Transcriptome analysis of spleen in chickens administered β -glucan derived from yeast cell wall

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Abstract

Background

Our previous study shown that oral administration of a product contained yeast cell wall polysaccharides enhanced immune responses elicited by Newcastle disease virus and changed microbial community of cecum in chickens.

Results

The present study was design to investigate the potential molecular mechanism in relation to the immunomodulation of β -glucan in chickens. Using RNA-sequence (RNA-seq) technique, we identified 198 differential expression genes (DEGs) in spleen in chickens after oral administration of β -glucan. In addition, these DEGs were significantly enriched in 205 Gene Ontology (GO) terms and 7 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Conclusions

β -glucan might regulate chicken immune system by regulating expression of genes involved in cognition, cytokines, binding, enzyme activities and multiple signaling pathway.

Background

Yeast cell wall polysaccharides, mainly composed of β -glucan and mannan-oligosaccharides (MOS), are derived from the cell wall of *Saccharomyces cerevisiae* [1]. They are extensively used as growth promoters, antimicrobial agents or immunomodulator in poultry for the purpose of improving production and health [2–4]. We have previously shown that oral administration of PW220, a product contained yeast cell wall polysaccharides, enhanced immune responses elicited by Newcastle disease virus and changed microbial community of cecum in chickens [5]. However, the underlying molecular mechanism remained obscure.

RNA sequencing (RNA-seq) is a high through-put technology that can be employed to detect quantitative gene expression and to analyze different expression profiles at the whole transcriptome level [6]. With the enhanced sensitivity and declining cost, RNA-seq has been widely used in livestock and poultry research [7–8]. Because the spleen is one of the dominating sites for priming of the primary immune responses, in the present study, transcriptome sequencing was performed to investigate the effect of β -glucan on gene expression in chicken spleen and to explore the potential signal transduction pathways.

Materials And Methods

Reagents

β -glucan with purity $\geq 70\%$ derived from yeast cell wall was provided by Angel Yeast Co. LTD (Yichang, China). PrimeScript™RT Master Mix and SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) were purchased from Takara Bio INC (Dalian, China).

Birds and Experimental Design

One-day-old White-feather silky chickens (male) were purchased from Sichuan Lihua Poultry Co., Ltd. (Chongqing, China) and randomly allocated into two groups. Group 1 was orally administered β -glucan at a dose of 20 mg/kg of BW (the dose was chosen based on a preliminary experiments) in drinking water for 7 d before vaccination; group 2 was administered saline as a control. On the day following administration of β -glucan, each bird was received intraocular and intranasal immunization with a live vaccine of NDV at a dose of 2×10^3 egg infectious dose (EID), according to the manufacturer's instruction. On 2 weeks post immunization, chickens were humanly killed by cervical dislocation and the whole spleen was immediately frozen and stored at -196°C in liquid nitrogen for sequence analysis.

RNA extraction and cDNA Library Construction

The total RNA of the spleen was extracted using TRIzol reagent (Thermo fisher Scientific). RNA was detected free of contamination and degradation using 1% agarose gel, and the RNA purity was estimated using a NanoPhotometer® spectrophotometer (IMPLEN, Westlake Village, CA, USA). RNA concentration was measured using a Qubit® RNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA), and RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). The results showed that the RNA was intact and free of DNA contamination. The libraries were sequenced using Illumina HiSeq platform with a PE150 strategy.

