The novel role of Yin Yang 1 in the acute rejection of liver allografts by activating dendritic cells

Yi Chen¹, Bin Wang¹, Hong Liping¹, Wubing He², and Lihong Chen³

¹Fujian Medical University
²Provincial Clinical Medical College, Fujian Provincial Hospital, Fujian Medical University
³School of Basic Medical Sciences of Fujian Medical University,

April 12, 2023

Abstract

Acute rejection that causes liver transplant dysfunction is the most common cause of mortality following liver transplantation. The potential role of Yin Yang 1 (YY1), a widely expressed zinc finger DNA-binding transcription factor, in acute rejection of liver allografts remains unknown. Here, we evaluated the effects and mechanisms of YY1 in an acute rejection using major histocompatibility complex (MHC) class II-mismatched rat liver transplantation model. On days 5 and 10 after liver transplantation, allografts showed elevated expression of YY1 in infiltrating inflammatory cells around the central vein of recipient livers accompanied by elevated levels of serum transaminase and proinflammatory cytokines. In vitro analysis showed that YY1-overexpressing DCs had higher expression of CD80, CD86, and MHC class II compared with the control group. Additionally, YY1-overexpressing DCs triggered naïve CD4+ T cells to produce high levels of intracellular cytokines IL-17 and TNF-γ. These results suggest that YY1 activates DCs and participates in the pathogenesis of acute rejection by polarizing naïve T cells to the inflammatory phenotype, making YY1 a candidate therapeutic target to avoid acute rejection after liver transplantation.

INTRODUCTION

Liver transplantation is the best treatment for end-stage liver disease, and successful liver transplantation prolongs patient survival (1). Compared with other transplantations, such as kidney and lung, immune rejection after liver transplantation is lower (2). Nevertheless, some patients will experience acute rejection, which will seriously reduce the survival rate of the donor liver. As a result, lifelong systemic administration of immunosuppressive drugs after transplantation is often chosen to achieve long-term survival of transplanted livers, although these drugs may cause malignant tumors or infections because of the immunosuppressed state (3). Therefore, revealing the mechanism underlying immune rejection and tolerance after liver transplantation is of great significance to diagnose and develop new treatment methods for acute rejection after liver transplantation.

Abnormal activation of various immune cells is involved in the mechanism of immune rejection in liver transplantation, among which donor-derived and recipient dendritic cells (DCs) have long been recognized as important factors in favor of direct, indirect, and semi-indirect pathways of antigen presentation that trigger acute liver allograft rejection (4, 5). Compared with other non-lymphoid organs, more interstitial DCs reside in portal tracts and around hepatic veins with some cells scattered interstitially between hepatocytes (6). Therefore, few donor-derived DCs are transferred to the recipient as passengers after liver transplantation. In the direct pathway of antigen presentation in acute rejection of liver transplantation, they migrate into recipient lymph nodes and present alloantigens on their MHC class I and II molecules to naïve T cells from recipients. The indirect pathway involves recipient DCs capturing and presenting alloantigens to naïve T-cells (7). In the semi-indirect pathway, recipient DCs gain non-self MHC molecules via cell-to-cell contact.
or fusion of exosomes from recipient DCs and then activate naïve T cells. Although less potent than the
direct pathway, indirect and semi-indirect pathways initiate acute rejection and contribute to later episodes
of rejection (8). In fact, the common result of the three pathways is accumulation of mature DCs followed
by differentiation of naïve T cells into Th1 and Th17 subtypes. Th1 cells secrete IL-1, IFN-γ, and TNF-α
after activation and play major roles in immune regulation, autoimmune disease, and transplant immune
rejection. Th17 cells are involved in acute immune rejection of the kidney, lung, heart, and other organs via
the release of proinflammatory cytokine IL-17. The crosstalk between DCs and naïve T cells during liver
transplantation is associated with abnormal expression of damage-associated molecular pattern molecules
and transcription factors encoding inflammatory mediators, which may be therapeutic targets and early
warning factors to prevent liver allograft rejection.

