Int J Clin Exp Med 2016;9(3):6413-6420
www.ijcem.com /ISSN:1940-5901/IJCEM0017441

Original Article

A study on the molecular mechanisms of *cicada slough* acting on IgA nephropathy

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Received October 8, 2015; Accepted December 28, 2015; Epub March 15, 2016; Published March 30, 2016

Abstract: *Cicada Slough* has been used widely in Traditional Chinese Medicine (TCM). Recently, its therapeutic potential in treatment of IgA Nephropathy (IgAN) has been showed. However, the underlying mechanisms of the action are not known. In this study, a systematic analysis was performed to elucidate the main regulation networks of its multi-targets and associated biological processes of *C. Slough* acting on IgAN. We found that *C. slough* mainly interact the imbalanced network of IgAN via regulation of innate immune system and inflammatory response system via proteins of MDM, REN, VDR, AGT, NOS2, JUN, ACE, SYK and TNF. This regulation network may provide a new insight for developing new therapy for IgAN, and thus further studies are called to adapt the formula of TCM and to enhance its effectiveness on IgAN.

Keywords: Traditional Chinese Medicine, cicada slough, IgA nephropathy, proteins, network

Introduction

IgA Nephropathy (IgAN) is now recognized to elevate the risk of cardiovascular disease as well as kidney failure and other complications [1, 2]. It is a leading cause of end-stage kidney disease in China [3]. Effective control of proteinuria may be a key strategy for treating IgAN [4]. Reninangiotensin-aldosterone system blockers, glucocorticoids, and immunosuppressants have often been used for primary glomerular diseases. Immunosuppressive therapies have usually been applied to treat patients with heavy proteinuria, but are not entirely suitable for patients with non-nephrotic-range proteinuria [5]. Moreover, treatments with glucocorticoids and immunosuppressants are usually long term, which can result in severe adverse effects and increase the risk of rebound [5, 6].

Traditional Chinese medicine (TCM), as a system of ancient medical practice, continues to play a critical role in maintaining health for the peoples of China, and is growing in popularity in Western countries [7]. Notably, TCM has promising effects on the control of proteinuria, protection of kidney function, and improvement in patients' clinical symptoms [8, 9]. For example, *Abelmoschus manihot*, a traditional Chinese herb, has increasingly been used to treat IgAN [10, 11]. Clinical studies have proven that *A. manihot* can reduce proteinuria and thus protect kidney function [12].

Cicada is an insect of the order Homoptera, suborder Auchen-orrhyncha, in the superfamily Cicadoidea [13]. *Cicada sloughs* are predominantly chitin, a polysaccharide that is a structural component found in the cell walls of fungi and in the exoskeletons of insects and other arthropods [14]. It is a common intergradient in many TCM prescriptions, such as Chantuisan, Chantuiying, sichantang and Chantuizhugansan. It has significant effects in treatment of nephiritis and control the proteinuria level. Previous studies have confirmed its anti-inflammatory activities [15], anti-oxidant activities via phar-

