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Intestinal DCs global gene expression dynamics affected by recombinant baker's yeasts saccharomyces cerevisiae

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Abstract

Background The baker's yeast, saccharomyces cerevisiae, has been widely used throughout our daily life in diverse aspects for thousands of years. The saccharomyces cerevisiae was found to specifically target the dendritic cells (DCs) in mammalian with a manner of antigen-receptor interaction as described previously. It is necessary to investigate the effect of the baker's yeasts on global gene expression dynamics of intestinal DCs and explore the possibilities of using baker's yeast as gene delivery vehicle to modulate animal's immune functions Results with a murine oral delivery model in vivo, we confirmed the feasibility of using budding yeast as gene delivery vehicle to the intestinal DCs using the Western blots. We then examined the transcriptome profile of the mouse intestinal DCs upon yeast stimulus. The enrichment analysis of unique transcripts indicated the beneficial role of yeast in modulating the DC-mediated adaptive immunity. Compared with previous study, we also found that a large fraction of the regulated genes is coincident with the response induced by other fungus, suggesting that the budding yeast induces a similar tailored unique genetic re-programming of DCs. Another analysis of transcriptome profile indicated that expression of β-catenin gene significantly changes DCs gene expression related to inflammatory response and cell adhesion. Conclusions Here, we defined the role of budding yeast on global gene expression of intestinal DCs, and confirmed the important role of β-catenin gene on the DCs-related inflammatory response, which provides a framework for the development of mucosa yeast-based DNA vaccine.

Background

As we all know, the fact that *saccharomyces cerevisiae* has a "generally regarded as safe status" (GRAS) is not normally recognized as a pathogen and can be easily cultured[1]. Because of their wide physiological diversity in a broad range of habitats, the baker's yeast, *saccharomyces cerevisiae*(*S. cerevisiae*), has been used in our daily life to produce carbonation and alcohol, leaven breads, and provide nutritional supplementation for thousands of years[2]. In addition to important roles in fermentation and wine making, yeast has many benefits for human health. Among them, the probiotic effect is the most widely known health effect, including prevention and treatment of intestinal diseases and immune regulation[3]. A number of studies have shown that *S. cerevisiae* can bring the following benefits: prevention of various enteric pathogens[4]; maintenance the integrity of the epithelial barrier [5, 6]; enhancement of anti-inflammatory effect[7, 8]; stimulation of immune response [9, 10]; nutritional promotion of intestinal mucosa [11, 12]. Therefore, further efforts to explore the exact mechanisms by which yeast can exert a beneficial health effect on the mammalian immune system through genetic methods are worth encouraging.

Since the first report discovering yeast targeting the dendritic cells (DCs) in the vaccine application [13], more interest of the DCs-mediated immune function has been attracted from scientists. Previous reports discovered that DCs reside in an immature state in most tissues, including the intestine tissue, where they recognize and phagocytose pathogens and other antigens[14]. And direct contact with many pathogens leads to the maturation of DCs, which is characterized by an increase in antigen presentation, expression

of costimulatory molecules, and subsequent stimulation of native T cells in lymphoid organs[15]. The extensive reprogramming of DCs during maturation prompted us to measure the corresponding changes in gene expression. Thereafter, through diverse methods (e.g. oligonucleotide microarrays), many reports have discovered the interaction mechanisms between many DCs subsets (bone marrow-derived DCs) and many pathogens, including some fungi species (e.g. *Candida albicans*)[16, 17]. But how intestinal DCs respond appropriately to the baker's yeasts *S. cerevisiae* they encounter daily remains ambiguous in genetic level.

Several studies have also shown that DCs are involved in the initiation of both innate and adaptive immunity[18]. The importance of DCs in initiating immune responses led us to investigate at a genetic level how intestinal DCs interact with yeasts. To further understand how yeast, affect intestinal DCs gene expression and to confirm the feasibility of using budding yeast cells as gene delivery vehicle to the intestinal DCs, we used a murine oral delivery model *in vivo* to pursue a whole genome transcriptomic analysis of the response of intestinal DCs to different recombinant yeasts.

We show that intestinal DCs suffer extensive genetic regulation upon diverse yeasts exposure, comprising 1442 transcripts (JMY-P group) and 2078 transcripts (JMY-Pcat group) compared with PBS group. A large fraction of these is similarly regulated at both exposures studied, comprising 1097 common unique transcripts and some of them have functions potentially related to T cell activation and adaptive immunity. And the JMY-Pcat group displayed another 944 DEGs other from the JMY-P group, which were mainly involved in "inflammatory response", "myeloid leukocyte activation", "leukocyte migration", "cell-cell adhesion" and "extracellular structure organization". A comparison of yeast-regulated genes with a set of genes previously identified as the common DC transcriptomic response to pathogens[16] revealed about 77.70% (108/139) genes expressions were in accordance with the former study, indicating a similar gene cluster related to DCs-derived immune response in diverse fugus. Only a small fraction of the regulated genes is reverse with the response induced by other fungus, suggesting that the budding yeast induces a tailored unique genetic re-programming of DCs. After oral administration, the isolated DCs was utilized to test the possibility of application of oral gene delivery platform. Up to now, this is the first report to systematically explore how *saccharomyces cerevisiae* modulate intestinal DCs-mediated immune response and successfully verified the feasibility of using budding yeast as gene delivery vehicle to modulate intestinal immune functions.

Results

Vector validation in HEK293T cells

We used *Xho*Ⅰ and *Not*Ⅰ enzymes (NEB, UK) to digest the JMB84-CMV-*β-catenin*-polyA vector, which was in accordance with the prediction (two bands: 5969 bp and 3030 bp). We then digested JMB84-CMV-*β-catenin*-HA-polyA vector with *Eco*RⅠ and *Nhe*Ⅰ enzymes (NEB, UK) (two bands: 7917 bp and 1118 bp). And we also used *Eco*RⅠ and *Nhe*Ⅰ enzymes (NEB, UK) to digest the JMB84-CMV-*β-catenin*-HA-T2A-GFP-polyA vector (two bands: 7917 bp and 1901 bp) (data not shown). We also determined the sequence accuracy

of these vectors with sequencing, in consistent with the released sequence in NCBI. Then we transfected the JMB84-CMV-*β-catenin*-HA-T2A-GFP-polyA vector into the HEK293T cell. 48 hours after transfection, clear fluorescence was observed which proved the GFP expression in cells (Fig. 1A), which proved the utility of this vector in subsequent experiments.

Detection of HA-tagged fusion protein expression in the CD11c+ DCs from orally administrated mice

After separating CD11c+ DCs from the orally administrated mice, we observed the representative HA-tagged fusion protein expression from the JMY-Pcat group (Fig. 1B(a)), while the control group was negative. We confirmed the feasibility of using budding yeast cells as gene delivery vehicle to the intestinal DCs. Notably, as displayed in the Fig. 1B(b), the mice fed with recombinant yeast containing JMB84-CMV-*β-catenin*-HA-polyA for five days showed the highest relative expression calibrated with reference protein (β-actin), indicating the time point with highest expression of target protein is 5-day after oral administration, which provides a foundation for development of oral DNA vaccine in the future.

Yeasts regulate 1097 common genes in CD11c+ DCs

Mice were treated with a diverse set of oral stimuli: PBS group, JMY31 yeast harboring JMB84 base vector (JMY-P group) and JMY31 yeast harboring JMB84-CMV-*β-catenin*-polyA vector (JMY-Pcat group). After 5-day orally administration, we separated intestinal CD11c+ DCs and isolated RNA, then fragmented RNA to synthesize cDNA and connected short fragments for the final sequencing. To investigate the genes and signaling pathways that are associated with DC responses to yeast stimuli, we performed a differential expression analysis between different groups. Treatment with different yeasts resulted in transcriptional changes in all DCs examined, albeit to differing degrees (Fig. 1C). According to the analysis result (Additional file 6, Table S3), a total of 18410 genes were screened, including 9488 induced genes and 8922 repressed genes between control and JMY-P group; a total of 18942 genes were screened, including 10807 induced genes and 8135 repressed genes between control and JMY-Pcat group; a total of 19188 genes were screened, including 11103 induced genes and 8084 repressed genes between JMY-P group and JMY-Pcat group.