Yin Yang 1 belonging to the GLI-Kruppel family is a member of the zinc finger class of DNA-binding
proteins and an evolutionarily highly conserved transcription factor. The N-terminal region of YY1 (1–100
aa) contains a transcriptional activation domain and is involved in electrostatic interactions with positively
charged proteins and nuclear localization (9, 10). The central region and the sequence near to the carboxyl
terminal region of YY1 is responsible for transcriptional repression (11). Furthermore, the C-terminal region
mediates DNA binding via four C2H2-type zinc finger motifs, by which YY1 interacts with and recruits a
diverse array of other transcription factors (12, 13). Therefore, YY1 functions as transcriptional activator
or repressor in many cellular processes depending on the chromatin context and environments. Several T
cell cytokine promoter regions, including IL-4 (14), IL-5, and IFN-γ (15), contain consensus YY1 binding
sites. Thus, YY1 may play a major role in inflammation-related diseases. In rheumatoid arthritis, YY1
positively promotes pathological Th17 cell differentiation by an interaction with T-bet and participates in
pathophysiologic process (16). Partial YY1 deficiency suppresses the expression of IL-4 and IFN-γ and
attenuates the differentiation of naïve T cells toward the Th2 phenotype (17). Although these studies
emphasize the important role of YY1 in T cell differentiation and its ability to release proinflammatory
factors, no studies have revealed whether YY1 is also involved in the maturation of DCs or crosstalk between
DCs and naïve T cells. Considering that Th1 and Th17-dominated immune responses are observed in acute
rejection of liver transplants, revealing the function of YY1 in these processes might explain the mechanism
shifting the Th1/Th2 balance to Th1 and accumulation of Th17 cells responsible for immune injuries after
liver transplantation.

In this study, we established a rat liver transplantation model and found increased expression of YY1 together
with accumulation of proinflammatory cells in allogeneic transplanted livers. In vitro analysis demonstrated
that overexpression of YY1 activated bone marrow-derived mouse DCs and consequently promoted differentiation
of CD4+ T cells to Th17 and Th1 subsets, which may play a major role in acute rejection of liver
transplants.

MATERIALS AND METHODS

Animals

Specific pathogen-free 6–8-week-old Lewis rats and Brown Norway (BN) rats (female; , 250–300 g, Laboratory
Animal Center of Fujian Medical University, Fujian, China) were used in the study. They were bred and
maintained under specific pathogen-free conditions at a constant temperature (20 ± 2 °C) with 50% ±
10% humidity. All experiments were conducted in compliance with the Ethics Committee of Fujian Medical
University (No. 2015-29) under the NIH Guidelines for the Care and Use of Laboratory Animals.

Orthotopic Liver Transplantation

Orthotopic liver transplantation was performed by the improved two-cuff method as described previously
(18, 19). In the allogeneic group, livers from 14 Lewis rats were orthotopically into corresponding BN rats
with a similar weight and age. In the syngeneic group, 14 pairs of Lewis rats were used as both donors and
recipients. As a control, 14 Lewis rats only underwent opening and closure of the abdomen under anesthesia
for 35 min to simulate the procedure and time of liver transplantation.
Hematoxylin and Eosin (H&E) staining and Immunohistochemistry

Grafted liver tissues were fixed in paraformaldehyde, embedded in paraffin, and serially sectioned at 4 μm thicknesses. Representative H&E staining was used to evaluate the degree of acute rejection. For immunohistochemistry, after deparaffinization, antigen retrieval was performed by heating in sodium citrate-EDTA antigen repair solution under high pressure. Endogenous peroxidase was blocked by 3% H₂O₂. Sections were probed with rabbit monoclonal anti-CD3 antibody (1:500; ab86883, Abcam, USA) or monoclonal anti-CD86 antibody (1:200; ab239075, Abcam) overnight at 4 °C. Subsequently, the sections were stained using a Streptavidin/Peroxidase Histostain Plus Kit (ZSGQ-BIO, Beijing, China) in accordance with the manufacturer’s instructions. Positive cells per field were analyzed and calculated by Image pro plus software 6.0 (USA).

Measurement of serum alanine aminotransferase and aspartate aminotransferase

At 5 and 10 days after transplantation, blood samples were collected from the caudal vein of recipient rats under ether anesthesia and centrifuged (5000 g for 15 min) to separate serum. The serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with commercial kits (C010-2, C009-2, and E003-2, Nanjing Jiancheng, Nanjing, China) in accordance with the manufacturer’s instructions.