Table 1. The list of shared GO terms and associated proteins

GO term	GO ID	IgAN *P* value	Chantui *P* value	IgAN	Chantui
Small molecule metabolic process	GO: 0044281	8.17E-08	3.18E-13	P12268, Q96PD7, P20618, Q99460, P28074, P20839, O75907, Q13200, P49721, P01019	P00480, P15104, Q06278, Q9NTJ5, P00491, Q92562, O00219, P32320, P35270, Q70JA7, Q08AM6, Q9Y2T3, P51687, Q92839, P07101, P00439, P47989, O95196, P04040, P30793, P23921, Q03393, Q16851, O60701, P06865, P36871, P07686, Q9Y2I7, Q9HCG7, P31350, P04062, P15291, P06744, P54802
Cellular nitrogen compound metabolic process	GO: 0034641	3.87E-07	0.003697	P20618, Q99460, P28074, Q13200, P49721	P00480, P15104, P51687, P07101, P00439
Gene expression	GO: 0010467	1.46E-05	0.033819	P11473, P20618, Q99460, P28074, Q13200, P49721	P52298, O75821, O75822, P52747, Q92989, Q13347, P05198, Q6P2Q9
Purine nucleobase metabolic process	GO: 0006144	0.000405	4.57E-05	P12268, P20839	P00491, Q9Y2T3, P47989, P04040
Positive regulation of cytokine secretion	GO: 0050715	0.000405	0.016832	P43405, P01375	O60603, Q9ULY5
Lipid storage	GO: 0019915	0.000475	0.019586	Q96PD7, O75907	P06865, P07686
Positive regulation of reactive oxygen species metabolic process	GO: 2000379	0.000633	0.025612	P04637, P01019	P01127, P47989
Cellular response to molecule of fungal origin	GO: 0071226	0.00165	0.010995	P43405	Q9BXN2
Nucleobase-containing small molecule metabolic process	GO: 0055086	0.001889	2.59E-07	P12268, P20839	P00491, P32320, Q9Y2T3, P47989, P04040, P23921, P31350
Positive regulation of interleukin-18 production	GO: 0032741	0.002474	0.016447	P01375	O60603
Leukocyte activation involved in immune response	GO: 0002366	0.002474	0.016447	P43405	Q9BXN2
Response to ether	GO: 0045472	0.00412	0.027263	Q00987	P07101
Defense response to bacterium	GO: 0042742	0.004542	0.030155	P43405, P35228	Q99698, P61626, Q9HC29
Cellular response to nicotine	GO: 0071316	0.005763	0.03796	P01375	P07101
Innate immune response in mucosa	GO: 0002227	0.005763	0.03796	P35228	Q9HC29
Fat pad development	GO: 0060613	0.006584	0.043264	Q96PD7	P52926
Positive regulation of humoral immune response mediated by circulating immunoglobulin	GO: 0002925	0.006584	0.043264	P01375	Q9HC29
Positive regulation of apoptotic process involved in mammary gland involution	GO: 0060058	0.007404	0.04854	P11473	P15291
Phospholipid metabolic process	GO: 0006644	0.008147	0.002263	Q96PD7, O75907	Q99698, Q9NTJ5, Q92562, Q08AM6, Q9Y2I7
Cellular protein metabolic process	GO: 0044267	0.010526	2.16E-09	P00797, P12821, P01019	O60645, Q9UBM8, Q9H4B7, Q06210, O95394, Q16222, O75821, O75822, Q96EK6, O60762, O94808, Q9BVK2, P07686, Q9Y2I7, Q99832, Q13347, P05198, P15291
Nitric oxide biosynthetic process	GO: 0006809	0.011494	0.002632	P35228	P35270, P30793
Response to lipopolysaccharide	GO: 0032496	0.013036	0.001155	P00797, P05412	P07101, P35225, Q9HC29, P30793, P30793, P05186
Cellular amino acid biosynthetic process	GO: 0008652	0.014755	0.004361	P01375	P15104, P00439
Negative regulation of growth of symbiont in host	GO: 0044130	0.024475	0.011875	P01375	O60603, Q9HC29
Innate immune response	GO: 0045087	0.024654	0.027018	Q00987, P43405, P05412	O00602, Q9BXN2, P04003, P01127, O60603, Q9BWS9, Q9HC29, Q8WWG1, Q15485

Positive regulation of mitosis	GO: 0045840	0.026086	0.013443	P01375	P01127, Q8WWQ0
Inflammatory response	GO: 0006954	0.036732	0.001044	P01375, P35228	Q06278, Q9BXN2, P36222, P61626, O60603, P35225, O15054, Q9Y258
Positive regulation of interferon-gamma production	GO: 0032729	0.038883	0.028872	P01375	P42681, P10809
Defense response to Gram-positive bacterium	GO: 0050830	0.047587	0.042114	P01375	O60603, Q9HC29
Positive regulation of interleukin-6 production	GO: 0032755	0.047587	0.004266	P01375	P10809, O60603, Q9HC29

Table 2. Biological processed involved by 6 proteins that associated with IgAN, but not affected by *Cicada slough*

Term	*P* Value	Genes
Actin filament bundle formation	0.007417	ADD2, ACTN4
Immune system process	0.043256	ADD2, MS4A1, IL16
Response to hypoxia	0.046578	PDLIM1, ACTN4

macological investigation [16]. However, molecular mechanisms of *C. sloughs* acting on nephiritis or IgAN remains unknown.

In this study, we have developed a comprehensive systematic approach for understanding the potential molecular mechanisms of *C. sloughs* acting on IgAN. An overview of regulation networks were generated with information gained from proteins, biological processes and protein-protein interactions. This information will be useful for understanding the underlying mechanisms about how *C. slough* regulate the imbalanced systems in IgAN patients.

Materials and methods

Description of ingredients in cicada slough and prediction of potential targets for cicada slough and IgAN-associated gene and protein selection

Targets of *Cicada slough*, was obtained in the TCM database (TMDB) a comprehensive collection of TCM-related information from different resources in English and Chinese, including HIT database, TCM@Taiwan, TCMD, TCMgeneDIT, Chinese traditional medicine herbs database and Drugbank, OMIM and PubChem. Then, well annotated IgAN-associated genes and proteins were collected from two IgAN related databases: OMIM (http://www.ncbi.nlm.nih.gov/omim) and IPA (www.ingenuity.com).

