Int J Clin Exp Pathol 2015;8(9):11356-11363
www.ijcep.com /ISSN:1936-2625/IJCEP0010732

Original Article

Functional analysis of *UMOD* gene and its effect on inflammatory cytokines in serum of essential hypertension patients

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Received May 27, 2015; Accepted June 29, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: Objective: The study aimed to investigate the function of uromodulin (UMOD) gene and its effect on inflammatory cytokines in serum of essential hypertension patients. Methods: The online database and software of computer were used for bioinformatics analysis on UMOD gene as well as the structure and function of its encoding proteins. Moreover, radioimmunoassay and enzyme linked immunosorbent assay was adopted to validate the content of urine *UMOD* protein of essential hypertension patients and their serum inflammatory cytokines. Results: As an alkaline and hydrophilic protein, UMOD has no transmembrane region, but it does have a signal peptide sequence. It is mainly located extracellularly, belonging to a secreted protein, whose secondary structure was based mainly on Random coil which account for 58.44%. According to function prediction, it is found that the *UMOD* protein has stress response which may be participate in the inflammatory reaction. It has been observed from the experiment which was designed on the basis of the correlation between inflammation reaction and essential hypertension that the content of urine UMOD protein of essential hypertension patients who is in stage I was (28.71±10.53) mg/24 h and when compared with the control group's content (30.15±14.10 mg/24 h), the difference was not obviously; The content of urine UMOD protein of essential hypertension patients who's in stage II and III was (18.24±6.12) mg/24 h and (9.43±3.16) mg/24 h, respectively, which were obviously lower than that of the control group (P<0.01). Additionally, the serum inflammatory cytokines, such as TNF-α, IL-6 and IL1-α content of essential hypertension patients were all markedly higher than that of control group (P<0.05). Conclusion: For essential hypertension patients, there's a close relationship between the expression level of UMOD gene and inflammatory cytokines, which were manifested as the negative correlation between the level of the gene's expression and inflammatory cytokines. That has certain reference value to realize the targeted treatment for essential hypertension through regulated blood pressure conversely in the view of expression level of inflammatory cytokines.

Keywords: *UMOD*, essential hypertension, inflammatory cytokines, gene ontology, protein function analysis

Introduction

Hypertension, sustaining high blood pressure disease, is often accompanied with systemic disease of functional change in heart, blood vessel, brain and kidney, etc. It was divided into essential hypertension and secondary hypertension. As a polygenic hereditary disease caused by the joint action of genetic and environmental factors, Essential hypertension (EH) is an independent risk factor which could bring about myocardial infarction, cardiac failure, cerebrovascular disease and chronic renal failure, and accounting for about 90% to 95% of the total number of patients with hypertension [2]. With the rapid development of molecular biology techniques and bioinformatics, discussion on EH susceptibility genes has become the hot topic of its pathogenesis research in both current and future studies. Currently, dozens of related genes of EH have been identified using candidate gene approach.

As an identified candidate gene that closely related to EH, uromodulin (*UMOD*) gene located on the short arm of chromosome 16p11-13 promoter region, consists of 11 exons (39777 bp). The protein, which was expressed by it also known as Tamm-Horsfall (THP), one of the mucin in the urine, was separated from the

urine via salting out method by Igor Tamm and Frank Horsfall in 1950 [3]. *UMOD* is primarily synthesized and secreted into the urine by thick ascending limb (TAL) of kidney tubules medullary loop and epithelial cells in the initial segment of the distal convoluted tubule. The daily secretion of the healthy is about 20~70 mg. It is the most abundant protein in normal urine. However, at present, the physiological function of *UMOD* protein is not very clear, and there are researches indicated that the *UMOD* gene knockout mice has decreased urine concentration ability while the incidence of urinary tract infections as well as kidney stones increased [4, 5], which conducted to the conjecture that the gene may be related to the lithangiuria formation, immune response, renal tubular epithelial cell protection. Up to now, more than 60 disease-causing mutations have been identified, and almost all of them are resulted by missense mutations. Mutation sites are mostly located No. 4, 5 and 8 exons. In addition, the vast majority of mutations occur in exon 4. All of them are involved the highly conservative base sequence in the evolution, and the tertiary structure of *UMOD was destroyed by that* and result in its functional loss.