Using significance analysis of multiclass testing type using a cut-off of $P < 0.05$, after excluding redundant DEGs, we found that JMY-P yeast and JMY-Pcat regulate a high number of unique genes or DEGs (1442 and 2078) at the RNA level at least twofold, considering both induced and repressed transcripts (Additional file 7, Table S4). We segregated the 1442 differentially regulated transcripts between those that are: (i) induced upon JMY-P treat (1062 genes, 73.6%) and (ii) repressed upon JMY-P treat (380 genes, 26.4%); And we also found that 2078 differentially regulated transcripts between those that are: (i) induced upon JMY-Pcat treat (1747 genes, 84.1%) and (ii) repressed upon JMY-Pcat treat (331 genes, 15.9%) (Fig. 2A). The intersection of these groups (JMY-P and JMY-Pcat) responses revealed a common set of 1097 highly regulated genes, which further proved the significant modulating role of

yeast on CD11c+ DCs. According to the comparison based on selected 1097 genes between these two groups, we found that the corresponding trend of regulated 1091 genes (99.5%) in intestinal CD11c+ DCs upon different yeasts treats is very similar at these two stimuli. The heatmap of these ESTs expressions and complete lists of the transcripts in each comparison are shown (Additional file 1, Fig S1; Additional file 8, Table S5). Such a large-scale change in gene expression demonstrated that CD11c+ DCs can undergo a marked transformation in their cellular phenotype upon yeast stimuli, which further indicating that yeast stimuli can regulate a high number of unique genes in CD11c+ DCs to modulate the DCs-mediated functions.

The budding yeast induces a similar and tailored unique genetic re-programming of DCs compared with other fungus

Compared with previous result upon *Candida albicans* stimuli, we found that about 77.70% (108/139) genes expressions were in accordance with the former study (Additional file 12, Table S9), indicating a similar gene cluster related to DCs-derived immune response in diverse fugus. The small fraction of the 39 reverse regulated genes suggested that the budding yeast induces a tailored unique genetic re-programming of DCs other from other fugus.

Yeast preferentially regulates DCs genes involved in T cell activation, cytokine production, adaptive immune response, positive regulation of immune response, Cytokine-cytokine receptor interaction, Natural killer cell mediated cytotoxicity and Hematopoietic cell lineage

Using the Gene Ontology systems of classification and the Kyoto Encyclopedia of Genes and Genomes (KEGG), we analyzed gene annotation for the total unique genes or ESTs that were regulated after yeast treatment. As might be expected, the host response is enriched for ESTs genes (1097) that are associated with the immune response, particularly those that encode cytokines and chemokines. In consideration of the *p-value* of different class (Fig. 2B and 2D), we found that the main biological process under yeast stimuli are: “T cell activation” (126 genes), “cytokine production” (126 genes), “adaptive immune response” (103 genes), “positive regulation of immune response” (113 genes), “myeloid leukocyte activation” (53 genes) and “leukocyte migration” (67 genes) (Fig. 2B and 2C; Additional file 9, Table S6). These class induced by the yeast stimuli, indicated the important boosting influence of yeast on the DCs-participated immune response. From a series of heatmaps for many terms (Additional file 2, Fig S2A), we found that many diverse genes dramatically increased upon the yeast treat, further demonstrating the important influence of yeast on the DCs-mediated immune response.

Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (Fig. 2B and 2D) and from a series of heatmaps for many terms (Additional file 2, Fig S2C), we found that yeast preferentially regulates the following metabolic pathways: “Cytokine-cytokine receptor interaction” (50 genes), “Natural killer cell mediated cytotoxicity” (32 genes) and “Hematopoietic cell lineage” (27 genes) (Additional file 9, Table S6), further demonstrating the yeast’s important influence on cytokine or chemokine signaling

pathway which actively participated into the immune response and Hematopoietic cell lineage-related metabolism.

Altogether, the data on functional annotation of yeast regulated transcripts of DCs show that the yeast exerts pressure on many cell functions including diverse metabolic pathways, but the major target was concentrated on Cytokine-cytokine receptor interaction and Chemokine signaling pathway, which plays very important roles in regulating the DCs-mediated immune response and T cell activation.

JMY-Pcat yeast regulates 944 genes in CD11c+ DCs compared with the JMY-P group

We then used the JMY-P group as control group and conducted significance analysis of multiclass testing type using a cut-off of $P < 0.05$, after excluding redundant DEGs, we found that JMY-Pcat yeasts regulate a high number of unique genes or ESTs (944) at the RNA level at least twofold, considering both induced and repressed transcripts. We segregated the 944 differentially regulated transcripts between those that are: (i) induced compared with JMY-P treat (802 genes, 85.0%) and (ii) repressed compared with JMY-P treat (142 genes, 15.0%) (Fig. 2A). Individual genes following different expression profiles and complete lists of the transcripts in each group are shown (Additional file 1, Fig S1B; Additional file 10, Table S7). Such a large-scale change in gene expression and the Circos plots (Fig. 3A) demonstrated that *β-catenin* truly expressed in intestinal DCs and *β-catenin* modulates a marked gene expression change on the intestinal DCs.

In addition, we found that the intersection of the two different comparisons (PBS vs JMY-P and JMY-P vs JMY-Pcat) revealed a common set of 388 highly regulated genes (Additional file 1, Fig S1C). We also found that the 18 selected genes expressions of JMY-Pcat group is relatively higher than other two groups within the 388 ESTs (Fig. 3E), which further proved the expression of *β-catenin* in DCs and the significant modulating role of *β-catenin* on CD11c+ DCs function.

JMY-Pcat yeast preferentially regulates DCs genes involved in inflammatory response, myeloid leukocyte activation, leukocyte migration, cell-cell adhesion, extracellular structure organization, positive regulation of cytosolic calcium ion concentration, and Cytokine-cytokine receptor interaction

Using the Gene Ontology systems of classification and the Kyoto Encyclopedia of Genes and Genomes (KEGG), we analyzed gene annotation for the total DEGs that were regulated in the comparison between JMY-P and JMY-Pcat treatments (Fig. 3B and 3C; Additional file 11, Table S8). We found that the main biological process under JMY-Pcat yeast regulation are: “inflammatory response” (70 genes), “myeloid leukocyte activation” (31 genes), “leukocyte migration” (39 genes), “cell-cell adhesion” (60 genes), “extracellular structure organization” (34 genes) and “positive regulation of cytosolic calcium ion concentration” (33 genes). From a series of heatmaps for many terms (Additional file 3, Fig S3), we found that many diverse genes dramatically increased upon the JMY-Pcat treat, further demonstrating the important influence of *β-catenin* on the DCs-mediated immune response, especially the inflammatory

response and leukocyte-related functions. Besides these categories, it should not be ignored that *β-catenin* participated into the “cell-cell adhesion”, “extracellular structure organization” and “positive regulation of cytosolic calcium ion concentration”. Together with the high number of genes with metalloproteinase activity, it suggested that JMY-Pcat treat had increased boosting role on regulating permeability of ion channels and activity of ion pumps. JMY-Pcat yeast also preferentially regulates the following metabolic pathways: “Cytokine-cytokine receptor interaction” (29 genes) (Additional file 11, Table S8).

Altogether, the data on functional annotation of JMY-Pcat yeast regulated transcripts of DCs show that the *β-catenin* exerts many promoting roles on many DCs functions including diverse metabolic pathways differ from the JMY-P yeast, but the major targets were concentrated on inflammatory response and leukocyte-related functions, which plays very important roles in regulating inflammation.

Cluster analysis of DEGs among the three comparative groups

Finally, we conducted the cluster analysis of DEGs among three comparative groups using the Metascape (http://metascape.org/). And it was found that every two groups shared many DEGs (Fig. 4A, 4B, 4C, 4D and 4E). Using the Gene Ontology systems of classification and the Kyoto Encyclopedia of Genes and Genomes (KEGG), these DEGs mainly concentrated on following categories: “T cell activation”, “inflammatory response”, “cytokine production”, “positive regulation of immune response”, “mononuclear cell migration” and “leukocyte activation involved in immune response”. It further demonstrated the yeast and *β-catenin* could influence the DC-mediated adaptive immunity and inflammatory response.

