Please provide specific point-to-point replies to each reviewer's comments.

Replies to reviewer#1's comments.

Reviewer #1 comments:

1、This study by Jianwen Jiang demonstrates alteration in the gut microbiota with higher doses of immunosuppressant. Endotoxemia was also noted with increasing pathogenic bacteria. These findings were nicely correlated with histological changes in the liver. So far the author deserves credit and if they can come up with a simple test which can correlate the changes in microbiotra to the optimal immunosuppressant dose, it will be of great use.

Reply to this comment:

Thanks to this reviewer for his or her very good suggestions. We find that compared to the FK506-H and FK506-L groups, FK506-M was optimal for maintaining immunosuppression and inducing normal graft function, the integrity of gut barrier, low plasma endotoxin levels and stable gut microbiota after liver transplantation in rat.

Moreover, the gut microbial changes with different FK506 dosage are related, compared to the FK506-H and FK506-L groups, Phylogenetic tree analysis identified crucial bacteria associated with the FK506-M, among them, seven of the 9 bacteria that were decreased corresponded to Bacteroidetes, while increased bacteria were of the Bifidobacterium species; the FK506-M increased Faecalibacterium prausnitzii and Bifidobacterium spp. and decreased Bacteroides-Prevotella and Enterobacteriaceae as assessed with quantitative PCR, which confirmed the crucial bacterial alterations identified through DGGE.

However, our experimental method to examination and analysis of gut microbiota is not simple to master easily, if we can find the key microbes changes by further investigation, it is possible to establish a quantative relationship such as ratio of specific strains of probiotics and harmful bacteria to predict the degree of gut microbiota dysbiosis caused by different dosage immuosupressior such as

FK506, it will be more valuable to help doctor to judge the degree of gut microbiota dysbiosis caused by the over-dose immuosupressior such as FK506, so, we need further researches to establish a simple test which can correlate the changes in microbiotra to the diferent immunosuppressant dose.
Thanks to this reviewer for his or her best suggestion, which will help us to do further research.

2、However extrapolating these well documented changes in the microbiota landscape to rejection is another story. One would expect the observed changes in microbiota with administration of immunosuppressant. This may further lead to up regulation of cytokine production and changes in the liver histology. Whether this changes are causing rejection is not studied in the present work. The authors postulate that various cytokines and LPS may be involved in the pathogenesis of rejection, this was not studied or documented.

Reply to this comment:

Thanks to this reviewer for his or her very good suggestions.We should add the information about what we have previously done the research on gut microbiota and liver acute rejection(AR) after orthotopic liver transplantation (OLT) in rat, the results of the article were published in Transplantation with title of "Intestinal microbial variation may predict early acute rejection after liver transplantation in rats" on 2014 (PMID:25321166). Please refer to the reference 15# of this article.
In our previous research,the OLT models in rats were established. Hepatic graft histology, ultrastructure, function, intestinal barrier function,serum cytokine and gut microbiota were tested. Intestinal microvilli loss and tight junction damage were noted, and intestinal barrier dysfunction during AR presented a decrease of fecal secretory immunoglobulin A (sIgA) and increase of blood bacteremia, endotoxin, and tumor necrosis factor-α(TNF-α). Real-time quantitative polymerase chain reaction(RT-PCR) results showed that genus Faecalibacterium prausnitzii and Lactobacillus were decreased, whereas Clostridium bolteae was increased during AR. Microbial diversity and species richness were decreased

during AR. Phylogenetic tree analysis showed that most of the decreased key bacteria belonged to phylum Firmicutes, whereas increased key bacteria belonged to phylum Bacteroidetes.

We have found that liver rejection reaction can lead to gut microbiota dysbiosis, up regulation of cytokine production and changes in the liver histology. We should succinctly introduce our formal results in introduction or discussion part of this article. Thanks to the remind of this reviewer.

4、*I would suggest that the authors rewrite the discussion section highlighting their findings of the changing the microbiota landscape related to immunosuppression in a dose dependant manner.*

Thank to this reviewer for his or her good suggestion, we have rewritten the discussion section to emphasize that FK506 affect the gut microbiota changes in a dose-dependent manner. We find that compared to the FK506-H and FK506-L groups, FK506-M was optimal for maintaining immunosuppression , inducing normal graft function, the integrity of gut barrier, low plasma endotoxin levels and stable gut microbiota after liver transplantation in rat. Moreover, the gut microbial changes with different FK506 dosage are related.

5、*Finally blood levels of Tacrolimus might have been helpful.*

In patients, routine monitoring of blood tacrolimus concentration (TC) was performed using the PRO-TracTM II Tacrolimus Elisa Kit (Diasorin, United States), however, there were no kit used for rat blood TC testing. According to our experimental experience in our Lab, the blood TC in rats can not be tested by the above kit used for human, it may be due to immunological reasons**.** Fortunately, according to clinical practice of LT, different dosages of FK506 are positively correlated to different blood concentrations across a specific dosage range, meanwhile, intragastric dosages of FK506 were more feasible and easier to control the blood TC in rat.

Reviewer #2: This paper studied the influence of different dosages of Tacrolimus (FK506) on gut microbiota after liver transplantation in rats. The researchers presented interesting data on the optimal dosage of FK506 that induces immunosuppression along with stability of the gut microbiota following LT. They studied the association between hepatic graft function and gut microbiota affected by immunosuppression. It is a quite interesting topic from the field of the basic research on a newly introduced factor, the gut microbiome affecting hepatic function. Given that gut microbiota has been associated with the progression of the hepatic diseases, it is worthy to find out what happens after LT with regards to immunosuppression which is crucial after LT.

Replies to reviewer#2's comments.

Thanks to this reviewer for his or her excellent appreciation for our research. Immunosuppressor must be used to prevent rejection after liver transplantation, but the use of Immunosuppressor inevitably brings about side effects, including immunosuppression, infection, and recurrence of the original disease such hepatitis B infection, abnormal metabolism, chronic graft dysfunction, if the dosage is not enough, the rejection will happen, these side effects are related to the use of immunosuppressor, however, the effect of immunosuppressor on the gut microbiota has not been reported yet.

We conducted such a research to confirm that an optimal immunosuppressor maintains good graft function and induces stable gut microbiota after liver transplantation in rat, the optimal immunosuppressor is a reasonable option in cases where immunosuppressor such as FK506 must be used. The effect of immunosuppressor on the structure and function of gut microbiota of liver graft recipients requires further in-depth study.

With the improvement of metagenomics and metabolomics techniques, the integrative study can further reveal that how gut microbiota participates in the side effects of immunosuppressor on recipients, and gut microbiota may be a target to reduce side effects of immunosuppressor. In addition, in order to further study the mechanism of the involvement of gut microbiota on the side effects of immunosuppressor in recipients, it is essential and very meaningful to do clinical

research.

Thanks to this reviewer for his or her high appreciation for our research, which will inspire us to do in-depth research.