In 2010, as a genome-wide association analysis (GWAS) showed, there is a significant correlation between blood pressure and the single nucleotide polymorphism of rs13333226 locus in *UMOD* gene promoter region, and people who carry G allelic fragments suffered lower incidence of hypertension and abnormal renal function [6-9]. Trudu et al. [10] found that after *UMOD* gene mutations, the expression of uromodulin increased, leading to salt-sensitive hypertension and renal damage. The research of Trudu et al. about the role of NKCC2 between urine regulation and hypertension established link of hereditary susceptibility between hypertension and chronic nephrosis. Studies of Graham LA et al. [11] have shown that the *UMOD* gene is the candidate gene of essential hypertension, which can be regarded as a novel drug target and provides a new way in the prevention and treatment of essential hypertension. However, as a new finding in studying essential hypertension, the specific functions or possible signal pathway of *UMOD* gene in the diagnosis and treatment of primary hypertension is still not clear. In order to get some functional information from the gene and protein sequential structure, this paper performed bioinformatics analysis of *UMOD* gene and the structure as well as the function of its proteins. The influence of *UMOD* gene on serum Inflammatory cytokines was investigated by the use of experiment, thus offering reference for the practical research to regulated blood pressure conversely along with the level change of inflammatory cytokines.

Materials and methods

Bioinformatics analysis of UMOD gene and encoding product

UMOD gene sequences came from Genbank (Accession: M17778.1). The ORF Finder program of NCBI was used to analyze *UMOD* gene open reading frame. The ProtParam, Compute pI/Mw and ProtScale software in ExPASy server was utilized to predict amino acid composition, molecular mass, isoelectric point, hydrophobicity/hydrophilicity, instability coefficient and fat coefficient etc. of *UMOD* protein. TMHMM software was put to use to predict transmembrane region of *UMOD* protein. Then, SignalP 4.1 Server tools were adopted to predict amino acid sequence signal peptide of the human *UMOD* encoded products. Afterwards, TargetP software was employed to predict localization of *UMOD* protein in cells. Hopfield neural network was used to predict the secondary structure of *UMOD* protein, and Protfun software and Gene Ontology database was used separately to predict the function of *UMOD* protein. Its interactional proteins were analyzed using STRING online database of protein interactions, and then the signaling pathways of the *UMOD* interactional proteins were analyzed in KEGG database.

Regulation of UMOD gene on essential hypertension patients

This study was carried out in accordance with the “Ethical Guidelines and Regulations” and the international standards for editors and authors press release.

EH case group: fifty-two EH patients who received treatment in hospital during September, 2011 to September, 2014 were enrolled as case group, among which were 28 males and 24 females, their age ranged within 54 to 80 years old and share an average age of 64.6 years old, an average blood pressure of 170±14/105±10 mm Hg as well. There are 13 essential hypertension patients who were in stage I, and 18 patients in stage II and 21

Table 1. The GO analysis results of UMOD gene

Acc	Taxon	Accession	Name
UMOD	Homo sapiens	GO:0048878	chemical homeostasis
UMOD	Homo sapiens	GO:0007588	excretion
UMOD	Homo sapiens	GO:0010033	response to organic substance
UMOD	Homo sapiens	GO:0072218	metanephric ascending thin limb development
UMOD	Homo sapiens	GO: 072233	metanephric thick ascending limb development
UMOD	Homo sapiens	GO:2000021	regulation of ion homeostasis
UMOD	Homo sapiens	GO:0072221	metanephric distal convoluted tubule development
UMOD	Homo sapiens	GO:0006968	cellular defense response
UMOD	Homo sapiens	GO:0008285	negative regulation of cell proliferation
UMOD	Homo sapiens	GO:1990266	neutrophil migration
UMOD	Homo sapiens	GO:0007159	leukocyte cell-cell adhesion
UMOD	Homo sapiens	GO:0007157	heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules

patients in stage III. All of them meet the WHO hypertension diagnosis and grading standard, and none of them suffered from disease like immunogenic, diabetes and hepatic renal dysfunction. Other 24 healthy volunteer were treat as control group, including 13 males and 11 females, their age were within 53 to 79 years old and the average age was 63.2 years old.

Content determination of UMOD protein

Sampling patients urine in 24 hours and the content of UMOD protein in it was tested through radioimmunoassay for both EH case group and control group. The kit was provided by Chinese Isotopes Company North Immunoreagent Institution, and the experimental procedures were carried out in strict accordance with the kit instructions.

Contents determination of serum inflammatory cytokines TNF-α, IL-6 and IL1-α

2 ml fasting venous blood from control group and case group were collected, respectively, and the blood was centrifuged for 10 minutes in speed of 3000 r/min, and then the supernatant was collected and preserved in -20°C for preparation. TNF-α, IL1-α and IL-6 levels in serum were detected by using enzyme-linked immunosorbent assay (ELLSA). The experimental procedures were carried out in strict accordance with the kit instructions, and the kit was purchased from Roche, Germany.

Statistical analysis

Notable difference analysis of experimental data were run via statistics: software SPSS17.0, and the measurement data were written as ($x \pm s$), then the t test was used to determine. There's statistical significance for the differences when $P < 0.05$.

Results

GO functional analysis of UMOD gene

Taking the function of gene and protein in cells as the measurement basis, Gene Ontology (GO) has provided three kinds of ontological categories: biological process, molecular function and cellular components. UMOD plays a vital role in biological process, molecular function and cellular components. As it shown, what was contained in **Table 1** is the GO analysis results of UMOD protein in biological process, and there is where it can be observed that the UMOD protein has such functions like maintaining systemic chemical equilibrium and cellular defense, which has further explanation about the crucial role of UMOD protein played in inflammatory reaction.

Physicochemical properties and structure analysis of UMOD of protein

Such bio-information software as ProtParam, Compute pI/Mw, ProtScale and TMHMM were used to analyze the physicochemical properties and structure of *UMOD* protein. The results indicated the atomic composition of UMOD protein was $C_{3011}H_{4654}N_{832}O_{952}S_{63}$; relative molecular weight: about 69760.86 Da; theoretical isoelectric point: 5.0; the unstable coefficient was 40.53; the fat coefficient was 70.69; total average hydrophobicity was -0.111. No typical spi-