Yeasts-mediated regulation of genes expression by DCs–confirmation using quantitative real-time polymerase chain reaction

Transcriptomic analysis shows that DC expression of many genes such as CD80, CD6 and CD40LG is induced with yeast treatment. To confirm the accuracy of RNA-seq, we performed quantitative real-time polymerase chain reaction (PCR) analysis (Fig. 5). For up-regulated genes, the relative expression levels in JMY-P group was significantly higher than that in the PBS group (including TLR9, CD6, CD80, IL12RB1, Gas1, ITGAE, Serpina3g, Cxcr3, CD3d, Gzmk, Serpinb6b, Ly6c2, IL10RA, Fcrl1, Rnase6, CD40lg, Klra7, Rhoh, Arhgap15 and Lair1), while expression levels in JMY-Pcat group was relatively higher than that in JMY-P group (including IL10RA, Rnase6, Tff2 and Cfd). For down-regulated genes, the relative expression levels in JMY-P group was significantly lower than that in the PBS group (Gsdmc3), while the expression level of JMY-Pcat group is lower than the JMY-P group (such as NeuroD2 and Spata17). Above results reinforced the accuracy of the RNA-seq.

Discussion

No specific or detailed investigation on the modulation of intestinal DCs gene expression and function by baker's yeast *saccharomyces cerevisiae* has been addressed, despite the importance of DCs for initiation and maintenance of the protective immunosurveillance[19]. To fill this gap, we comparatively examined responses generated in intestinal CD11c+ DCs upon encounter with different yeasts, in consideration of the supposed, differential modulation role of the two yeasts in immune response and other pathways.

We used the murine model to accomplish oral administration of the baker's yeast *saccharomyces cerevisiae*. Abundant yeasts can guarantee the enough interaction between the DCs and yeasts in intestine area. 5-day after administration, we separated the CD11c+ DCs and conducted the RNA-seq to examine the different response in the transcriptional level.

As might be expected, the common host response upon encounter with the yeast is enriched for genes that are associated with the immune response, particularly those that encode cytokines and chemokines. We identified and grouped several functional groups of gene products according to the part in which they function. Functional groups of genes are described in more detail below.

Gene that mediates DCs activation and maturation. There is a known fact that APCs (Macrophages and DCs) play diverse important roles in adaptive immunity[14, 20]. We found that the phagocytosis of yeast by intestinal DCs can induced highly-expressed CD19 providing the first signal for activating the T leukomonocyte and enhance the antigen-presenting ability, and some marker genes displayed higher expressions, such as ICAM-1, ICAM-3, CD80 and CD40, further indicating the maturation and activation of DCs after engulfing the yeast. Activated APCs can use pattern recognition to distinguish the "self" or "non-self" to initiate different adaptive immunity (humoral immunity or cellular immunity)[21] and the immune system can increase some effectors expression of the APCs to enhance the capture and presentation abilities[22]. Our results further proved the enhancing role of yeast on the activation and maturation of DCs, which can provide a foundation for explaining the boosting role of yeast on T cell activation and cytokine production.

Gene that activates the adaptive immunity. The group of genes that is most strongly and most consistently upregulated consists almost entirely of those encoding cytokines or chemokines. These processes mainly concerned on the DCs-Lymphocytes interactions. As we all know, lymphocytes (B cells or T cells) are activated by antigen-specific receptors on the cell surface[23], which causes the cells to proliferate and differentiate into specific effector lymphocytes. For example, activated B cells can produce antibodies-producing cells, and some activated T cells become cytotoxic T cells. [24, 25]. In this study, for the APCs-T cells interaction, the DEGs include CD2, CD3, CD4, CD6, CD8α1, CD8β1, CD40L, CD72, CTLA-4, IL-1β, IL-16 and ICOS; for the APCs-B cells interaction, the DEGs include CD19, CD40, CD79α, CD79β and CD80. In addition, there are some other chemokines being expressed highly, which participate into the proliferation and migration of T helper cells[26], such as Cxcl1, Cxcl2, Ccl3, Ccl4, Ccl5 and Ccl22; some chemokines receptors were also found much higher expressed in activated DCs, including Cxcr3, Cxcr4, Cxcr5, Cxcr6, Ccr2, Ccr5, Ccr6, Ccr7, Ccr9 and Cx3cr1. All these increased expressions indicated that the cooperation of chemokine and the coupling receptors may initiate the

directional migration of target cells. Additionally, the immunological synapse plays an important role in participating the interaction between DCs and T cells[27], which can promote the binding ability of TCR and MHC complex, the signal transduction of T cells and the effector function of T cells. Herein, CD3, ICAM-1, ICAM-5, LCK, ZAP70, LAT, VAV1, Was, Rac2, Fyn and Fyb, are the most DEGs participating into the formation of immunological synapse upon encounter with yeast. There are also some increasingly expressed transcription factors involving in the development and activation of leukomonocytes, including NFAT (Nfatc1 and Nfatc1), Relt, Stat4, ETS-1, PAX5, BATF, IRF4, GATA-2 and GATA-3. Induction of signaling genes and transcription factors may be involved in the process of allowing DCs to receive regulatory signals from lymphatics and lymph nodes. In a word, the yeast plays the beneficial role of in modulating the adaptive immunity through diverse pathways.

Gene that mediates inflammation. The strongest, most persistently up-regulated set of genes consists almost entirely of genes encoding cytokines called inflammatory/chemokine cytokines. This set contains genes encoding pro-inflammatory mediators, such as TNFAIP3, IFNγ, IL1β and IL18RAP, and PTGS2 enzyme (epoxidase-2), involved in the production of prostaglandins.

Other common host-response-gene. This set of genes includes genes encoding OAS (2',5'-oligo adenylate synthetase)1h, OAS2 and IFIM1. As expected, similar with *Candida albicans* exposure[16], some same genes are upregulated in DCs stimulated with yeast, which further describing the important role of yeast in regulating IFN-stimulated function of the DCs. What the fact is equally important is the dramatically increased expressions of the TLR family genes upon yeast exposure, suggesting an intensive capacity of DCs to communicate with other cells of both the innate and adaptive arms of the immune system. A class of cytokine receptors (IL10RA, IL12RB1, IL12RB2, IL1R2, IL21R, IL22RA2, IL27RA, IL2RB, IL2RG, IL7R, and IL9R) were also induced. The expression of these receptors may allow DCs to respond to lymphocyte-derived interleukins within the lymph node.

To better understand the overlap of the total transcriptome regulation of DC cells after yeast stimulation with changes caused by other fungus, and the similarity of regulation of DC cell maturation, we compared a series of orthologs genes that are regulated by a fungus in human monocyte-derived DCs[16] to the yeast-regulated genes in this study. About 77.70% (108/139) genes expressions were in accordance with the former study (Additional file 12, Table S9), indicating a similar gene cluster related to DCs-derived immune response in diverse fugus. More importantly, we found 38 genes had a reverse expression trend combined with this report, and it further validated that the DCs detect diverse microorganism and induce tailored specific immune responses.

We then confirmed the DEGs expressions of separated DCsin JMY-Pcat group with the comparison analysis between the two groups: JMY-P and JMY-Pcat. As we all know, β-catenin is a bifunctional protein involved in the regulation and coordination of cell-cell adhesion and gene transcription [28], which is a known subunit of the cadherin protein complex and functions as a signal transducer in the Wnt signaling pathway[29]. Mutation and overexpression of β-catenin is associated with the development of many cancers, including hepatocellular carcinoma, colorectal cancer, lung cancer, malignant breast

tumors, ovarian cancer, and endometrial cancer[30]. Changes in β-catenin localization and expression levels are also associated with a variety of heart diseases [31], including dilated cardiomyopathy. In the process of adherens junction, β-catenin molecules are also recruited by cadherins onto their intracellular regions. β-catenin, in turn, associates with another important protein, α-catenin that directly binds to the actin filaments[32]. The β-catenin–α-catenin complex can thus physically bridge cadherins with the actin cytoskeleton[33]. Based on our analysis, we also identified and grouped several functional groups of gene products according to the part in which they function upon encounter with the JMY-Pcat yeast. Functional groups of genes are described in more detail below.