Western blot analysis

Grafted liver tissues were collected, followed by total protein extraction using RIPA Lysis and Extraction Buffer (Thermo, USA). After quantification by a BCA kit (23250, Thermo), 60 μg of proteins was separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. The membrane was blocked with 5% milk and then incubated with primary antibodies against YY1 (1:1000) or β-actin (1:2000) overnight at 4 °C. Subsequently, the membrane was washed with PBS and incubated with peroxidase-conjugated secondary antibodies. Signals were visualized by enhanced chemiluminescence (Pierce, Rockford, IL, USA) in accordance with the manufacturer’s instructions. The band signal was semi-quantified with ImageJ (USA) to evaluate target protein expression.

RNA extraction and Quantitative real-time PCR

Grafted liver tissues were ground into a tissue homogenate and then total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA). A PrimeScript 1st Strand cDNA Synthesis Kit (Takara, Japan) was used to generate first-strand cDNA. Subsequently, real-time PCR (RT-PCR) was carried out using TransStart Top Green qPCR SuperMix (TransGen, Beijing, China). The primers were as follows: YY1 gene, 5’-GAGTCCACGTCTGTGCAGAAT-3’ (forward) and 5’-CGTCGAAGGGGCACATAG-3’ (reverse); 18S (internal control), 5’-AGAAACGGCTACCACATCC-3’ (forward) and 5’-CACCAGACTTGCCCTCCA-3’ (reverse). The cycle threshold (Ct) was recorded and relative expression of the YY1 gene was analyzed by the 2^-Ct method after being normalization to GAPDH.

Tandem mass tag-based proteomic analysis

Frozen grafted liver tissues were lysed with RIPA lysis buffer (Beyotime Biotechnology, Beijing, China), ground in a tissue homogenizer, and then sonicated by a high-intensity ultrasonic processor. After centrifugation, the supernatant was collected and transferred into a Pierce Top 12 Abundant Protein Depletion Spin Column (Thermo Fisher Scientific, Waltham, MA) to remove proteins with high abundance ratios. Subsequently, a BCA Protein Assay Kit was used to quantify the protein concentration. Then, 200 μg protein from each sample was alkylated by successive incubation with 5 mM dithiothreitol and 11 mM iodoacetamide. The supernatant was washed twice with 8 M urea (pH 8.5), followed by centrifugation at 12,000 g for 20 min, and then replaced with TEAB solution for proteolytic digestion. After digestion by trypsin, 100 μg of proteins was labeled with a TMT10-plex Isobaric Label Reagent Set (Thermo Fisher Scientific) in accordance with the manufacturer’s instructions. The labeled peptides were fractionated by high pH reversed-phase high-performance liquid chromatography (HPLC) (Shimadzu, Tokyo, Japan) and then subjected to nano-electrospray ionization, followed by tandem mass spectrometry in a Q Exactive mass spectrometer (Thermo Fisher Scientific) coupled to the high-performance liquid chromatography. Raw data were processed using Proteome Discoverer 2.2 (Thermo Fisher Scientific). Proteins with fold changes of >1.20 or <0.83 and an
adjusted significance level P-value of <0.05 between two comparable groups were considered differentially expressed proteins.

**Functional enrichment analysis**

To determine whether YY1 affected cell functions, we identified a gene set that was highly correlated with YY1 by Parson correlation analysis. Proteins with correlation coefficients of >0.5 or < -0.5 and an adjusted significance level P-value of <0.05 between two comparable groups were considered highly correlated genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis was performed on the highly correlated genes by ClusterProfiler, which visualized functional profiles of genes and gene clusters.

**Separation and cultivation of rat bone marrow-derived dendritic cells**

Bone marrow-derived dendritic cells (BMDCs) were generated from Lewis rats. Briefly, bone marrow-derived dendritic cells were isolated by flushing hind limbs with PBS and then filtering the cells through a 40 μm cell strainer. The harvested cells were resuspended and cultured in RPMI-1640 medium containing 10% (v/v) FBS (Life Technologies), 10 mM glutamine, and penicillin/streptomycin. After culture at 37 °C with 5% CO2 for 3 h, non-adherent cells were removed and the remaining adherent cells were harvested and cultured in RPMI-1640 medium with 20 ng/ml recombinant GM-CSF and 10 ng/ml IL-4 (PeproTech, USA) for 7 days before use. During cultivation, the culture medium was replaced on days 3 and 5.