Gene ontology (GO) and pathway enrichment analysis for IgAN associated proteins and potential targets of cicada slough

We used Database for Annotation, Visualization and Integrated Discovery (DAVID, version 6.7) for GO enrichment analysis. The enrichment score was calculated using hypergeometric enrichment algorithms [17]. The EASE (Expression Analysis Systemic Explorer) score was set to the default value [18]. We also performed pathway enrichment analysis using pathway data obtained from the FTP service of KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/). *P*-values of the KEGG pathway were calculated using the Fisher exact test. Pathways with *P*-value < 0.05 were taken as significantly enriched.

Comprehensive regulation network construction

First, we searched the STRING database [19] with 99 query proteins (74 of *C. slough* targets and 25 IgAN associated proteins) based on direct interactions. A core network with 186 interaction pairs of 83 proteins with score > 400 were obtained, proteins that do not connected with the core network were defined as orphans. Each orphan was then connected with the core network through the best shortest path found in the STRING database. PPI network were visualized using force directed layout cytoscape 3.1.

Results and discussion

Information of ingredients in cicada slough and prediction of potential targets for cicada slough and IgAN-associated gene and protein selection

The *Cicada slough* has 170 annotated potential targets based on TMDB database, however, they do not overlapped with the 25 known IgAN associated proteins. Since the *C. slough* does not act directly on these 25 proteins, it should interact with the IgAN imbalanced network defined by these 25 proteins, and through proteins that interact with these 25 proteins or belong to same biological processes. Therefore, we performed gene ontology enrichment analysis for target sets of cicada slough and IgAN associated proteins, respectively, and compared them for shared biological processes.

Gene ontology (GO) and pathway enrichment analysis for IgAN associated proteins and potential targets of cicada slough

It results 30 GO terms that shared by *C. slough* and IgAN (**Table 1**). 74 target proteins of *C. slough* were obtained, each of them are co-occurred in at least one shared GO terms. Moreover, 19 IgAN associated proteins were