Genes that mediate inflammatory response. Core stimulated genes are as follows: Ccl1, Ccl3, Ccl4, Ccl7, Ccr3, Chi3l3, Cxcl2, Cxcl3, Cxcl5, Cxcl9, Fcer1a, Il13, Il1a, Il1b, Il23a, Il27, Il4, Il5ra, Itih4, Nfkbid, Tlr11, Tlr8, Siglece and so on. These core immune-related DEGs again proved the importance of *β-catenin* in modulating the immune response, especially the “inflammatory response”. As we all know, the inflammatory response has a tight relationship with the leukocyte activation and leukocyte migration. Based on our analysis, for the “leukocyte migration”, the significant DEGs include: Adora3, Cd37, Cxcl5, Dnase1l3, Dock2, Fcer1a, Fcgr4, Fyb, Gata2, Havcr1, Havcr2, Il13, Il18rap, Il4, Lbp, Lilrb3, Tff2, Tlr8, Tnfsf9, Ttbk1 and so on; for the “leukocyte migration”, there include many stimulated DEGs as follows: Adora3, Ccl1, Ccl3, Ccl4, Ccl7, Ccr1l1, Ccr3, Cd34, Cxcl2, Cxcl3, Cxcl5, Cxcl9, Il1a, Il1b, Il23a, Il4, Itga2b, Itga4, Itgb3, Lbp, Mmp9, Pik3cg, Tnfsf14, Tnfsf4. These stimulated DEGs further proved the boosting role of β-catenin on inducing inflammatory response.

Genes that mediate the adhesion function. From the GO and KEGG analysis, we also found some DEGs related to the cell-cell adhesion and extracellular structure organization. For the cell-cell adhesion category, the DEGs are as follows: Adam19, Rltpr, Cd163l1, Cd34, Cd4, Cd93, Cdh11, Cdh22, Cdh24, Cdh9, Cdk5r1, Cdkn2a, Ctla4, Ctnnd2, Gata1, Havcr2, Hlx, Icam4, Igsf21, Il1b, Il23a, Il4, Itga2b, Itga4, Itgad, Itgax, Itgb3, Nfkbid, Pdgfra, Rasgrp1, Serpinb8, Tnfsf14, Tnfsf4, Tnfsf9 and so on. Herein, cadherin superfamily (Cdh11, Cdh22, Cdh24, Cdh9 and Cdk5r1) are integral membrane proteins that mediate calcium-dependent cell-cell adhesion; integrins (Itga2b, Itga4, Itgad and Itgax) are heterodimeric integral membrane proteins involving into the adhesion and cell-surface mediated signaling; Ctnnd2, has a critical role in neuronal development, particularly in the formation and/or maintenance of dendritic spines and synapses[34, 35]; and also functions as a transcriptional activator when bound to ZBTB33[36]; Cdk5r1, is a neuron specific activator of CDK5. The complex p35/CDK5 is required for neurite outgrowth and cortical lamination[37] and involved in dendritic spine morphogenesis by mediating the EFNA1-EPHA4 signaling. For the extracellular structure organization, the stimulated DEGs are as follows: Col15a1, Col1a1, Col28a1, Col5a2, Col5a3, Colq, Ets1, Flrt2, Itgb3, Lcat, Loxl1, Loxl3, Mmp11, Mmp9, Pdgfra, Tnfrsf11b and so on. Of which, matrix metalloproteinase (MMP) proteins (Mmp11 and Mmp9) are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling. These regulated gene families further proved the important role of *β-catenin* on actins arrangement for DCs, which was hoped to be involved in “leukocyte migration”, “cell-cell adhesion”, “extracellular structure organization”, “positive regulation of cytosolic calcium ion concentration”. Other from these genes, there are some gene families which should be targeted. Alpha-

actinins (ACTN2 and ACTN3), which helps to anchor the myofibrillar actin filaments; Pcdhga5, Pcdhga7, Pcdhgb1, Pcdhgb4 and Pcdhgb5 are integral membrane proteins that mediate calcium-dependent cell-cell adhesion. But the specific and detailed network of this process remains unclear and need more efforts to discover it.

Much more than this, as described previously, *saccharomyces cerevisiae* can be an effective oral DNA vaccine vehicle targeting for intestinal cells and provoke strong immune response in different animal species[38-40]. Our results further proved the utility of oral yeast-based gene delivery vehicle targeting for intestinal DCs directly, which lays a solid foundation for the development of yeast-based DNA vaccine.

Conclusions

In a word, we confirmed the beneficial role of yeast on modulating the intestinal DCs functions and proved the feasibility of using budding yeast cells as gene delivery vehicle to the intestinal DCs directly. Moreover, we further validated the important modulating role of *β-catenin* on the DCs-mediated immune response and other functions.

Methods

Vectors construction and verification

Based on the pcDNA3.1(-) vector, we amplified the CMV promotor and polyA fragment using the following primers: CMV-F/CMV-R and PA-F/PA-R; then referred on the *β-catenin* sequences (NM_001098209.1) from NCBI database, we amplified the *β-catenin* fragment with special primers: B-F/B-R templated on cDNA of HEK293T cells. Next, we conducted overlap PCR to get the CMV-*β-catenin*-polyA cassette, which was templated on CMV, *β-catenin* and polyA fragments. Finally, CMV-*β-catenin*-polyA cassette and JMB84 base vector (maintained in our lab) were digested with *Sac*I / *Not*I and *Xho*Ⅰ/ *Not*Ⅰ, separately; then we conducted gel extraction and ligated them with T4 DNA ligase to get the vector JMB84-CMV-*β-catenin*-polyA. In the following step, to get the *β-catenin*-HA fragment, we made two continuous-step PCR reaction using primers Beta-F/ HA-R1 and Beta-F/ HA-R2 based on the JMB84-CMV-*β-catenin*-polyA vector. JMB84-CMV-*β-catenin*-polyA vector and *β-catenin*-HA fragment were digested with *Nhe*Ⅰ and *Eco*RⅠ simultaneously. After gel extraction, we ligated above DNA fragments with T4 DNA ligase to get JMB84-CMV-*β-catenin*-HA-polyA vector.

To insert the GFP reporter gene into the JMB84-CMV-*β-catenin*-HA-polyA vector, we first amplified the *β-catenin*-HA fragment templated on the JMB84-CMV-*β-catenin*-HA-polyA vector using special primers Beta-F/Beta-R; meantime, based on the pLenti-T2A-GFP vector, we amplified the T2A-GFP fragment with primers: T2A-GFP-F/T2A-GFP-R. Then overlap PCR was conducted to get the *β-catenin*-HA-T2A-GFP cassette using *β-catenin*-HA and T2A-GFP fragments as templates. Finally, we digested this cassette and JMB84-CMV-*β-catenin*-polyA vector with *Eco*RⅠ and *Nhe*Ⅰ enzymes; then we made the gel extraction of above cassettes and ligated them with T4 DNA ligase to get JMB84-CMV-*β-catenin*-HA-T2A-GFP-polyA

vector. All above vectors were digested by special enzymes and determined by sequencing (data not shown). All primers sequences were displayed in Additional file 4, Table S1. We then transfected the vector JMB84-CMV-*β-catenin*-HA-T2A-GFP-polyA into the HEK293T cells to verify the successful expression of *β-catenin*.

Culture and transformation of yeast

The yeast *S. cerevisiae* strain JMY31 (*MATa, ade2-1; ura3-1; his3-11; trp1-1; leu2-3,112; can1-100*) was cultured in YPDA medium before transformation. The naked vector JMB84 and above two newly-constructed vectors (including JMB84-CMV-*β-catenin*-polyA and JMB84-CMV-*β-catenin*-HA-polyA) were transformed into JMY31 as described previously[38]. And the positive transformant was selected and cultured with SD media supplemented with leucine, tryptophan, adenine and histidine (Sigma, America). Plasmids from yeast were extracted via Yeast Plasmid Extraction Kit (Omega, America) by standard procedure and plasmid accuracy was determined via enzymes digestion (data not shown).

