Reviewer's report

Title: In Vivo Experimental intervertebral disc Degeneration Induced by Bleomycin in the Rhesus Monkey

Version: 2 Date: 14 July 2014

Reviewer: Juquan J.S. Song

Reviewer's report:

The current study is to establish a mild intervertebral disc degeneration animal model in rhesus monkey with bleomycin injection. Authors observed that a time course of radiology and MRI image changes of IVD in rhesus monkey from 1 to 15 months after bleomycin locally injection. Compared to histological morphology and genomic changes at 15 months, they confirmed that bleomycin caused IVD degeneration in rhesus monkeys.

The animal model is very interested and provides a new tool for IVDD research. There is a concern of bleomycin application. Several procedures in method also need to be clarified with typos correction in the paper. The manuscript needs to be revised carefully and polished by a native English writer.

The concerns from reviewer are listed as below and divide them into:

- Major Compulsory Revisions
  1. Authors applied Bleomycin for the current animal model. First, how did authors determine the dose of bleomycin in the study? Bleomycin is associated with DNA strand breaks, with pulmonary fibrosis and lung function impairment. Are authors aware of the side effect of bleomycin in the current model? Did authors examine pathophysiologic changes of lung tissue during the current? Bleomycin diminished cavelolin -1 expression in lung tissue, and caveolin-1 elevated in NP cells from human degenerate discs. Does bleomycin application potentially affect the mechanic pathway study for future therapeutic strategy development? Authors should address those concerns in the revised version.
  2. A recent publication from Wan ZY (Int J Clin Exp Pathol, 2014) demonstrates that TNF, IL1beta, TGF and SMAD increased in human degenerative samples. Authors might provide some data of inflammation response and TGF/smad pathway correlated with the current model.

- Minor Essential Revisions
  1. The reviewer is confused about the content of abstract which needs to be revised carefully
     a. Move the objective of study (at Line 38) into the section of Background.
     b. There were confusions about animals with reagent rejection, please clarify and correct them. In the method at line 121, injection locations are L3-4 and L5-6; at line 124, injection volume is 2.0ml.
c. At line 55, correct negative symbol “r=-0.740”

d. The conclusion makes confusion between statements of a slow progressive IVDD animal model and early stage of MRI effectiveness. Please restate the conclusion.

2. In the method section, there are several procedures need to be clarified.

a. 6 male and 4 female rhesus monkeys were enrolled in the study. Were there activity behaves and body weight different between two genders? If so, please provide the information.

b. Please clarify and revise the sentence of “and written informed consent was obtained from all subjects” in line 107.

c. Please confirm and correct the agent delivery location and volume dose.

d. Please provide the detail of animal position when taking roentgenograms procedure.

e. Please provide information of light microscopy for taking histologic images

f. Is there another way such as histologic morphology to assess the number of capillaries in the cartilage endplate at 15 months directly instead of measuring vWF gene expression?

3. In Result section,

a. Please clarify the sentence in line 112 “7 IVDs in rhesus monkeys”

b. Data presented is somewhat overlapped in figure 6 and table 3. Please reorganize data presentation form.

c. In figure 5, please mark and specify the IVD location for each treatment

d.

4. Authors claimed the current model is closely to mimic clinical patients. According to age converting to human, it stands for young adults, is it the purpose for this model? Please justify the reason of animal aging selecting in the current study?

- Discretionary Revisions

Please scale the histology figure3

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'