**Establishment of YY1-overexpressing imDCs and surface marker analysis**

The YY1 gene was amplified by PCR to confirm the sequence using the following primers: 5'-AGGTCGACTCTAGACGATCCGCCACCATGGCCTCGGGCGACACCCTC-3' (forward) and 5'-TCCTTGTAGTCCATACCCCTGTTGTTTTTGCGTTGACGTG-3' (reverse). The PCR product was separated by agarose gel electrophoresis and DNA with a band size of 1277 bp (YY1) was purified using a Gel Extraction Kit (NA1111, Sigma, St. Louis, USA). After digestion with BanH1 and AgeI, the vector named GV492 (Genechem, Shanghai) was incubated with the PCR product in the presence of T4 DNA Ligase (2011A, Takara). Subsequently, the inserted YY1 gene in the linked plasmid was sequenced and then transfected into 293T cells to produce lentiviruses. Cultured DCs were infected with the GV492-YY1 virus in the presence of 0.8 μg mL-1 polybrene (Sigma). DCs infected with the GV492 virus, and TNF-α- or PBS-pulsed DCs were used as the control. At 2 days post-transduction, DCs were resuspended, washed, and stained with a FITC-conjugated monoclonal antibody against CD86, PE-conjugated monoclonal antibody against MHC II. Expression of surface markers was measured with by fluorescence-activated cell sorting (FACS; BD Biosciences, USA).

**Cytokine profile of the T cell response stimulated by YY1-overexpressing DCs in vivo**

To assess naïve T cell polarization induced by YY1-overexpressing DCs, DCs transduced with GV492-YY1, DCs transduced with the GV492 virus, and TNF-α- or PBS-pulsed DCs were cultured for 72 h. Naïve CD4+ T cells were obtained from the spleens of healthy Lewis rats by MACS using a mouse CD4+ T cell isolation kit (130-100-008, Miltenyi Biotechnology, Germany). In total, 5 × 10^4 DCs were coincubated with 5 × 10^5 naïve CD4+ T cells in each well of a round-bottom 96-well plate for 8 h at 37 °C in the presence of 0.7 ml/ml GolgiStop (BD Biosciences), 50 ng/ml phorbol 12-myristate 13-acetate (Sigma), and 750 ng/ml ionomycin (Sigma). Surface staining was performed with anti-CD4-FITC on ice for 30 min. Subsequently, a Fixation/Permeabilization solution (Cytofix/Cytoperm kit, BD, Germany) was used to suspend cells, and intracellular cytokine staining was performed using anti-IFN-γ-APC and anti-IL-17-PE antibodies. The cell suspension was analyzed by flow cytometry (BD Biosciences).

**Statistical Analysis**

Continuous variables are presented as means ± standard deviation (SD). Statistical significance was calculated by one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test using GraphPad Prism version 6.0 software (GraphPad Software, San Diego, CA, USA). p< 0.05 was considered statistically significant.
RESULTS

Establishment and evaluation of a rat liver transplantation model

After the liver of Lewis rats was orthotopically transplanted into BN or Lewis rats, grafted liver tissues and serum samples from recipient rats were extracted for H&E staining and serum aminotransferase determination, respectively. As shown in Figure 1A, grafted liver tissues from the allogeneic group displayed typical features of acute cellular rejection, including severe portal inflammation, endotheliitis, destruction of bile ducts, and mixed proinflammatory cell infiltration. The allograft group showed severe acute rejection, whereas the control group showed no endotheliitis, bile duct injury, or mixed proinflammatory cell infiltration. Serum ALT and AST levels in the allogeneic group were significantly higher than in the other two groups at 5 and 10 days after transplantation (all $P < 0.05$) (Figure 1B).

Inflammatory cell infiltration and cytokines in recipient rats

To classify the main type of proinflammatory cells and cytokines, immunohistochemistry and ELISAs were performed. Immunohistochemistry showed that proinflammatory cells in the allogeneic group were mainly CD3+ and CD86+ cells, and infiltration of CD3+ T cells and CD86+ cells was not obvious in syngeneic or sham groups, indicating that T and dendritic cells were the major components (Figure 1A). Consistent with the time course of the number of proinflammatory cells, IL-16 and IFN-γ levels in the allogeneic group significantly increased at 10 d after transplantation (Figure 2B).

