived neurotrophic factor (GDNF) [47]. In addition, the up-regulated cAMP induced by trk receptor signaling plays a key role in improving axonal outgrowth of peripheral nerve. Testosterone has been suggested as one of the agents that show consideration promise for the treatment of peripheral nerve injury [48]. In our study, the JUN protein was predicted to be the target for testosterone and pathway analysis showed that cAMP signaling pathway was a significant pathway involved with JUN. Thus, we suggested that testosterone may improve the recovery of facial paralysis by targeting JUN involved in cAMP signaling pathway.

Testosterone and ASD co-exist in human plasma [49]. In our study, the interactive docking of 1FOS-ASD was predicted. ASD is found to show proliferative effect on androgen-sensitive LNCaP cells [50], while there was rare evidence for the therapeutic role of ASD on facial paralysis. Our data show that ASD
targeting 1FOS may play a key role in treating facial paralysis. However, further analyses are urgently needed.

In conclusion, this work hypothesized testosterone as the effective component of She-xiang which may advance the recovery of facial paralysis by targeting FUN, MAPK and cAMP signaling pathway; docking of ASD and 1FOS might play a critical role in facial paralysis treated by She-xiang. The future work can take this as a breakthrough to continue studying the mechanism and improving clinical outcomes of She-xiang in treating facial paralysis.

Declarations

Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the institutional ethics committee of Baoshan Branch of Shuguang Hospital, Shanghai University of Traditional Chinese Medicine (Approval number 201809-03), and followed the tenets of the Declaration of Helsinki.

Consent for Publication

Consent from all participants permitting their bioinformation for publication has been collected by the researchers.

Availability of data and materials

The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Competing Interests

The author declares that there are no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

Author Contributions

Conception and design: ZhiDdan Liu, Lan Shen; Administrative support: ZD Liu; Provision of study materials or patients: Xiaoyan Li; Collection and assembly of data: Xiaoyan Li, Chuang Zhao; Data analysis and interpretation: Xiaoyan Li, Chuang Zhao, Jiamiao Wang; Manuscript writing: All authors; Final approval of manuscript: All authors.

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References

1. Owusu JA, Stewart CM, Boahene K. Facial Nerve Paralysis. *Med Clin North Am.* 2018; 102:1135-1143.
2. Chan MK, Toribio JA, Podadera JM, et al. Incidence, cause, outcome and possible risk factors associated with facial nerve paralysis in dogs in a Sydney population (2001-2016): a retrospective study. *Aust Vet J.* 2020;98(4):140-147.
3. Somasundara D, Sullivan F. Management of Bell's palsy. *Aust Prescr.* 2017; 40:94-97.
4. Seiff SR, Carter SR. Facial nerve paralysis. *Int Ophthalmol Clin.* 2002; 42:103-112.
5. Papan C, Kremp L, Weiß C, et al. Infectious causes of peripheral facial nerve palsy in children—a retrospective cohort study with long-term follow-up. *Eur J Clin Microbiol Infect Dis.* 2019; 38:2177-2184.
6. Zhang W, Xu L, Luo T, et al. The etiology of Bell's palsy: a review. *J Neurol.* 2020;267(7):1896-1905.
7. Madhok VB, Gagyor I, Daly F, Somasundara D, et al. Corticosteroids for Bell's palsy (idiopathic facial paralysis). *Cochrane Database of Systematic Reviews.* 2016; 7:CD001942.
8. Gagyor I, Madhok VB, Daly F. Antiviral treatment for Bell's palsy (idiopathic facial paralysis). *Cochrane Database of Systematic Reviews.* 2015;11:CD001869.
9. Sullivan F, Daly F, Gagyor I. Gagyor, Antiviral agents added to corticosteroids for early treatment of adults with acute idiopathic facial nerve paralysis (Bell Palsy). 2016;316: p. 874-875.
10. Holland NJ, Bernstein JM. Bell's palsy. *BMJ Clin Evid.* 2014; 2014. pii: 1204.
11. Lee SY, Seong J, Kim YH. Clinical Implication of Facial Nerve Decompression in Complete Bell's Palsy: A Systematic Review and Meta-Analysis. *Clin Exp Otorhinolaryngol.* 2019; 12:348-359.
12. Dashtdar M, Dashtdar MR, Dashtdar B, et al. The Concept of Wind in Traditional Chinese Medicine. *J pharmacopuncture.* 2016; 19: 293-302.
13. Rastogi S, Pandey MM, Rawat AK. Traditional herbs: a remedy for cardiovascular disorders. 2016; 23:1082-1089.
14. Chan ES, Bautista DT, Zhu Y, et al. Traditional Chinese herbal medicine for vascular dementia. *Cochrane Database of Systematic Reviews.* 2018; 12: CD010284.
15. Li J, Cao GY, Zhang XF, et al. Chinese Medicine She-Xiang-Xin-Tong-Ning, Containing Moschus, Corydalis and Ginseng, Protects from Myocardial Ischemia Injury via Angiogenesis. *Am J Chin Med.* 2020; 48:107-126.
16. Gu YL, Hao FQ. Twenty-eight cases of facial neuritis treated with Strychnos-Moschus bones strengthen plaster. *J South Med Uni.* 1995; 18: 144.

