Integrated network pharmacology and proteomics to reveal the cognitive improvement effect of Wuzang Wenyang Huayu decoction on vascular dementia

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To the Editor: Vascular dementia (VD) is a clinical disease characterized by cognitive dysfunction and intellectual decline caused by various cerebrovascular factors. The high incidence of VD in middle-aged and elderly people has become a major medical and social problem worldwide.[1] Since its specific pathogenesis has not been fully elucidated, clinical practice is still focused on improving cognition and symptomatic treatment, such as piracetam and donepezil.[2] Alternatively, with high effectiveness and few side effects, traditional Chinese medicine has drawn medical attention and become a hotspot in the medical research field.

Wuzang Wenyang Huayu decoction (WZWYHYD) is, in our clinical experience, effective in treating VD; it has a good effect on cognitive improvement of vascular decoction. The components of WZWYHYD contains (gram): *Rhizoma typhonii* (Fuzi) 15 g, *Rhizoma zingiberis* (Ganjiang) 15 g, *Radix morindae officinalis* (Bajitian) 15 g, *Ramulus cinnamomi* (Guizhi) 15 g, *Rhizoma pinelliae preparatum* (Banxia) 15 g, *Acorus tatarinowii* (Shichangpu) 15 g, *Radix notoginseng* (Sanqi) 15 g, *Herba epimedii* (Yinyanghuo) 15 g, *Radix ginseng* (Shengshaishen) 15 g, and Radix et rhizome rhei (Dahuang) 6 g. Currently, the development of network pharmacology has inspired new explorations to elucidate Traditional Chinese Medicine therapeutic mechanisms. Moreover, with the application of proteomics technology in drug research, network pharmacology combined with proteomics analysis technology provides skills for analyzing protein changes and therapeutic mechanisms in the development of disease.

This study was approved and monitored by animal experiments ethical review committee of the Guangxi University of Chinese Medicine, Nanning, China. First, in this study we established a rat model of VD using bilateral total carotid artery occlusion, divided into a VD model group, high and low dose Chinese medicine groups, and a Western medicine (Wes) group, with a sham operation group as the control group. For 2 weeks, the control group and the model group were given the same amount of 0.9% sodium chloride solution every day; the high-dose (VD-WY-H) and low-dose (VD-WY-L) groups were given WZWYHYD 5 g/kg and 1.25 g/kg, respectively; and the Western medicine group was given piracetam dispersible tablets 0.15 g/kg every day. The Morris water maze test was used to evaluate the improvement effect of WZWYHYD on cognitive function. By calculating the escape latency [Supplementary Figure 1, http://links.lww.com/CM9/A911] and distance ratio [Supplementary Figure 2, http://links.lww.com/CM9/A911] of rats, it was found that rats in the model group had obvious cognitive impairment, indicating that our modeling was successful, and we found that the cognitive function of rats was significantly improved in the VD-WY-H and Wes group. Next, we performed hematoxylin-eosin staining on the hippocampal tissue in each group and found that the hippocampal tissue damage was consistent with the behavioral results, and the damage in the VD-WY-H and Wes groups was significantly reduced [Supplementary Figure 3, http://links.lww.com/CM9/A911].

Then, we used the Chinese Medicine System Pharmacology to collect 65 active components of WZWYHYD [Supplementary Table 1, http://links.lww.com/CM9/A907] and the SwissTargetPrediction platform to predict the protein targets of active components. Finally, disease targets were collected through the Therapeutic Target Database, Online Mendelian Inheritance in Man, and the GeneCards database and intersected with the predicted targets from the SwissTargetPrediction platform to obtain 78 potential targets [Supplementary Table 2, http://links.lww.com/CM9/A908]. Through Gene Ontology, Kyoto Encyclopedia of Genes and Genomes [Figure 1A, B], and protein protein interaction network [Supplementary Figure 4, http://links.lww.com/CM9/A911] analyses, it was found that the mechanism of effect is mainly related to oxidative