Transcriptome analysis

Transcriptome sequencing, sequence assembly, and data analysis are provided by Novogene Bioinformatics Technology Co. Ltd. (Beijing, China). The transcriptome analysis process was performed as follows: (a) mRNA was purified from total RNA using poly-T oligo-attached magnetic beads; Fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5X). (b) First strand cDNA was synthesized using random hexamer primer and Moloney Murine Leukemia Virus (M-MuLV) Reverse. Transcriptase (RNase H-); Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. (c) Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities; After adenylation of 3' ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. (d) In order to select cDNA fragments of preferentially 250 ~ 300 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then 3 μl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37°C for 15 min followed by 5 min at 95°C before PCR. Then PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and

Index (X) Primer. At last, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system. TruSeq PE Cluster Kit v3-cBot-HS (Illumina) was employed to carry out the cluster of the index-coded samples. Then, the sequencing was executed on an Illumina Novaseq platform, and 150 bp paired-end reads were produced. Reference genome and gene model annotation files were downloaded from the genome website (ftp://ftp.ensembl.org/pub/release-98/fasta/gallus_gallus/ and ftp://ftp.ensembl.org/pub/release-98/gtf/gallus_gallus/). Using HISAT2 (v2.0.5) to build the index of the reference genome and align paired-end clean reads with the reference genome. Feature Counts v1.5.0-p3 was used to count the reads numbers mapped to each gene, and then Fragments Per Kilobase per Million(FPKM) of each gene was calculated basing the length of the gene and its reads count. DESeq2 R package (1.16.1) was used to exporting the differential expression between the H with the control group. Genes with $P < 0.05$ and $|\log_2(\text{foldchange})| > 1$ were defined as differential expression genes (DEGs).

Real-time Quantitative PCR Validation

Two DEGs that were up-regulated and four DEGs that were down-regulated in the comparison H vs. Control were selected to validate the transcriptome sequencing results using RT-qPCR. PrimeScript™ RT Master Mix (Takara, Dalian, China) was used to convert RNA into cDNA on a T100™ thermal cycler (Bio-Rad, USA). Information of primers were provided in the supplementary materials table 1. The Chicken β -actin was served as the internal control gene. RT-qPCR with SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (Takara, Dalian, China) on selected genes was carried out on a Multiple Real-Time PCR System (AB, USA). A relative quantitative method ($2^{-\Delta\Delta CT}$) was employed to evaluate the quantitative variation. All samples were analyzed in triplicate.

Results And Discussion

HI

The effect of YP on serum HI titers was shown in Fig. 1. The results suggested that G70 significantly increased HI titers at 1 ($P > 0.05$) and 2 ($P < 0.01$) weeks post first immunization when compared with the chickens only immunized with vaccine.

RNA sequencing data analysis

To detect differences between H and Control, RNA-seq of splenic samples was performed using the Illumina sequencing platform. Four individual samples were included for each group, and they were marked as C1, C2, C3, C4 and H1, H2, H3, H4, respectively. As shown in supplementary materials Table 1, RNA sequencing of the 8 spleen libraries produced 24.1G raw data. The C1, C2, C3, C4, H1, H2, H3 and H4 libraries contained 45,591,492, 47,424,782, 46,193,492, 54,403,376, 47,118,476, 45,899,262, 45,841,884 and 45,504,438 raw reads. After removing adaptor, ambiguous and low-quality sequences, 44,050,198, 45,449,884, 44,261,112, 52,097,846, 45,542,404, 44,566,290, 44,113,172, and 44,026,960 clean reads were

remained. The percentage of clean reads among raw reads was greater than 96%, the sequencing reads from the eight libraries were aligned to a reference database, which consisted of the *Gallus gallus* genome, using HISAT2. More than 95% of the clean reads mapped to this database. In particular, 40921384 (92.9%), 41302100 (90.87%), 40771075 (92.11%), 46905784 (90.03%), 42444112 (93.2%), 40213444 (90.23%), 40922895 (92.77%) and 40971972 (93.06%) reads from the C1, C2, C3, C4, H1, H2, H3 and H4 libraries, respectively, uniquely mapped to the reference database.

Differentially expressed genes

As shown in Fig. 2A, there were 198 differentially expressed genes in total, including 47 up-regulated and 151 down-regulated genes. Detailed description of DEG will be shown in supplementary material Table 2. The distribution of differentially expressed genes was depicted in the heatmap (Fig. 2B). The clustered biological replicates of DEGs indicated good reproducibility of treatment.