17. Zhao MQ. Thirty-two cases of facial neuritis treated by Acu-point application. *Si-chuan J Tradi Chin Med.* 1996; 4: 54.

18. Wang LY, Chen XQ. Clinical observation and analysis of 96 cases of facial neuritis treated with the combination of Chinese and Western Medicine. *J Int Chin West Med.* 1997; 6: 617-618.

19. Meng SJ, Hu CH, Zhang HL, et al. Effect of acupoint burying with She Xiang combined with physical therapy on facial paralysis. *J New Chin Med.* 2013; 45: 134-136.

20. Xue R, Fang Z, Zhang M, et al. TCMID: traditional Chinese medicine integrative database for herb molecular mechanism analysis. *Nucleic Acids Res.* 2012; 41: D1089-D1095.

21. Liu Z, Guo F, Wang Y, et al. BATMAN-TCM: a Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine. *Sci Rep.* 2016; 6: 21146.

22. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics.* 2010; 26:139-140.

23. Ashburner M, Ball CA, Blake JA, et al. Gene Ontology: tool for the unification of biology. *Nat Genet.* 2000; 25:25-29.

24. Yu G, Wang LG, Han Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. 2012; 16: 284-287.

25. Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* 2000; 28: 27-30.

26. Szklarczyk D, Franceschini A, Kuhn M, et al. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids Res.* 2010; 39: D561-8.

27. Szklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* 2014; 43: D447-52.

28. Shannon P, Markiel A, Ozier O, et al. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Res.* 2003; 13:2498-2504.

29. Berman HM, Kleywegt GJ, Nakamura H, et al. The Protein Data Bank archive as an open data resource. *J Comput Aided Mol Des.* 2014; 28:1009-1014.

30. Kim S, Chen J, Cheng T, et al. PubChem 2019 update: improved access to chemical data. *Nucleic Acids Res.* 2018; 47: D1102-D1109.

31. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem.* 2009; 30:2785-2791.

32. Bassil N, Alkaade S, Morley JE. The benefits and risks of testosterone replacement therapy: a review. *Ther Clin Risk Manag.* 2009; 5:427-448.

33. Cauley JA, Lucas FL, Kuller LH, et al. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. *Ann Intern Med.* 1999; 130: 270-277.
34. Hankinson SE, Eliassen AH. Endogenous estrogen, testosterone and progesterone levels in relation to breast cancer risk. *J Steroid Biochem Mol Biol.* 2007; 106: 24-30.

35. Rooyackers OE, Nair KS. Hormonal regulation of human muscle protein metabolism. *Annu Rev Nutr.* 1997; 17: 457-485.

36. Hansen-Smith FM, Carlson BM. Cellular responses to free grafting of the extensor digitorum longus muscle of the rat. *J Neurol Sci.* 1979; 41: 149-173.

37. Kuiper GG, Carlsson B, Grandien K, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. 1997; 138:863-870.

38. Bielecki B, Mattem C, Ghomari AM, et al. Unexpected central role of the androgen receptor in the spontaneous regeneration of myelin. *Proc Natl Acad Sci U S A.* 2016; 113: 14829-14834.

39. Kujawa KA, Emeric E, Jones KJ. Testosterone differentially regulates the regenerative properties of injured hamster facial motoneurons. *J Neurosci.* 1991; 11:3898-3906.

40. Kujawa KA, Kinderman NB, Jones KJ. Testosterone-induced acceleration of recovery from facial paralysis following crush axotomy of the facial nerve in male hamsters. *Exp Neurol.* 1989; 105:80-85.

41. Angel P, Karin M. Specific members of the Jun protein family regulate collagenase expression in response to various extracellular stimuli. *Matrix Suppl.* 1992; 1: 156-164.

42. Thanos PK, Okajima S, Tiangco DA, et al. Insulin-like growth factor-I promotes nerve regeneration through a nerve graft in an experimental model of facial paralysis. *Restor Neurol Neurosci.* 1999; 15:57-71.