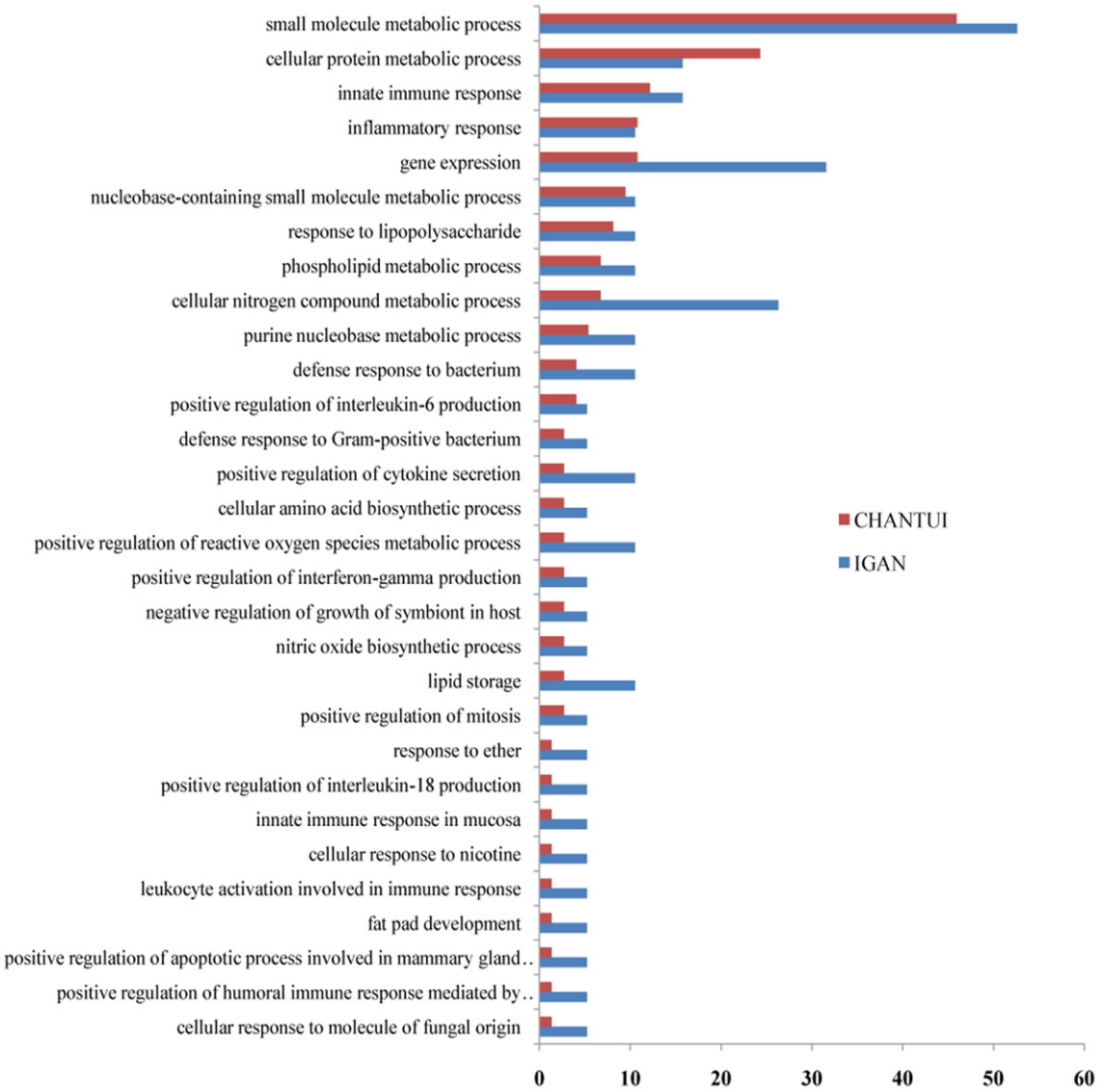

Figure 1. Association diagram of shared GO. X axis indicates number of target proteins, Y axis are shared GO terms ordered by enrichments score of the IgAN.

included in these biological processes. The rest of 6 IgAN associated proteins were found in neither the shared GO, nor the extended protein-protein interactions data (**Table 2**). It suggests that *C. slough* could affect the main pathogenesis system of IgAN via regulation of these set of proteins that are co-players in the same network.

As shown in the enrichment analysis (**Figure 1**), the *C. slough* mainly affects the IgAN via innate immune response and inflammatory response systems (52 targets proteins and 13 IgAN associated proteins).

Core regulation network

The pathogenesis of IgAN was a multi-level co-regulation network. Abnormality of proteins in various processes could lead to disease, including immune defect, viral process, inflammatory response, B cell and lymphocyte proliferation, hypoxia, fatty acid homeostasis, etc. Functionally, we found 74 (43.5%) of all annotated targets of *C. slough* are potentially linked with IgAN. As shown in the network (**Figure 2A**), these proteins are PPI partners of the IgAN associated proteins, most of the targets were interacted with the inflammatory and innate