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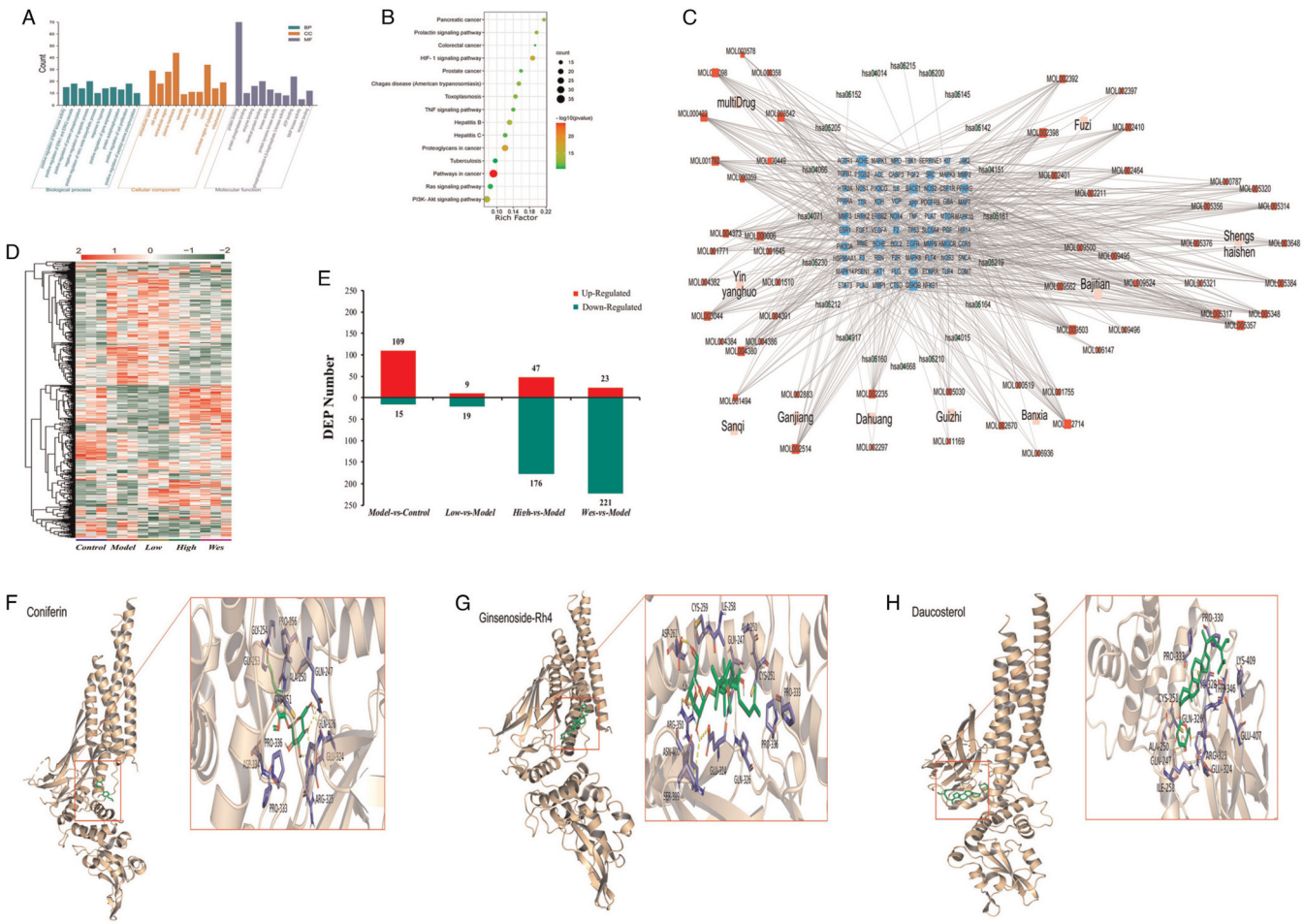

Figure 1: Wuzang Wenyang Huayu decoction improves the cognitive impairment of VD rats by inhibiting the expression of STAT3 protein and its related pathways. Network pharmacology results of Wuzang Wenyang Huayu decoction: GO analysis (A), KEGG pathway (B), Compounds targerts Pathways network (C); Proteomic results; Clustering heat map of differential proteins (D); The number of different proteins compared with other group (E); Molecular docking results: conifeinm (F), ginsenoside-rjh4 (G) and daucosterol (H) have strong binding energy with STAT3 protein. VD: Vascular dementia.

stress. For example, it is mainly positive regulation of MAP (Mitogen-activated protein) kinase activity in biological processes, and HIF-1 signaling pathway, that are crucial to the body's response to hypoxia.[3]

After intraperitoneal anesthesia with chloral hydrate (360 mg/kg), the hippocampal tissue protein was extracted and detected by LC-MS (Liquid chromatograph-Mass spectrometer) /MS; data are available via ProteomeXchang with identifier PXD028968. A total of 3692 differentially expressed proteins were identified [Supplementary Table 3, http://links.lww.com/CM9/A909]; the cluster heat map [Figure 1D] and the number of differential proteins were compared among four groups; analyzing the results of the comparison, we can see that most of the differential proteins in the High/Model and Wes/model groups were down-regulated, and a few were up-regulated, indicating that the High-dose group and Western medicine group mainly played a therapeutic role by inhibiting the expression of some proteins [Figure 1E].

By setting the screening conditions as ratio >1.5, we further screened 56 proteins that were significantly differentially expressed in the High-dose group [Supplementary Table 4, http://links.lww.com/CM9/A910]. Compared with the potential target proteins screened by network pharmacology, we obtained the intersection target STAT3. Modern research has also shown that inhibiting the expression of STAT3 can reduce the expression of ß-amyloid levels to protect against ischemic brain injury, which is consistent with our experimental conclusions.[4]

Finally, according to the component-target-pathway network [Figure 1C], we found the corresponding active components of the STAT3 target were coniferin in Banxia, ginsenoside-Rh4 in Raw ginseng, and daucosterol in Dahuang, and verified by molecular docking. It was found that coniferin, ginsenoside-rh4, and daucosterol had a strong affinity for the STAT3 protein [Figure 1F-H]. These results show that coniferin, ginsenoside-rh4, and daucosterol in WZWYHYD can improve cognition and treat VD by inhibiting the STAT3 protein and its related pathways.

In general, through the combined analysis of network pharmacology and proteomics, we found that coniferin, ginsenoside-Rh4, and daucosterol play an important role in the treatment of VD by inhibiting the expression of the

STAT3 protein. These findings also provide a new strategy for studying the effective components and treatment mechanism of Chinese medicine. In addition, further study is needed to ascertain whether the active ingredients play a therapeutic role through the HIF-1 signaling pathway.

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Conflicts of interest

The authors confirm that there are no conflicts of interest.

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