43. Yang B, Ji C, Kang J, et al. Trans-Zeatin inhibits UVB-induced matrix metalloproteinase-1 expression via MAP kinase signaling in human skin fibroblasts. *Int J Mol Med.* 2009; 23:555-560.

44. Cao Y, Li K, Fu KY, et al. Central sensitization and MAPKs are involved in occlusal interference-induced facial pain in rats. *J Pain.* 2013; 14:793-807.

45. Fang F, Liu CY, Zhang J, et al. Involvement of MAPK ERK activation in upregulation of water channel protein aquaporin 1 in a mouse model of Bell’s palsy. *J Mol Neurosci.* 2015; 56: 164-176.

46. Cao J, Xiao Z, Jin W, et al. Induction of rat facial nerve regeneration by functional collagen scaffolds. 2013; 34: 1302-1130.

47. Teng FY, Tang BL. Tang, Axonal regeneration in adult CNS neurons—signaling molecules and pathways. *J Neurochem.* 2006; 96:1501-1508.

48. Chan KM, Gordon T, Zochodne DW, et al. Improving peripheral nerve regeneration: from molecular mechanisms to potential therapeutic targets. *Exp Neurol.* 2014; 261:826-835.

49. Rivarola MA, Migeon CJ. Determination of testosterone and androst-4-ene-3,17-dione concentration in human plasma. 1966; 7: 103-117.

50. Laplante Y, Poirier D. Proliferative effect of androst-4-ene-3,17-dione and its metabolites in the androgen-sensitive LNCaP cell line. *Steroids.* 2008; 73:266-271.

**Figures**
Figure 1

The volcano plot and heatmap differentially expressed genes. The differentially expressed genes in the whole blood samples of 5 facial paralysis patients, compared with 5 healthy controls. (A) The volcano plot of differentially expressed genes was visualized by ggplot2 software based on significant p values and fold change. (B) The heatmap of differentially expressed genes was analyzed by heatmap 2 in R. The gene expression profiles were significantly different between patients and controls.
Figure 2

Venn diagram for the overlapped proteins by cross-validation. The protein targets related with facial paralysis were predicted based on BATMAN and CTD database. Then, the overlaps with differentially expressed genes were analyzed by Venn analysis. Finally, 33 overlaps were identified.
Figure 3

Top 20 significant GO biological processes of protein targets. The proteins of interest were subjected to GO function enrichment analysis by clusterprofiler. The significant GO terms with p value $\leq 0.05$ in biological process category were analyzed. Top 20 GO terms were listed. Vertical axis represents GO terms or pathways, horizontal axis indicates gene ratio enriched in a given GO term or pathway. The size of the nodes represents the ratio of enriched genes and the color closer to red indicates the p values closer to 0.
Figure 4

The significant pathways of protein targets. The significant pathways for proteins with \( p \leq 0.05 \) were also analyzed with clusterProfiler in R. Vertical axis represents GO terms or pathways, horizontal axis indicates gene ratio enriched in a given GO term or pathway. The size of the nodes represents the ratio of enriched genes and the color closer to red indicates the \( p \) values closer to 0.
Figure 5

Protein-protein interaction network The protein pairs with combined score > 0.4 were retrieved from STRING database and the protein-protein interaction network was constructed by Cytoscape software. Yellow dot, up-regulated target protein; blue square, down-regulated protein.
Figure 6

Pharmacology network of herb She-xiang The integrated network with active components of herb She-xiang, their protein targets, and related pathways was constructed by cytoscape software. Red rhombus, active ingredient; yellow dot, up-regulated target protein; blue square, down-regulated protein; green hexagon, pathway.
Interactive docking prediction of key target protein and effective component The 3D structure of key target protein was obtained from Protein Data Bank database and the molecular structure of effective components were predicted by PubChem Compound database. The docking possibility of key protein and effective component was predicted by AutoDock software. (A) Molecule structure of Androst-4-Ene-3,17-Dione (ASD); Green, C; gray, H; red, O. (B) Linear 3D structure of FOS-JUN complexes (1FOS). (C) Surface 3D structure of FOS-JUN complexes (1FOS). (D) Global graph of molecule docking of 1FOS and ASD. Opaque white, 3D model of 1FOS; translucent molecule, ASD. (E)local map of molecular docking model. Translucent molecule indicates ASD ligand. Spheres and secondary structure fragments represent hydrogen bond or atoms with intimate contact with ASD ligand. Affinity is -7.9 and the interaction sequences between 1FOS and ASD include LYS267, ASN271, DC26 and DT14.

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