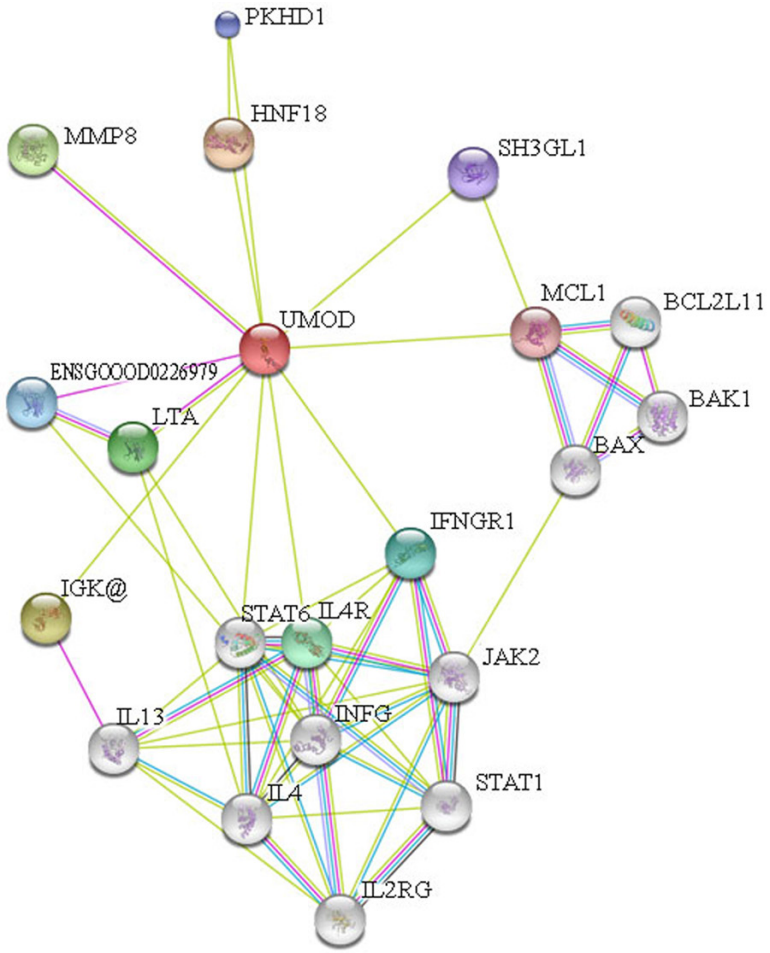

Figure 1. Analysis figure of UMOD protein cross-linking function.

ral transmembrane region in *UMOD* protein, and the possibility to function outside the cell was nearly 100%. Thus, it was speculated as an alkaline and unstable hydrophilia protein which contains a signal peptide sequence, located extracellularly, belonging to a secreted protein. In its secondary structure, Alpha helix accounted for 23.44%, extended strand accounted for 18.12%, Random coil accounted for 58.44%.

Prediction of UMOD protein function

The prediction results of *UMOD* protein which obtained through software Protfun were as followed. Functional category displayed *UMOD* protein had functions of purines and pyrimidines (Prob = 0.541, Odds = 2.227). Enzyme/nonenzyme displayed that *UMOD* protein was lyase (Prob = 0.057, Odds = 1.211). Gene Ontology category displayed that *UMOD* protein had stress response (Prob = 0.188, Odds = 2.140).

Analysis of UMOD protein cross-linking function

The interacting proteins of *UMOD* were analyzed using STRING protein interaction online database, and the results were show in **Figure 1**. It was indicated that what interacting with *UMOD* protein were mainly ten kinds of protein, they are IGK@, MMP8, ENSG00000226979, PKHD1, HNF1B, IL4R, IFNGR1, SH3GL1, MCL1 and LTA. Molecular signaling pathway analysis showed that these above mentioned proteins participated in inflammatory bowel disease, NF-κB signaling pathway, TNF signaling pathway, cytokines-cytokine receptor interaction, PI3K-Akt signaling pathway, etc. The results of present researches show that both inflammatory bowel disease and TNF signaling pathway were relative to the level of TNF-α [12]; the activated NF-κB canonical signaling gave rise to the increased level of IL-6 [13] and the restrained of it could result in the decreased level of IL-6 [14], in addition, there's a close link between the signaling pathway of cytokines-cytokine receptor interaction and the level of TNF-α [15]; the level of TNF-α and IL-6 deduced when P13k-Akt signaling pathway was activated [16]; when it combined with the prediction results of *UMOD* protein function, the important role *UMOD* protein played in the inflammatory response should be speculated.