Mice and oral administration

C57BL/6 mice aged 7 weeks were purchased from the Animal Breeding and Research Center of Xi'an Jiaotong University, China. The mice were housed under standard conditions of room temperature and dark-light cycles and water was available *ad libitum*. All the experimental procedures with mice used in the present study had been given prior approval by the Experimental Animal Manage Committee of Northwest A&F University (2011-31101684). All the operations and experimental procedures were complied with the national standard of Laboratory animal-Guideline for ethical review of animal welfare (GB/T 35892-2018) and Guide for the Care and Use of Laboratory Animals: Eighth Edition [41].

For optimization of oral administration formula and confirm the feasibility of using budding yeast as gene delivery vehicle, mice were randomly divided into five groups, and each group contained six female individuals. Four groups were orally administrated with 1×10^8 yeast[42] containing JMB84-CMV-*β-catenin*-HA-polyA vector suspended in 100ul PBS buffer for 3, 4, 5 and 7 days separately once a day. The control group was fed with the same volume of yeast containing naked vector. Before oral administration, mice need fasting treatment for 12h. After limited feeding time, mice were sacrificed for intestinal CD11c+ DCs separation post-anesthesia[39]. Then the best formula was determined via the Western blots through detecting the amount of HA-tagged fusion proteins.

Once determined the best formula, another batch of mice were randomly divided into three groups, and each group contained six female individuals. Mice from different groups were orally administrated with 1×10^8 yeast containing JMB84-CMV-*β-catenin*-polyA vector (named as JMY-Pcat) suspended in 100ul PBS buffer for 5 days separately once a day. The other two groups were fed with the same volume of yeast containing naked vector (named as JMY-P) and PBS (named as PBS).

Euthanasia/sacrifice methods

After 5 days' administration, mice were sacrificed for intestinal CD11c+ dendritic cells separation post-anesthesia, the detailed method was as following: according to previous reports[43], we chose intraperitoneal injection to perform this experiment. This is usually the most ideal method when it can be performed without causing fear or pain to the animal. When appropriately administered, an acceptable injection of the euthanasia agent can result in a smooth loss of consciousness before the heart and/or respiratory function ceases, minimizing pain and distress in the animal. The injectable euthanasia agent is Nembutal ie sodium pentobarbitone and it was administered at 150 mg/kg to accomplish the anesthesia. Overdose using this anaesthetic solution at 3-5 times. After that, we ensured the death by cervical dislocation or by opening the chest to collapse lungs.

Separation and purification of intestinal CD11c+ DCs

According to previous report[39], we chose CD11c as a marker of choice for intestinal DCs. Each group was collected for removal of the mesentery and fat of the intestine and colon, and the procedure was performed in a pre-chilled PBS solution. The intestinal wall and colon were washed with PBS containing 10% fetal calf serum before cutting the sample into tiny tissues. Then, each group was added with 20 ml of Digestion I (PBS containing 1 mM EDTA, 1 mM dithiothreitol, 10% fetal bovine serum) to digest the sample. After incubating for 40 minutes at 37 ° C on a shaker at 155 rpm, the digested fragments were centrifuged at 1500 g for 5 min. After washing twice with PBS, 20 ml of Digestion II (PBS containing 100 $U\,l^{-1}$ collagenase IV, 10% fetal bovine serum) was further added to the sample for further digestion, and incubated at 37 ° C for 40 minutes on a shaker at 155 rpm. After the digested cells were filtered using a stainless-steel cell sieve, the cell suspension was washed twice with PBS. After discarding the supernatant, 400 µl buffer (PBS and 0.5% bovine serum albumin, 2 mM EDTA) and 100 µl anti-CD11c-conjugated magnetic cell sorting beads (Miltenyi Study, Bergisch, Germany) were added per 10^8 cells. After incubation at 4°C for 30 min, intestinal DC cells were subjected to magnetic cell sorting from mixed cells using a MACS separation column (Miltenyi Biotec, Bergisch, Germany).

Western blots

After separating the intestinal CD11c+ DCs, total proteins were extracted by using the RIPA lysis buffer (Beyotime, China) according to the instruction. We then added the appropriate loading buffer into the sample and proteins were denatured in boiling water for 10 min. Protein was separated on 10% SDS-PAGE acrylamide gels and transferred onto a PVDF membrane (Millipore, USA). Then we blocked the membrane with 5% nonfat milk and washed it six times with TBST (20mM Tris-HCl, 150mM NaCl, 0.05% v/v Tween 20). HA-tagged fusion protein was detected using mouse anti-HA tag monoclonal antibody (1:1000; Sigma, America) overnight. The membrane was washed six times again and then we incubated it with the horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody (1:3000; Beyotime, China) secondary antibody. After removal of the unwanted antibodies by washing the sample with TBST, the

specific signal was detected by Leica DM2500M (Leica Microsystems GmbH, Wetzlar, Germany) with a chemiluminescent substrate.

RNA sequencing and data analysis

Another batch of mice were randomly divided into three groups, and each group contained six female individual mice. They were fed with three formula, including PBS (named as PBS), the recombinant yeast with base vector (named as JMY-P) and the recombinant yeast containing JMB84-CMV-*β-catenin*-polyA vector (named as JMY-Pcat). After 5 days' administration, mice were sacrificed for intestinal CD11c+ DCs separation. Total DCs RNA from different groups were extracted from every sample using the Trizol reagent (Invitrogen, CA, USA). The total RNA quantity and purity were analysis by Nanodrop 2000 (Thermo, MA, USA). After the total RNA quality detection and DNase I treatment, we used magnetic beads (Invitrogen, CA, USA) to isolate mRNA. After mixing with the buffer, the mRNA is divided into short fragments. Then we synthesized the cDNA using the mRNA fragment as a template. The short fragment was then purified and resolved with EB buffer and single nucleotide A (adenine) was added for end repair. In addition, we use specific adapters to connect short segments together. After agarose gel electrophoresis, the appropriate fragment was selected as a template for PCR amplification. In the QC step, the quality and quantity of the sample library was identified using the Agilent 2100 Bioanaylzer and the ABI StepOnePlus Real-Time PCR system. At last, all DNA fragments was sequenced using Illumina HiSeqTM 2000 (LC Sciences, USA). High quality clean data obtained by quality control from raw data, were aligned to the reference sequence using SOAPaligner/SOAP2. According to the distribution and coverage of the reads, quality of reads was confirmed. Gene expression levels were normalized by considering the RPKM value (reads per kilobase of the exon model per million mapped reads). Based on the expression levels, the significant unigenes that were differentially expressed (DEGs) between every two experiment groups were identified "|fold change|≥2" and "*P-value*< 0.05" as threshold to judge[44].

GO enrichment analysis and Pathway enrichment analysis

Based on the gene ontology database (http://www. geneontology.org/), this part of the experiment analyzes the function of DEGs between different groups through GO analysis. GO analysis is a common method for large-scale gene function enrichment research. In this study we used the R package clusterProfiler for GO enrichment analysis. The *p value* < 0.05 and the FDR value < 0.05 were used as the cutoff criterion for GO enrichment analysis [45].

KEGG is a commonly used bioinformatics database that includes information about biochemical pathways. In this study, the R package clusterProfiler was used to perform KEGG pathway enrichment analysis, and some pathways with significant differences were screened. The p-value < 0.05 was used as the cutoff criterion for KEGG enrichment analysis. Network enrichment analysis was performed using Metascape (http://metascape.org/).

Quantitative real-time PCR

RNA was transcribed into cDNA using PrimeScript® RT reagent Kit (Takara, Dalian, China) according to the manufacturer's instructions. We used the SYBR Green-based quantitative real-time PCR (qRT-PCR) to quantify the mRNA levels of selected genes in different groups using the lyceraldehyde-3-phosphate dehydrogenase (GAPDH) as reference gene. Genes and primers for qRT-PCR were presented on Additional file 5, Table S2. The LightCycler 480 Probes Master and the LightCycler 480 System (Roche Applied Science, Penzberg, Germany) was carried out as previously described. PCR was performed using 2 μl diluted cDNA in a 20 μl final reaction mixture (15 min at 95°C, 55 cycles of 15 seconds at 95°C, 30 seconds at 55°C, 30 seconds at 70°C, melting curve at 60–97°C with the specific product at 74–77°C). Data were analyzed using the $2^{-\Delta\Delta Ct}$ method as previously described[46] and all reactions were done in triplicate.