Expression of YY1 protein and mRNA in Liver Tissues of Recipient Rats

To measure YY1 protein and mRNA expression in recipient rats, grafted liver tissues and serum samples were collected from recipient rats at 5 and 10 days after transplantation. Immunohistochemical analysis showed that YY1 was expressed in the nucleus of infiltrated proinflammatory cells around the liver portal area (Figure 3A). Furthermore, YY1+ cells were obviously increased in the allogeneic group compared with the sham and syngeneic groups. Western blot analysis showed that YY1 expression increased gradually over time in the allogeneic group (Figure 3B), which was similar to YY1 mRNA expression (Figure 3C). TMT-based proteomic analysis showed that YY1 protein expression in the allograft group was higher than that in the syngeneic group, which further indicated the major role of YY1 in allograft rejection (Figure 3D). KEGG pathway analysis identified 10 significantly enriched pathway, such as endocytosis and leukocyte transendothelial migration. These two pathways are involved in the process of exogenous antigen uptake in dendritic cells and the inflammatory response induced by leukocytes (Figure 3E).

Overexpression of YY1 triggers maturation of DCs

Immunohistochemical staining and proteomics analysis showed that YY1 was upregulated in DCs and infiltrating immune cells, and the gene set that was highly correlated with YY1 was enriched in the process of exogenous antigen uptake in dendritic cells and the inflammatory response induced by leukocytes. To explore the effect of YY1 overexpression on maturation of DCs, naïve DCs were isolated and cultured in vitro, followed by transduction with a lentivirus carrying GV49-hYY1 or an empty control vector. The YY1 gene was cloned into the GV492 vector and then expressed in DCs (Figure 4A). The features of DCs in various groups analyzed by microscopy are displayed in Figure 4B. Irregular protrusions and villi-like structures were observed on the surface of DCs, especially in YY1-overexpressing and TNF-α-pulsed DCs. FACs showed higher expression of CD80, CD86, and MHC II both in YY1-overexpressing and TNF-α-pulsed DCs, indicating that YY1 triggered maturation of DCs (Figure 4B).

YY1-overexpressing DCs induce naïve CD4+ T Cells towards a proinflammatory phenotype

YY1 overexpression promoted DC maturation, but whether they were functional was unknown. Therefore, flow cytometry was used to assess whether YY1-overexpressing DCs induced naïve CD4+ T Cells toward a proinflammatory phenotype. Stimulated DCs from bone marrow of BN rats were used as stimulating cells and mixed with CD4+ T cells from the spleen of Lewis rats. Flow cytometry was used to measure the secretion of IL-17 and IFN-γ. DCs were cocultivated with naïve CD4+ T cells from Lewis rats for 72 h in
the presence of Con-A. The cytokine profiles showed that T cells incubated with YY1-overexpressing DCs showed high levels of inflammatory cytokine IL-17 and IFN-γ, which was consistent with TNF-α-pulsed DC cells (Figure 5A, B). These results showed that YY1-overexpressing DCs promote differentiation of CD4+ T cells to Th1/Th17.

DISCUSSION

YY1 is involved in various diseases by serving as both an activator or repressor of the expression of downstream genes depending on the chromatin context. This study revealed the potential role of YY1 in acute rejection after liver transplantation. An MHC class II (MHC II)-mismatched rat liver transplantation model was established and aberrant expression of YY1 was observed in proinflammatory cells in liver grafts of the allogeneic group. Furthermore, in vitro analysis revealed that YY1 overexpression induced DC maturation, which subsequently triggered the differentiation of naïve T cells toward Th2 and Th17 subsets. Experimental rats include many kinds of inbred and transgenic strains with a definite genetic background, and therefore the rat heterotopic liver transplantation model has been widely chosen to research transplant rejection mechanisms. In this study, acute liver rejection in the rat heterotopic liver transplantation model was reflected by severe portal inflammation, endotheliitis, destruction of bile ducts, mixed proinflammatory cell infiltration, and elevated serum levels of transaminase (ALT/AST) and proinflammatory cytokines. Acute liver rejection mainly occurs within the first month, particularly 5–7 days after liver transplantation (20). Therefore, we observed pathological changes and time-dependent expression of YY1 at 5 and 10 days after transplantation. Except for hepatocytes and bile duct injuries, dense inflammatory cell infiltration was observed around the central vein of the recipient liver in the allogeneic group compared with the syngeneic group. After semiquantitative analysis, we found that the inflammatory cells mainly consisted of CD86+ DCs and CD3+ T cells, which emphasized their major roles in acute liver rejection. Additionally, YY1 expression was elevated in the nucleus of the infiltrated inflammatory cells and the variation in expression was in consistent with the immune cell infiltration among the three groups. Considering that activated donor-derived DCs participated in function of T cells in acute liver rejection, these results revealed the potential role of YY1 in the relationship between abnormal activation of immune cells and acute liver rejection.