Gene Ontology classification and KEGG enrichment analysis

According to the Gene Ontology (GO) classification system, the differentially expressed genes were classified into three main functional categories: biological process, cellular component and molecular function. Figure 3 displayed top 10 GO terms in three categories. Genes involved in the “humoral immune response”, “response to chemokine”, “antimicrobial humoral response”, “defense response to bacterium”, “antimicrobial humoral immune response mediated by antimicrobial peptide”, “defense response to Gram-negative bacterium”, “defense response to Gram-positive bacterium” were predominant in the biological process category. Moreover, a significant proportion of the genes were involved in “C-C motif chemokine receptor (CCR) chemokine receptor binding”, “chemokine receptor binding”, “lipopolysaccharide binding” in the category of molecular function. In addition, “mitochondrial respiratory chain complex I”, “NADH dehydrogenase complex”, “respiratory chain” were the predominant enriched terms in the cell components category.

Immunomodulatory effect of polysaccharides from yeast cell wall have been demonstrated effective to change immune response and altered gut microbiota composition in chickens [9–10]. In our previous study, a yeast cell wall product contained β -glucan and MOS was found effectively to enhance intestinal IgA response to NDV vaccination and modulated the cecum microbiota by oral route [5]. However, only a few reports analyzed the mRNA expression of spleen after oral administration of yeast polysaccharides. RNA-seq based transcriptome analysis is a tool that allows a deep understanding of complicated physiological pathways (Fig. 4). β -glucan may exhibit its effects on the immune function of chicken by differentially expressed genes in relation to innate and adaptive immunity in lymphocyte. Several pattern recognition receptors target β -glucan were enriched on the surface of immune cells [11]. After recognition of β -glucan, these receptors stimulate tyrosine kinase and nuclear factor κ B, which consequently induced secretion of proinflammatory cytokines and activate immune reaction [12–15]. Recently, transcriptome analysis was used to study the mechanism of a vegetable oil adjuvant in mice [16]. They found DEGs in

spleen were enriched in immune response related GO terms such as “humoral immune response”, “antimicrobial humoral response” and “defense response to bacterium”, which were consistent with our results.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the differentially expressed genes was also performed. The results indicated that these genes were mainly classified into seven pathways included “Neuroactive ligand-receptor interaction”, “Gap junction”, “MAPK signaling pathway”, “ABC transporters”, “Biosynthesis of amino acids”, “Drug metabolism”, “Protein export”. These results indicated that β -glucan regulate immune response through multiple pathways. Similar results were also reported by others [17–18].

Interestingly, genes encoding cathelicidin including CATH3, CATH1, CATH2, and genes encoding beta-defensin such as AvBD6, AvBD7, AvBD1, and AvBD4 were significantly down-regulated in the comparison H vs. Control. Host Defense Peptides (HDPs) are important effector molecules of the innate immune system [19]. These peptides have been found in a wide range of animals from mammals to birds. The chicken has four cathelicidins, including CATH1, CATH2, CATH3 and CATHB1, which were able to efficiently kill a variety of bacteria [20]. Avian beta-defensins (AvBDs), also known as gallinacins, are small cationic peptides having three cysteine disulfide bonds between their cysteine residues and play essential roles in the innate immune system [21]. The decreased expression of cathelicidins and beta-defensin might be explained by the feedback regulation, in which the population of *Bacteroides* and *Parabacteroides* were reduced by yeast cell wall polysaccharide [5]. Similar results were found in other studies. Yu reported that supplementation of yeast β -glucan in broiler chicken inhibited *Salmonella* infection and reduced HDPs expression by quantitative real-time PCR analysis [22].

Confirmation of differentially expressed genes by qRT-PCR

We verified the 6 DEGs which were up- or down-regulated using real-time quantitative PCR. The result showed that the RT-qPCR data were consistent with the RNA-seq data in general, indicating the reliability of the sequencing results (Fig. 5).