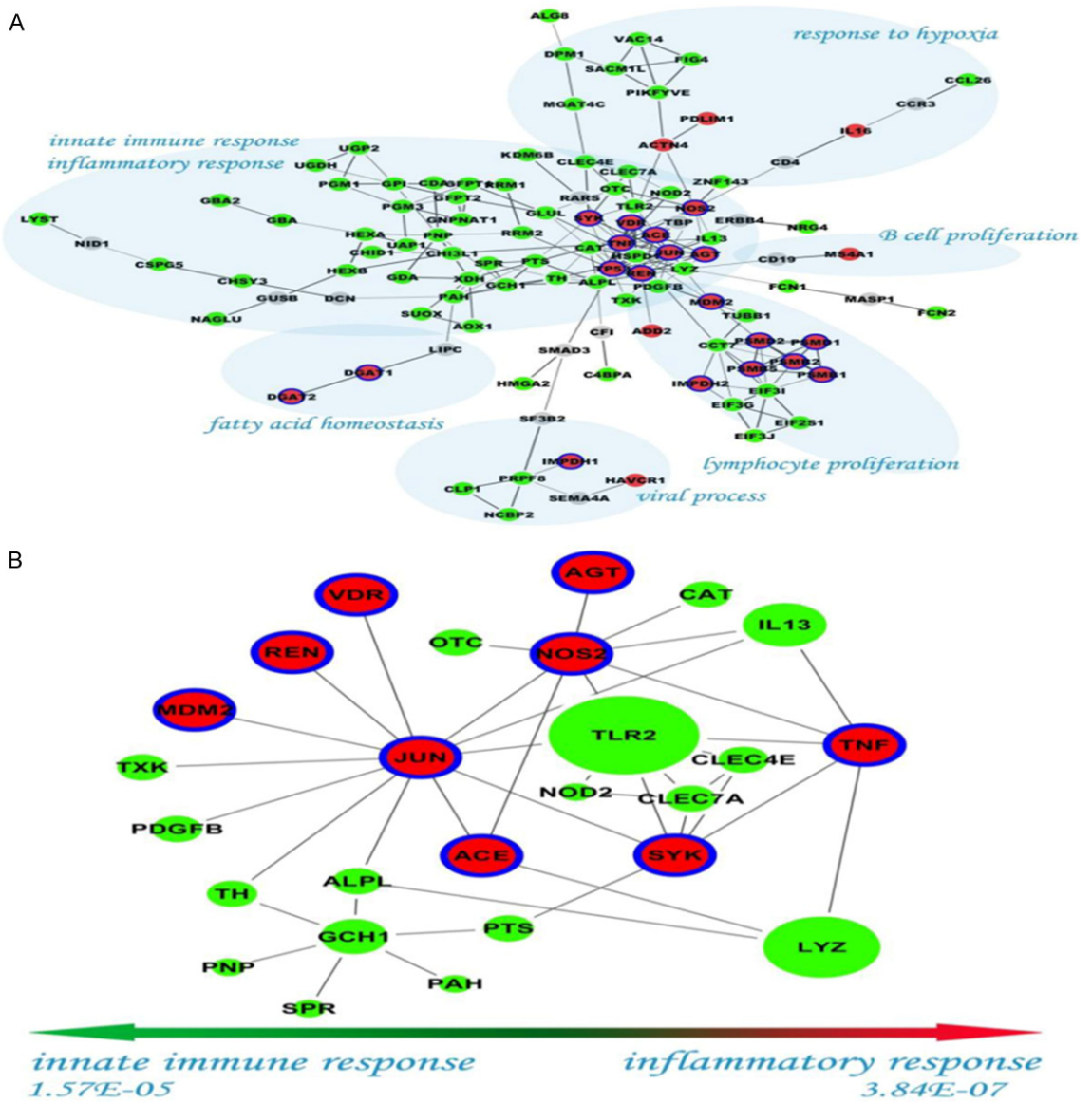

Figure 2. A. A comprehensive regulation network shows how the *Cicada slough* affects IgAN. B. Shared core regulation network of innate immune response and inflammatory response systems. Red filled nodes are IgAN associated proteins. Green filled nodes are annotated target proteins in the TCM database. Nodes with blue border line were proteins that shares biological processes. Edges indicate protein-protein interaction between two nodes.

immune system, suggesting a plausible regulation processes of pharmaceutical effects of *C. slough*.

As shown in **Figure 2B**, nine proteins (MDM, REN, VDR, AGT, NOS 2, JUN, ACE, SYK and TNF) are mainly responsible for failure of the immune systems in IgAN, and thus being centered in the regulation networks. The hub proteins are JUN, SYK, TNF, NOS2 and ACE. NOS 2 and SYK are also involved in inflammatory response, suggesting a cascade signaling pathway responsible for the IgAN development. Furthermore, NOS2 produces nitric oxide (NO) what is a messenger molecule with diverse functions throughout the body. Indeed, Xu et al, [16] has discovered inhibition activity of *C. slough* NO production and inducible expression of pro-inflammatory factors.

In conclusion, in this study, based on multi-level systems biology approach, we have success-

fully uncovered that the *C. slough* mainly interact the imbalanced network of IgAN via regulation of innate immune and inflammatory response system, through a set of key associated players in IgAN development such as NOS 2, JUN, TNF and ACE. The regulation network may provide a new insight for developing new therapy for IgAN. Overall, this study was the first to explore the molecular network of *C. slough*s acting on IgAN, and further studies are called to adapt the formula of TCM and to enhance its effectiveness on IgAN.

Acknowledgements

This study was supported by Ministry of Scientific technology of China Grant (2010070-05), National Natural Science Foundation of China Grant (81173219), Shanghai Science & Technology Commission Grant (12ZR1432400, 14401972203 and 15401930100), the project of Shanghai Municipal Commission of Health and Family Planning (201440488), Shanghai Key Laboratory of Traditional Chinese Clinical Medicine (14DZ2273200), Chinese Medicine development three years project (ZY3-JSFC-2-1029) and senior Chinese Integrative Medicine talent cultivation project (ZYSNXD012-RC-ZXY003) of Shanghai Municipal Health Bureau, and Innovative Research Team in Universities, Shanghai Municipal Education.

Disclosure of conflict of interest

None.

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