Statistical analysis

One-way analyses of variance (ANOVAs) were used to analyze all data and to identify differences among treatments, followed by an unpaired, two-tailed t-test. All the statistical analyses were performed using GraphPad Prism v5.01 (GraphPad, USA). The *P value* less than 0.05 and less than 0.01 were considered statistically significant and extremely significant, respectively.

Abbreviations

DCs: dendritic cells; *S. cerevisiae*: *saccharomyces cerevisiae*; DEGs: differentially expressed genes; GO: Gene Ontology; qRT-PCR: quantitative real-time PCR; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Declarations

Ethics approval and consent to participate

All the experimental procedures with mice used in the present study had been given prior approval by the Experimental Animal Manage Committee of Northwest A&F University. All the operations and experimental procedures were complied with the national standard of Laboratory animal-Guideline for ethical review of animal welfare (GB/T 35892-2018) and Guide for the Care and Use of Laboratory Animals: Eighth Edition [41].

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this article and its supplementary information files. Plasmids are available upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZZY designed the whole experiment. HBQ and ZTT performed the most of experiments and analyzed the data. LXY and ZR, performed the left experiments. GW, ML and XK participated into the data analysis. HBQ and ZTT wrote the first draft and all authors revised the paper and approved the final version to be published.

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Figures

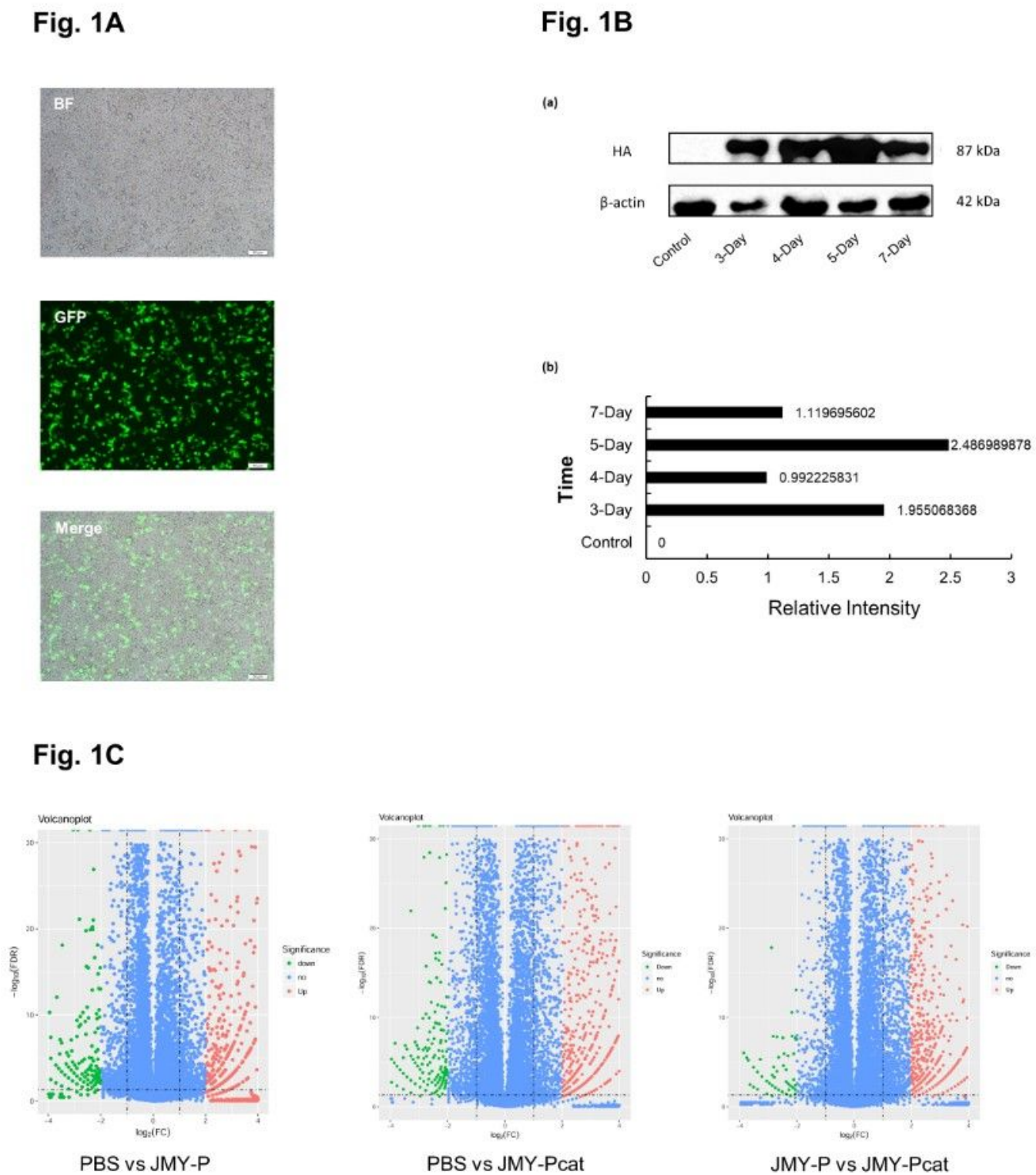

Figure 1

Vector validation and gene delivery confirmation. 1A, fluorescence detection. After 48h, clear green fluorescence was observed under fluorescent microscope. BF: bright field; GFP: green fluorescence; Merge: merge above two pictures. 1B, Western blots detection and intensity analysis. (a) HA proteins detection by Western blots. HA-tagged fusion protein was 87 kDa and β-actin protein was 42 kDa. There were five groups: control, 3-day, 4-day, 5-day and 7-day. (b) gray intensity analysis. The relative intensity stands for HA expressions calibrated with reference protein (β-actin). This analysis was conducted with ImageJ software. 1C, the volcano plot used for displaying the DEGs distribution, including PBS vs JMY-P, PBS vs JMY-Pcat and JMY-P vs JMY-Pcat groups.

Fig. 2A

Samples compared	Unique genes/ESTs		
	Total	Induced	Repressed
PBS vs JMY-P	1442	1062	380
PBS vs JMY-Pcat	2078	1747	331
Common	1097	937/943	160/154
JMY-P vs JMY-Pcat	944	802	142