In summary, this study identified 198 DEGs in spleen in chickens after oral administration of β -glucan. GO and KEGG pathway enrichment uncovered 205 significantly enriched GO terms and 7 KEGG pathways. β -glucan might regulate chicken immune system by changing expression of genes involved in cognition, cytokines, binding, enzyme activities and multiple signaling pathway.

Abbreviations

RNA-seq, RNA-sequence; DEGs, differential expression genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MOS, mannan-oligosaccharides; BW, body weight; NDV, Newcastle disease virus; EID, egg infectious dose; M-MuLV, Moloney Murine Leukemia Virus; FPKM, Fragments Per Kilobase per Million; CCR, C-C motif chemokine receptor; CATH3, cathelicidin-3; CATH1, cathelicidin-1;

CATH2, cathelicidin-2; CATHB1, cathelicidin-B1; HDPs, Host Defense Peptides; AvBDs, avian beta-defensins; AvBD6, avian beta-defensin-6; AvBD7, avian beta-defensin-7; AvBD1, avian beta-defensin-1; AvBD4, avian beta-defensin-4; HDPs, Host Defense Peptides

Declarations

Ethics approval and consent to participate

The animal management and study protocol were evaluated and approved by the Southwest University Committee on Animal Care and Use (IACUC-20200701-01). All animal behavior and care procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable

Availability of data and materials

The raw sequence data have been submitted to NCBI SRA (Accession: SAMN16965324, SAMN16965325, SAMN16965329, SAMN16965330, SAMN16965351, SAMN16965352, SAMN16965353, SAMN16965354).

Competing interests

The authors declare no competing financial interests.

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Authors' Contributions

Writing-review and editing: Liting Cao, Jianrong Zhang and Shicheng Bi. Writing-original draft: Huan Huang and Fuxing Gui. Formal analysis: Wei Xu and Shicheng Bi. Methodology: Liting Cao and Wei Xu and Shicheng Bi. All authors have read and agreed to the published version of the manuscript.

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References

1. Schiavone M, Dejean S, Sieczkowski N, et al. Integration of biochemical, biophysical and transcriptomics data for investigating the structural and nanomechanical properties of the yeast cell wall[J]. *Front Microbiol*, 2017, 8:1806.
2. Baurhoo B, Ferket PR, Zhao X. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers[J]. *Poult Sci*, 2009, 88(11):2262–2272.
3. Xiao R, Power RF, Mallonee D, et al. Effects of yeast cell wall-derived mannan-oligosaccharides on jejunal gene expression in young broiler chickens[J]. *Poult Sci*, 2012, 91(7):1660–1669.
4. Xue GD, Wu SB, Choct M, et al. Effects of yeast cell wall on growth performance, immune responses and intestinal short chain fatty acid concentrations of broilers in an experimental necrotic enteritis model[J]. *Anim Nutr*, 2017, 3(4):399–405.
5. Bi S, Zhang J, Qu Y, et al. Yeast cell wall product enhanced intestinal IgA response and changed cecum microflora species after oral vaccination in chickens[J]. *Poult Sci*, 2020, 99(12):6576–6585.
6. Zhao S, Zhang Y, Gamini R, et al. Evaluation of two main RNA-seq approaches for gene quantification in clinical RNA sequencing: polyA + selection versus rRNA depletion[J]. *Sci Rep*, 2018, 8(1):4781.
7. Teixeira SA, Ibelli A, Cantao ME, et al. Sex Determination Using RNA-Sequencing Analyses in Early Prenatal Pig Development[J]. *Genes (Basel)*, 2019, 10(12).
8. Yuan L, Wang Y, Ma X, et al. Sunflower seed oil combined with ginseng stem-leaf saponins as an adjuvant to enhance the immune response elicited by Newcastle disease vaccine in chickens[J]. *Vaccine*, 2020, 38(33):5343–5354.
9. Cox CM, Stuard L H, Kim S, et al. Performance and immune responses to dietary beta-glucan in broiler chicks[J]. *Poult Sci*, 2010, 89(9):1924–1933.
10. Park SH, Lee SI, Ricke SC. Microbial populations in naked neck chicken ceca raised on pasture flock fed with commercial yeast cell wall prebiotics via an Illumina MiSeq Platform[J]. *Plos One*, 2016, 11(3):e151944.
11. Goodridge HS, Wolf AJ, Underhill DM. Beta-glucan recognition by the innate immune system[J]. *Immunol Rev*, 2009, 230(1):38–50.
12. Bode K, Bujupi F, Link C, et al. Dectin-1 binding to annexins on apoptotic cells induces peripheral immune tolerance via NADPH oxidase-2[J]. *Cell Rep*, 2019, 29(13):4435–4446.
13. Xu J, Liu D, Yin Q, et al. Tetrandrine suppresses betaglucan induced macrophage activation via inhibiting NFkappaB, ERK and STAT3 signaling pathways[J]. *Mol Med Rep*, 2016, 13(6):5177–5184.
14. Kankkunen P, Teirila L, Rintahaka J, et al. (1,3)-beta-glucans activate both dectin-1 and NLRP3 inflammasome in human macrophages[J]. *J Immunol*, 2010, 184(11):6335–6342.
15. Masuda Y, Togo T, Mizuno S, et al. Soluble beta-glucan from *Grifola frondosa* induces proliferation and Dectin-1/Syk signaling in resident macrophages via the GM-CSF autocrine pathway[J]. *J Leukoc Biol*, 2012, 91(4), 547–556.