Yin Yang 1, which is a widely expressed zinc finger DNA-binding transcription factor, interacts with various kinds of transcription factors and genes and control their transacting ability or expression level in a pleiotropic manner (21). In inflammation-related diseases, such as rheumatoid arthritis (16) and ulcerative colitis (22), YY1 tends to promote the pathophysiological process by interacting with associated regulators. For example, YY1 plays a major role in the pathological mechanisms of RA by controlling the pathology of Th17 cells through binding to the promoter region of transcription factor T-bet and interacting with T-bet protein (16). Regulatory T cells are vital to maintain immune homeostasis, whose differentiation and function are regulated by an important transcription factor named Foxp3 (23). In a dextran sulfate-induced colitis model, YY1 increased regulatory T cells, reduced Foxp3 expression, and inhibit their suppressive function in immunity reaction (22). In this study, immunohistochemical staining and proteomics analysis showed that YY1 was upregulated in DCs and infiltrating immune cells, and the gene set that was highly correlated with YY1 was enriched in the processes of endocytosis and leukocyte transendothelial migration. These data indicate the potential role of YY1 in allograft rejection because these two pathways are involved in exogenous antigen uptake by dendritic cells and the inflammatory response induced by leukocytes, including T cells. As an important part of adaptive and innate immune systems, activated DCs and T cells are also involved in inflammation-related diseases, including acute allograft rejection (24). Because YY1 expression was elevated in the nucleus of infiltrating inflammatory cells, including DCs and T cells, we hypothesized that YY1 might trigger activation of DCs and participate in the pathogenesis of acute allograft rejection of liver transplants by polarizing naïve T cells to the inflammatory phenotype. As expected, YY1-overexpressing DCs showed more irregular protrusions and villi-like structures on their surface and had higher expression of CD80, CD86, and MHC II compared with the control groups, indicating that YY1 promoted the maturation of DCs. After incubation with naïve T cells, YY1-overexpressing DCs induced naïve CD4+ T cells to produce high level of intracellular cytokines IL-17 and TNF-γ). Th17 cells are proinflammatory and induce autoimmunity,
which inhibit the function of regulatory T cells. The flexibility between induced regulatory T cells and Th17 cells may affect the differentiation of CD4\(^+\) T cells, and therefore may alter the direction of the immune response (25, 26). Thus, our data support the theory that YY1 triggers activation of DCs and then promotes the production of Th17 cells, which subsequently or simultaneously polarize naïve T cells to the inflammatory phenotype. Unfortunately, the molecular mechanism governing the effects of YY1 on DCs was not investigated in this study. Furthermore, the upstream regulators that control YY1 expression remain unknown.

In conclusion, our results revealed the expression features of YY1 during acute rejection of liver transplants. Overexpression of YY1 activates rat bone marrow-derived DCs to a mature status characterized by expressing CD40, CD86, and MHC II on their surface. The mature DCs induced by YY1 overexpression polarized naïve CD4\(^+\) T cells to a Th1 or Th17 phenotype and cause immune injury, indicating that YY1 might be a therapeutic target to prevent liver allograft rejection.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

**ETHICS STATEMENT**

The animal experiment was reviewed and approved by the Institutional Animal Care and Use Committee of Fujian Medical University.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

L.C. and W.H. conceived and designed the experiments. Y.C., B.W., and L.H. performed the experiments. Y.C., B.W., and H.L. analyzed the data. Y.C., L.C., and W.H. wrote the manuscript. All authors reviewed the manuscript.

**FUNDINGS**

This study was supported by Natural Science Foundation of Fujian Province (Fujian Provincial Natural Science Foundation): (Grant number: 2020J01605, 2020J011076); Joint Funds for the innovation of science and Technology by Fujian Province (Grant number: 2021Y9003); Fuzhou health technology project (Grant number: 2021-S-wq27); Science and Technology Planning Project of Fuzhou(2022-S-011).