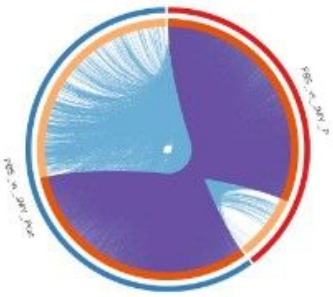

Fig. 2B

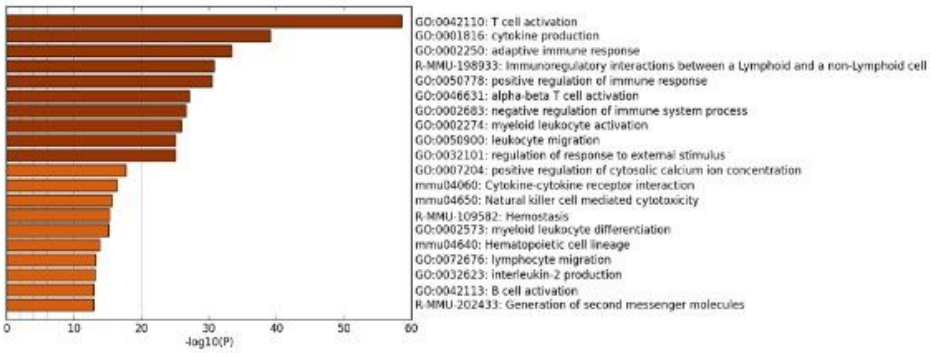

Fig. 2C

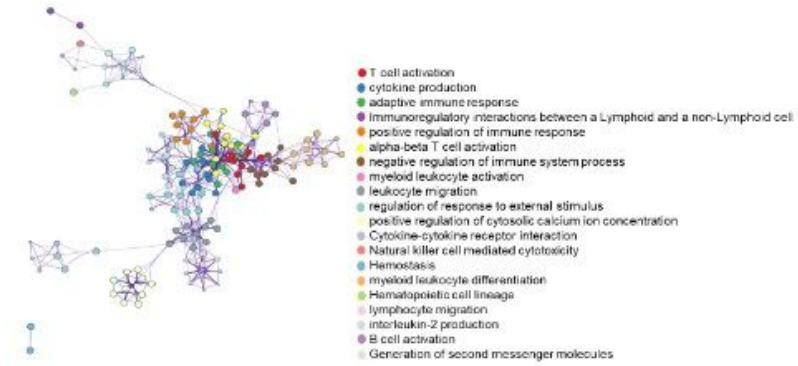

Fig. 2D

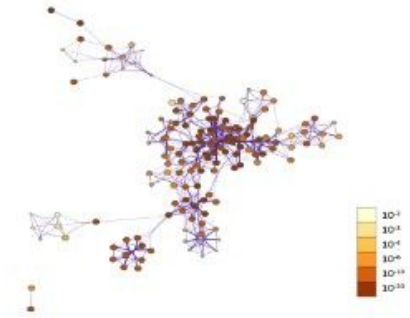

Figure 2

DCs gene expression profiling upon yeast JMY-P. 2A, left lane, total number of unique genes/ESTs differentially regulated by different yeasts in intestinal DCs. Total numbers of genes induced or repressed are presented for all comparisons of any two experimental groups; right lane, circos plots showing interaction between two comparisons. The shared genes are linked by purple lines, and the different genes falling into the same term are linked by blue lines. 2B, heatmap of enriched terms across input

gene lists, colored by p-value. 2C, network of enriched terms: colored by cluster ID, where nodes that share the same cluster ID are typically close to each other. 2D, network of enriched terms: colored by p-value, where terms containing more genes tend to have a more significant p-value.

Fig. 3A

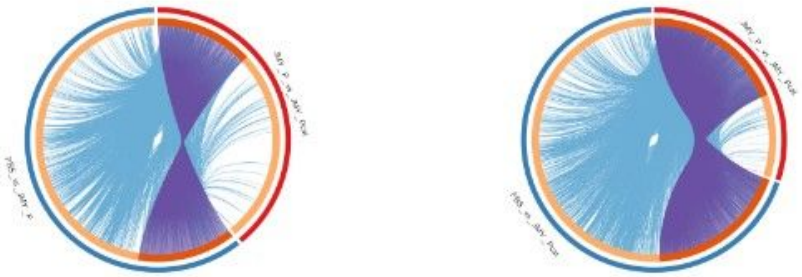

Fig. 3B

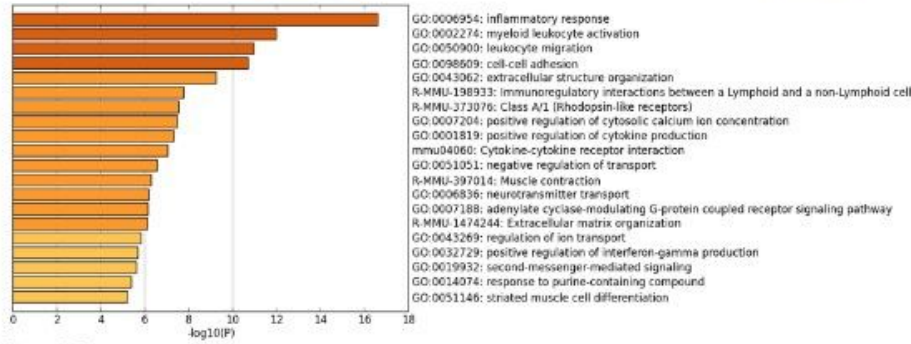

Fig. 3C

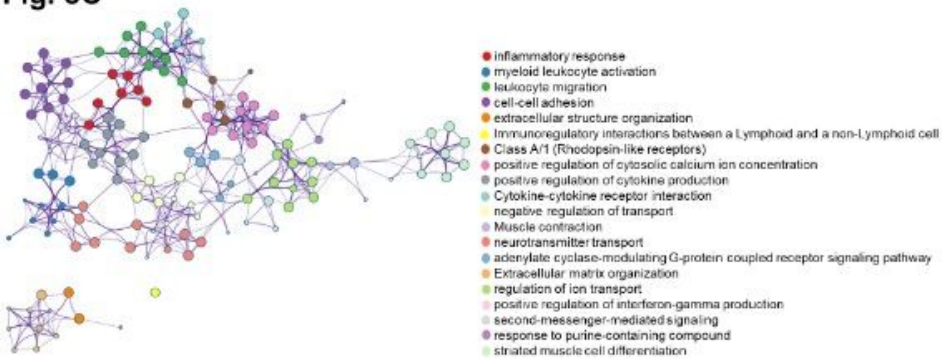

Fig. 3D

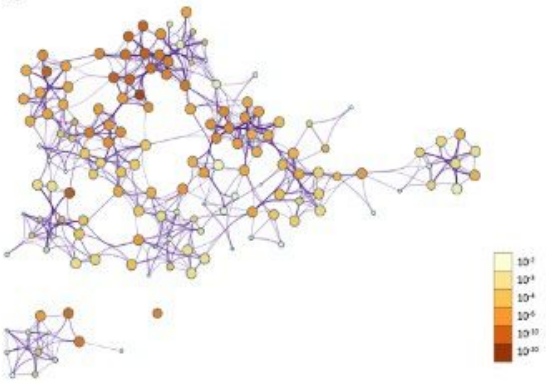

Fig. 3E

Gene ID	PBS	JMY-P	JMY-Pcat	
Actn2	0.001	2.0909404	8.5305262	
Adcy7	0.0234165	6.0229409	24.843273	
Ccl3	0.001	54.320531	259.25639	
Ccl4	0.2126593	37.902732	161.34187	
Cd33	0.001	0.7288664	5.2066091	
Cd34	0.0546861	0.6711889	3.5779052	
Clec2i	0.001	0.6193227	3.2010848	
Cxcl2	0.1323475	5.0143407	25.569497	
Igfbp5	0.001	0.1045253	0.4208989	
Il10ra	0.001	0.9963152	4.3060631	
Il18rap	0.001	0.42901	2.0627145	
Itgae	0.0747301	50.924437	207.92015	
Lair1	0.001	1.557168	6.6911693	
Mmp25	0.001	0.1360564	2.1260523	
Mmp9	0.0225012	0.9005457	3.8218552	
Nfkbid	2.531492	16.591069	70.275496	
Ptpn5	0.0457346	1.1348485	4.7523859	
Tnfsf14	0.001	1.0063994	4.2660874	

Figure 3

DCs gene expression profiling upon yeast JMY-Pcat. 3A, circos plots showing interaction between two comparisons. The shared genes are linked by purple lines, and the different genes falling into the same

term are linked by blue lines. 3B, heatmap of enriched terms across input gene lists, colored by p-value. 3C, network of enriched terms: colored by cluster ID, where nodes that share the same cluster ID are typically close to each other. 3D, network of enriched terms: colored by p-value, where terms containing more genes tend to have a more significant p-value. 3E, the expressions comparison of 18 selected genes among three treatment.