Detection of urine UMOD protein in essential hypertension patients

The detection results of urine UMOD protein in essential hypertension patients were shown in the **Table 2**. It can be observed from it that the content of urine *UMOD protein of* essential hypertension patients who is in stage I was (28.71±10.53) mg/24 h and when compared with the control group's content (30.15±14.10

Table 2. The detection results of urine UMOD protein in Essential hypertension patients

Group		Cases	The content of UMOD protein mg/24 h
Control group		24	30.15±14.10
EH group	Stage I	13	28.71±10.53
	Stage II	18	18.24±6.12$^{**,\Delta\Delta}$
	Stage III	21	9.43±3.16$^{**,\Delta\Delta}$

Note: when comparing to control group the **means P<0.01, comparing to stage I the $^{\Delta\Delta}$means P<0.01.

mg/24 h), the difference was not significant; The content of urine UMOD protein of essential hypertension patients who are in stage II and III was (18.24±6.12) mg/24 h and (9.43±3.16) mg/24 h, respectively, which were obviously lower than that of the control group (P<0.01). Besides, the content of urine UMOD protein of patients in stage II and III has dramatic decrease when comparing to that of control group and patients in stage I (P<0.01).

Content differences of serum inflammatory cytokines in essential hypertension patients

The level of serum inflammatory cytokines TNF-α, IL-6 and IL1-α from both EH case group and control group were shown in the **Figure 2**. It can be noticed that the content of serum inflammatory cytokines TNF-α, IL-6 and IL1-α of EH patients was obviously higher than that of the control group (P < 0.05). Combining the functional analysis for UMOD gene and its protein which mentioned in the last chapter, the conclusion can be drawn as the UMOD gene brings enormous influence to EH patients' multiple inflammatory cytokines during its expression.

Discussion

Essential hypertension is one of the common and frequently-occurring diseases which has severely damaged people's health, it is the disease of blood under supplied or relatively under supplied in important organs like heart, brain and kidney, which could blame it on systemic arteriolar constriction and the increased blood viscosit caused by many reasons as psychic factors. The blood pressure got rise to satisfy the supply requirement of organs through the blood regulation mechanism. The pathology of EH was not clear by now, and the excavation of EH susceptibility gene developed rapidly. As a new-found candidate gene with a high relevance to EH [17], UMOD gene has provide a new research direction for the prevention and treatment of EH.

Essential hypertension is a systemic metabolic disorder disease. In a sense, it is also a low-grade systemic inflammatory reaction, taking the form of the abnormal inflammatory cytokines and the activation of inflammatory signaling pathways [18]. Inflammation factors play a vital role in the occurrence, development and outcome of hypertension. What's worse, they are closely related to the occurrence and development of many cardiovascular diseases [19], while hypertension will promote the inflammation response through its bloodstream biomechanical stimulation [20]. In this paper, bioinformatics methods were used for prediction and analysis of *UMOD* protein structure and hence its function. We conclude that *UMOD* protein was a secreted protein and the proportion of Random coil in its secondary structure was the highest, reaching 58.44%. Additionally, after the functional prediction and analysis of UMOD protein, it turns out that it may be involved NF-κB signaling pathway, TNF signaling pathway, cytokine-cytokine receptor interactions and other signaling pathways. As a series of present researches about inflammatory cytokines has proved, a number of molecules in each stage of inflammatory response, including: TNF-α, IL-1β, IL-2, IL-6, IL-8, IL-12, etc. were regulated by these signaling pathways. The activation of signaling pathway can strengthen transcription of inflammation factors and enhance inflammatory signals [21]. In this study, after the note analysis UMOD function through GO database, it has been found that UMOD protein takes part in cellular defense, which means *UMOD* gene play an important role in the inflammatory response. Several studies indicated that it is obviously that some cytokines and inflammatory cytokines have close relevance to the occurrence as well as the development of hypertension. Inflammatory cytokines, a biologically active peptide which were synthesized and excreted after the injury or stimulation of immunocyte and non immune cells, had the central function in inflammatory reaction [22]. At present, the report about regulating the level of inflamma-

