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CLINICAL STUDY

Transcriptome analysis of blood stasis syndrome in subjects with hypertension

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Abstract

OBJECTIVE: To screen for mRNAs associated with blood stasis syndrome and to explore the genetic mechanisms of blood stasis syndrome in hypertension.

METHODS: This study involved groups of patients with hypertension and blood stasis, including those with *Qi* deficiency, *Qi* stagnation, cold retention and heat retention; as well as hypertensive patients without blood stasis and healthy individuals. Human umbilical vein endothelial cells were co-cultured with the sera of these healthy individuals and patients with blood stasis syndrome. Total RNA was extracted from these cells and assessed by a high-throughput sequencing method (Solexa) and digital gene expression. Differentially expressed genes among these six groups were compared using whole genome sequences, and mRNAs associated with blood stasis syndrome identified. Differences in gene use and gene ontology function were analyzed. Genes enriched significantly and their pathways were determined, as were network interactions, and encoded proteins. Gene identities were confirmed by real-time polymerase chain reactions.

RESULTS: Compared with cells cultured in sera of the blood stasis groups, those culture in sera of healthy individuals and of the non-blood stasis group showed 11 and 301 differences, respectively in stasis-related genes. Genes identified as differing between the blood stasis and healthy groups included activating transcription factor 4, activating transcription factor 3, DNA-damage inducible transcription factor 3, Tribbles homolog 3, CCAAT/enhancer binding protein-β, and Jun proto-oncogene (JUN). Pathway and protein interaction network analyses showed that these genes were associated with endoplasmic reticulum stress. Cells cultured in sera of patients with blood stasis and *Qi* deficiency, *Qi* stagnation, heat retention, and cold retention were compared with cells cultured in sera of patients with the other types blood stasis syndrome. The comparison showed differences in expression of 28, 28, 34, and 32 specific genes, respectively.

CONCLUSION: The pathogenesis of blood stasis syndrome in hypertension is related to endoplasmic reticulum stress and involves the differential expression of the activating transcription factor 4, activating transcription factor 3, DNA-damage inducible transcription factor 3, Tribbles homolog 3, CCAAT/enhancer binding protein-β, and JUN genes.

Key words: Hypertension; Blood stasis; RNA, messenger; Endoplasmic reticulum stress

INTRODUCTION

Hypertension is a highly prevalent clinical syndrome characterized by high arterial pressure that can seriously damage the heart, brain, and kidneys. In traditional Chinese medicine (TCM), blood stasis is believed to play an important role in the occurrence and development of hypertension. Current studies on the mechanism of blood stasis syndrome have been limited to observing changes in blood, focusing on the relationship between vascular changes and the initiation of blood stasis syndrome. Moreover, blood stasis biology has been associated with changes in vascular endothelial cell (VEC) function,[1] with VEC damage closely associated with the initiation and development of blood stasis syndrome.[2,3] Endoplasmic reticulum stress (ERS) has been shown to damage VECs,[4] indicating that ERS may induce blood stasis by causing endothelial cell injury.

Our research group has focused on a blood stasis syndrome model, including the effects of VEC injury and mechanisms of repair, as well as on the effects of drugs and the molecular / genetic mechanisms underlying blood stasis. We and others have found that different types of blood stasis have different biochemical foundations, but also have common pathological and physiological characteristics.[5-10] Because of the complex pathogenesis of blood stasis syndrome, including the effects of various factors, screening for and identifying biomarkers associated with this syndrome may help in its diagnosis and treatment. An established model of endothelial cell injury-blood stasis syndrome was used to screen for genes associated with the initiation of hypertension-related blood stasis syndrome among groups of hypertensive patients, with and without blood stasis syndrome, and healthy individuals. The genetic pathways of identified genes were investigated to determine the molecular mechanism underlying the development of blood stasis syndrome in hypertension.

MATERIALS AND METHODS

Three groups of individuals were analyzed. The first group included 40 patients (21 males, 19 females), of mean age (66 ± 7) years, diagnosed with blood stasis syndrome with hypertension from December 2011 to June 2012 at the Second Hospital affiliated with Guangzhou Medical College. Their mean systolic and diastolic blood pressures were (157 ± 13) and (104 ± 7) mm Hg, respectively. TCM syndrome differentiation rules have identified four types of patients with blood stasis syndrome plus hypertension: blood stasis syndrome with *Qi* deficiency, *Qi* stagnation, cold retention, and heat retention. Ten patients were included in each subgroup.

Another group of 12 patients [6 males, 6 females, of mean age (65 ± 9) years] had hypertension but without blood stasis. Their mean systolic and diastolic b; ppd pressures were (166 ± 8) and (104 ± 8) mm Hg, respectively. These patients were recruited from the cardiovascular department, the outpatient TCM department, and patients hospitalized at the First Affiliated Hospital of Jinan University. Patients in this group did not differ significantly from the group of hypertensive patients with blood stasis syndrome in blood pressure, sex distribution, mean arterial pressure, or average age (each $P > 0.05$).

The third group consisted of 30 healthy volunteers (17 males, 13 females), recruited from the Department of Traditional Chinese Medicine, School of Medicine, Jinan University. Their blood pressures were within normal ranges and differed significantly ($P < 0.01$) from blood pressures in hypertensive patients, both with and without blood stasis syndrome.

High blood pressure was defined according to World Health Organization / International Hypertension Alliance guidelines (1999),[11] and blood stasis syndrome was diagnosed according to the 2011 revised criteria.[12] Patients with target organ damage, diabetes, or severe cardiovascular, cerebrovascular, or renal disease were excluded.

Serum collection

All included individuals were instructed to fast and not take any drugs overnight prior to a blood draw. Forearm venous blood was aseptically collected into sterile, non-anticoagulation vacuum tubes, allowed to coagulate, and centrifuged at 2784´g at 4 ℃ for 15 min. Serum was removed, placed in an aseptic Eppendorf tube (Gibco, Guangzhou, China), and incubated in a water bath at 56 ℃ for 30 min to inactivate serum complement. Finally, these samples were stored at − 20 ℃.

Establishing a cell model

The cell model of hypertension and blood stasis,[13] involved the use of human umbilical vascular endothelial cells (HUVECs; CRL-1730), obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). These cells were cultured in Dulbecco's Modified Eagle's medium (DMEM); (Gibco) containing 10% fetal bovine serum at 37 ℃ in a 5% CO_2 incubator. To each culture flask was added serum, to a concentration of 10%, plus 90% F12K medium (Gibco), and the cells were cultured at 37 ℃ in a 5% CO_2 incubator for 24 h.

The cells were collected, and total RNA was extracted with Trizol (Invitrogen, Carlsbad, CA, USA). RNA quality was assessed by agarose gel electrophoresis.

mRNA sequence analysis and gene screening

mRNA transcriptome sequencing was performed using second-generation IIlimina Solexa sequencing technology by the Shanghai Megiddo Biotechnology Co., Ltd. (Shanghai, China), The original statistical data were assessed while retaining high-quality sequences. Statistical analyses of each fragment per kilobase of exons per million fragments mapped (FPKM), as well as the transcript value in the expressed samples, were compared.