Fig. 4A

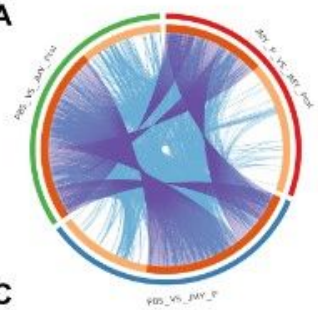

Fig. 4B

Samples compared	Unique genes/ESTs
Set1	876
Set2	87
Set3	347
Set4	221

Fig. 4C

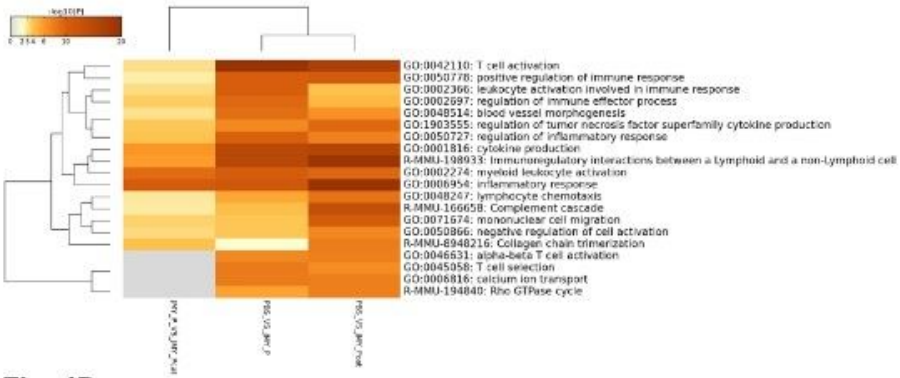

Fig. 4D

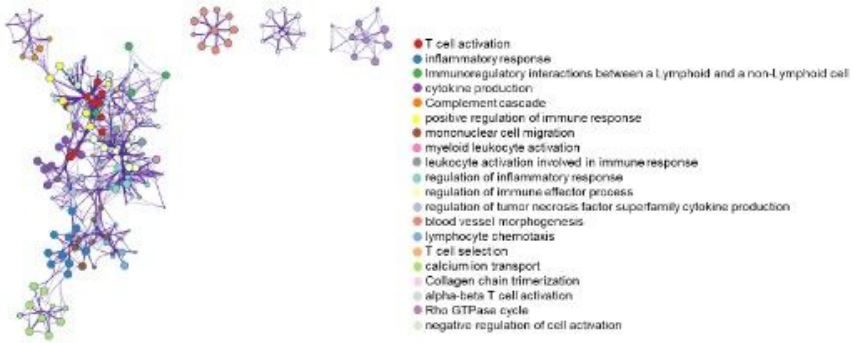

Fig. 4E

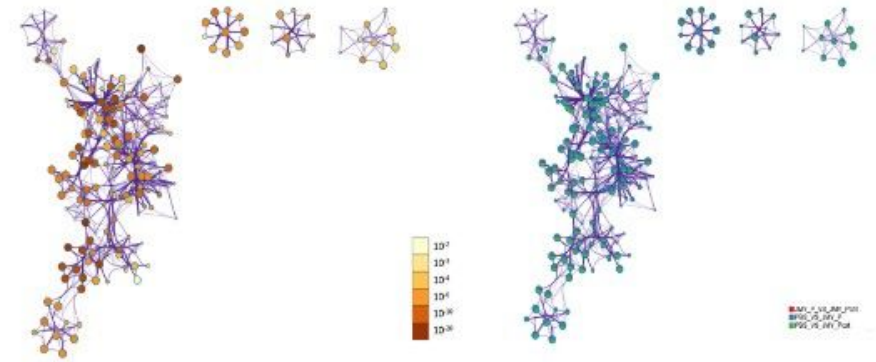

Figure 4

DCs gene expression profiling upon different yeast treatments. 4A, circos plots showing interaction between two comparisons. The shared genes are linked by purple lines, and the different genes falling into the same term are linked by blue lines. 4B, the intersection DEGs from each two comparisons. Set1: pbs vs JMY-P and pbs vs JMY-Pcat; Set2: pbs vs JMY-P and JMY-P vs JMY-Pcat; Set3: pbs vs JMY-Pcat and JMY-P vs JMY-Pcat; Set4: pbs vs JMY-P and pbs vs JMY-Pcat and JMY-P vs JMY-Pcat. 4C, heatmap of enriched terms across input gene lists, colored by p-values.4D, network of enriched terms: colored by cluster ID, where nodes that share the same cluster ID are typically close to each other.4E, left lane, network of enriched terms: colored by p-value, where terms containing more genes tend to have a more significant p-value; right lane, network of enriched terms represented as pie charts, where pies are color-coded based on the identities of the gene lists

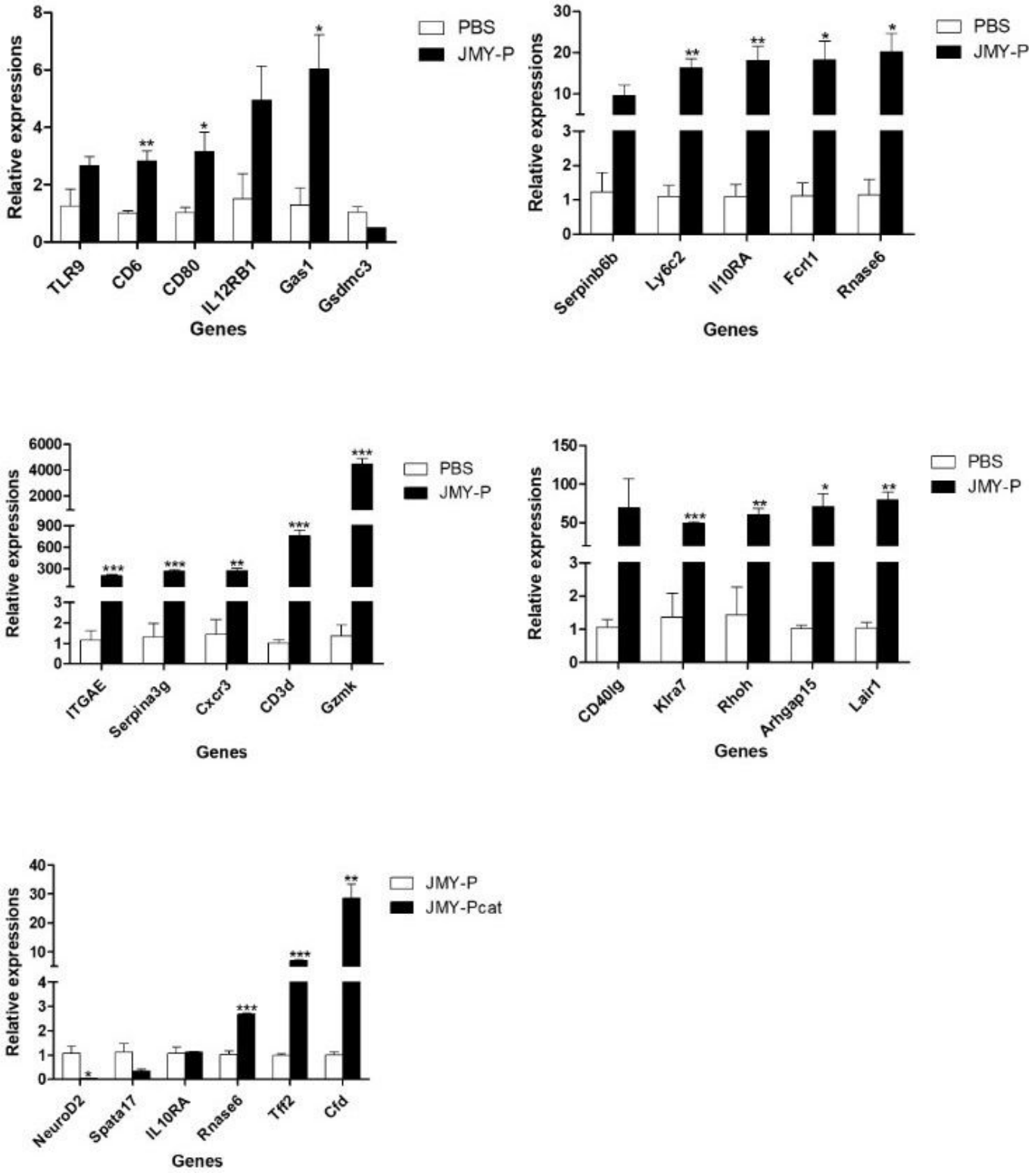

Figure 5

Real-time quantitative. There were 25 genes being selected, including TLR9, CD6, CD80, IL12RB1, Gas1, ITGAE, Serpina3g, Cxcr3, CD3d, Gzmk, Serpinb6b, Ly6c2, IL10RA, Fcrl1, Rnase6, CD40lg, Klra7, Rhoh, Arhgap15, Lair1, Tff2 and Cfd. The black bar stands for relative expression of JMY-P or JMY-Pcat groups; the white bar stands for PBS or JMY-P groups. “*” indicated significant difference (P-value<0.05); “**” or “***” indicated extremely significant difference (P-value<0.01).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.docx
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- supplement3.xlsx
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- supplement10.tif
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