16. Cui X, Wang Y, Maqbool B, et al. Early IgG response to Foot and Mouth Disease Vaccine formulated with a vegetable oil adjuvant[J]. *Vaccines (Basel)*, 2019, 7(4):143.
17. Jin X, Zhang M, Yang Y. Saccharomyces cerevisiae beta-glucan-induced SBD-1 expression in ovine ruminal epithelial cells is mediated through the TLR-2-MyD88-NF-kappaB/MAPK pathway[J]. *Vet Res Commun*, 2019, 43(2):77–89.
18. Wang S, Zhou H, Feng T, et al. beta-Glucan attenuates inflammatory responses in oxidized LDL-induced THP-1 cells via the p38 MAPK pathway[J]. *Nutr Metab Cardiovasc Dis*, 2014, 24(3):248–255.
19. Cuperus T, Coorens M, van Dijk A, et al. Avian host defense peptides[J]. *Dev Comp Immunol*, 2013, 41(3):352–369.
20. Hamad SK, Kim S, El-Kadi SW, et al. Comparative expression of host defense peptides in turkey poult[J]. *Poult Sci*, 2017, 96(7):2083–2090.
21. Yoshimura Y. Avian beta-defensins expression for the innate immune system in hen reproductive organs[J]. *Poult Sci*, 2015, 94(4):804–809.
22. Shao Y, Wang Z, Tian X, et al. Yeast beta-d-glucans induced antimicrobial peptide expressions against Salmonella infection in broiler chickens[J]. *Int J Biol Macromol*, 2016, 85:573–584.

Figures

Figure 1

HI titers. The data were expressed as the Log2 of the highest dilution that exhibited complete inhibition of hemagglutination. The values were represented as mean ± SE.

A

B

Figure 2

Summary of RNA-Seq data. (A) List of differentially expressed genes, (B) Clustering map of the DEGs

Figure 3

Top10 enriched GO terms in biological process, cellular component and molecular function categories

Figure 4

KEGG pathway analysis

Figure 5

RT-qPCR confirmation of selected DEG candidates. Data are expressed as mean $\pm$ SE.

Supplementary Files

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