**REFERENCES**


Figure Legends

Figure 1. Establishment and evaluation of a rat liver transplantation model. Donated livers from Lewis rats were transplanted into BN and Lewis rats in the allogeneic and sham groups, respectively. The opening and closure of the abdomen under anesthesia was performed in Lewis rats in the sham group. A. Histopathology analysis of orthotopic livers from recipient rats. (magnification, ×200). The grafted liver tissues was obtained and stained with H&E. Compared with the allogeneic and sham groups, liver tissues in the allogeneic showed most severe rejection reflected by severe portal inflammation, endotheliitis, destruction of bile ducts, and mixed inflammatory cells infiltration. B. Concentration of ALT and AST in the serum of recipient rats. In 5 and 10 days after transplantation, serum samples were obtained from the recipient rats in the three groups in order to detect the concentration of ALT and AST. Recipient rats have the highest serum concentration of ALT and AST among the three groups. N=5 in each group. All experiments were performed three times and data are presented as the mean ± SD. *p < 0.05, **p < 0.01; ns, not significant.

Figure 2. Inflammatory cells infiltration and cytokines in recipient rats. Inflammatory cell infiltration in grafted liver tissues and representative inflammatory cytokines in serum samples were analyzed with immunohistochemistry and ELISA at 5 and 10 days after surgery. A. CD 3 and CD 86 positive inflammatory cells in liver grafts (magnification, ×200). The remarkable numbers of CD 3 and CD 86 positive inflammatory cells could be observed in the allogeneic groups, which was also proved by statistical chart below. B. Serum concentrations of IL-6 and TNF-α in recipient rats. Serum AST and ALT levels in the allogeneic group were highest among the three groups at 5 and 10 day after surgery. N=5 in each group. All experiments were performed three times and data are presented as the mean ± SD. *p < 0.05, **p < 0.01; ns, not significant.

Figure 3. The localization and expression pattern of YY1 protein in liver grafts. The extracted tissues were analyzed with immunohistochemistry, western blot and qRT-PCR in order to evaluate the YY1mRNA and protein levels. A. YY1 protein measured by immunohistochemistry (magnification, ×400). YY1 mainly appeared in the nucleus of the infiltrating immune cells in the allograft group, which was stained with brown. B,C. Expression of YY1 protein and mRNA in grafts. YY1 protein and mRNA expression in the allogeneic group was increased with time and reaches the highest among the three groups at 10 d after surgery. D. YY1 protein expression level between the allograft and syngraft group identified by proteomics. Proteomics analysis showed that YY1 protein expression of allograft group was higher than that of the syngraft group. E. KEGG pathway enrichment analyses. KEGG pathway analysis identified 10 significantly enriched pathways, including endocytosis and leukocyte transendothelial migration. N=5 in each group. All experiments were performed three times and data are presented as the mean ± SD. *p < 0.05, **p < 0.01; ns, not significant.

Figure 4. Features and expression of surface markers on YY1 overexpressed DCs. Cultured imDCs were transfectioned with lentivirus carrying PCDH-CMV-EGFP- hYY1, or an empty control vector at a multiplicity of infection (MOI). Meanwhile, imDCs was pulsed with PBS and TNF-α as the negative and positive control. After cultured for 8 d, cells was collected for flow cytometry assay. A. Gene expressing YY1 was successfully cloned into GV492 vector and then transferred into DCs reflected by green fluorescence (magnification × 400). After transduction, YY1 was successfully expressed in DCs B. DCs under light microscopy (magnification × 400) were showed in the left panel and the YY1 overexpressed and TNF-α pulsed DCs showed irregular shapes with uneven projections formed clusters. Flow cytometry revealed higher expressions of CD80, CD86 and MHCII both in the YY1 overexpressed and TNF-α pulsed DCs. The statistical analysis of expression of surface markers on DCs was showed in the lower pannel. All experiments were performed three times and
data are presented as the mean ± SD. *p < 0.05, **p < 0.01; ns, not significant.

**Figure 5.** Cytokine secretion of YY1 overexpressed CD4+ T cells evaluated by flow cytometry. CD4+ T cells were separated form spleens in rats and also transfected with lentivirus carrying PCDH-CMV-EGFP-hYY1, or an empty control vector. PBS and TNF-α stimulated DCs were served as the negative and positive control, respectively. Representative staining and percentages of IFN-γ(A) and IL-17(B)-producing CD4+ T cells. YY1 overexpressed and TNF-α stimulated CD4+ T cells showed higher levels of intracellular cytokine IFN-γ and IL-17 compared the other two group. All experiments were performed three times and data are presented as the mean ± SD. *p < 0.05, **p < 0.01; ns, not significant.