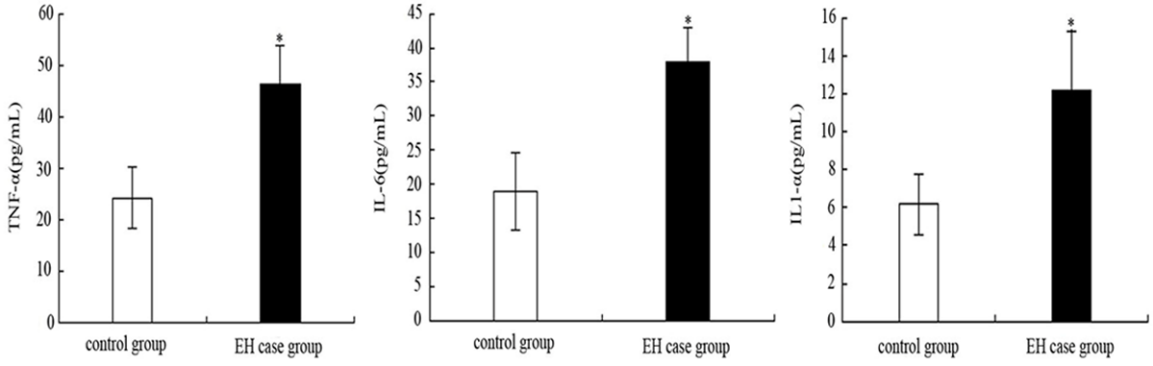

Figure 2. The level of TNF-α, IL-6 and IL1-α in serum from both EH case group and control group.

tory cytokines to control blood pressure is quite rarely. In this study, the EH patients' level of inflammatory cytokines is obviously higher than that of control group, which is in accordance with the domestic research finding that the level of inflammatory cytokines in serum of EH patients increased with the rising of blood pressure. It is such inflammatory cytokines as TNF-α, IL1, IL-6 that functioned among variety of them [23]. Serum inflammatory cytokines TNF-α and IL-1 can promote the proliferation of smooth muscle cells from a variety of ways, thereby enabling calcium ions within the smooth muscle cells rise quickly, causing the constriction of blood vessels and then the elevation of blood pressure [24]. Serum IL-6 can change the rheological properties of leukocytes, make them adhere to vascular endothelium more easily, increase the vascular resistance and increase the number of platelets. In addition, it may also interact with angiotensin II in the blood vessels and elevate the blood pressure [25, 26]. A study of one hundred and ninety six healthy people conducted by Bautista et al. [27] found that IL-1 and TNF-α levels was positively correlated to systolic blood pressure and diastolic blood pressure, suggesting that IL-1 and TNF-α may be the independent risk factors of hypertension and inflammatory reaction was indirectly involved in the pathogenesis of essential hypertension.

The results of this study show that the content of urine UMOD protein of essential hypertension patients who is in stage I was (28.71±10.53) mg/24 h and when compared with the control group's content (30.15±14.10 mg/24 h), the difference was not significant; The content of urine UMOD protein of essential hypertension patients who's in stage II and III was (18.24±6.12) mg/24 h and (9.43±3.16) mg/24 h, respectively, which were obviously lower than that of the control group (P<0.01). Besides, the content of TNF-α, IL-6 and IL1-α in serum of EH patients has dramatic increasing when comparing to that of control group (P<0.05).

To sum up, this study predicated *UMOD* gene and its encoding proteins' structure and function using bioinformatics methods, meanwhile, the negative correlation between the expression level of UMOD gene and the level of inflammatory cytokines were verified via the valid experimental data. All of that presaged that through simple antibacterial and anti-inflammatory medicine we can regulate EH conversely from the view of level of inflammatory cytokines, which has reference value for the prevention and treatment of EH.

Acknowledgements

The authors thank the Medical Engineering Technology & Data Mining Institute of Zhengzhou University for their invaluable technical assistance. Moreover, the authors would like to thank Zhengzhou Central Hospital for providing us the experimental base. This work was supported by grant: 20120900, 20130459 and 132102310332.

Disclosure of conflict of interest

None.

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