November 01, 2019

Le Zhang
Science Editor, Editorial Office
Baishideng Publishing Group Inc
World Journal of Stem Cells

Dear Le Zhang:

Thank you very much for reviewing our article entitled “Comparison between the Therapeutic Effects of Differentiated and Undifferentiated Wharton's Jelly Mesenchymal Stem Cells in Rats with Streptozotocin-induced Diabetes”. A point-by-point response to each of the comments of the reviewer was prepared and submitted with revised manuscript. We carefully proofread the manuscript to minimize typographical, grammatical, and bibliographical errors. We sincerely hope that you and your editorial committee would consider our paper for publication in the “World Journal of Stem Cells”.

Yours sincerely

Pei-Jiun Tsai, M.D., Ph.D.

Department of Critical Care Medicine, Veterans General Hospital, Institute of Anatomy and Cell Biology, School of Medicine, National Yang Ming University,
201 Shih-Pai Road Section 2, Taipei, Taiwan 112, R.O.C.
Tel: +(886)-2-28712121, ext: 7021;
Fax: +(886)-2-28757890;
E-mail: pjtsai0230@gmail.com; pjtsai@vghtpe.gov.tw

Responses to the Reviewer 1:

Major points

1. In Fig.2 the undifferentiated hWJ-MSCs produced neither insulin nor C-peptide, but in vivo hyperglycemia was improved in Fig.3A and C. What is the mechanism for this discrepancy? Did the undifferentiated hWJ-MSCs were differentiated to insulin-producing cells (IPCs) in pancreatic tissues?

   Response: Thanks for the Reviewer's comment.
   As shown in the Figure 5, we found cells that were only with red (human nucleus) fluorescence without green fluorescence as well as a few cells were marked with red (human nucleus) fluorescence and very weak green fluorescence in the pancreas of undifferentiated hWJ-MSCs treatment rats. These meant that the undifferentiated hWJ-MSCs transplanted via the portal vein could also home to and survive in the pancreas. And a few of them could be differentiated into IPCs in rat pancreatic tissues. Besides, the undifferentiated hWJ-MSCs could significantly reduce the insulitis which was shown in the Figure 6. The undifferentiated MSCs treatment could also dramatically diminish the inflammatory response in STZ induced diabetic rats as shown in Figure 4. Thus **we proposed that the major mechanism of hWJ-MSCs to improve the hyperglycemia status in vivo was significantly improvement of islet infiltration and restoration of immune balance in diabetic rats** while the minor the mechanism may be directly differentiated into insulin-producing cells (IPCs) in pancreatic tissues. We are continuing to study more detail about the mechanisms of hWJ-MSCs treatment.

   According to the Reviewer's comment, we have revised the description in RESULT as follow：
   In page 15, paragraph 2, line 8:
   in the pancreas. In the pancreas of undifferentiated hWJ-MSCs treatment rats, we found cells that were only with red (human nucleus) fluorescence without green fluorescence as well as a few cells were marked with red (human nucleus) fluorescence and very weak green fluorescence. These meant that the undifferentiated hWJ-MSCs transplanted via the portal vein could also travel to and survive in the pancreas. And a few of them could be differentiated into IPCs in rat pancreatic tissues (Figure 5).

2. The undifferentiated hWJ-MSCs improved the insulitis in Fig.6 better than differentiated insulin-producing cells (IPCs). The treatment with hWJ-MSCs

gave rise to the up-regulation of serum IL-4 and TGF-b in Fig.4C and D. I think both IL-4 and TGF-b are inflammatory cytokines. How do the authors explain the mechanisms of decreased inflammation in pancreas treated by hWJ-MSCs better than IPCs treatment?

Response: Thanks for the Reviewer's comment.
According to the literatures, major anti-inflammatory cytokines include interleukin (IL)-1 receptor antagonist, IL-4, IL-10, IL-11, IL-13, and transforming growth factor (TGF)-β.

- Torre D, Tambini R, Aristodemo S, Gavazzeni G, Goglio A, Cantamessa C, Pugliese A, Biondi G. Anti-inflammatory response of IL-4, IL-10 and TGF-beta in patients with systemic inflammatory response syndrome. Mediators Inflamm. 2000;9(3-4):193-5.
- Shomyseh Sanjabi, Lauren A. Zenewicz, Masahito Kamanaka, and Richard A. Flavell. Anti- and Pro-inflammatory Roles of TGF-β, IL-10, and IL-22 In Immunity and Autoimmunity. Curr Opin Pharmacol. 2009 Aug; 9(4): 447–453. PMID: 19481975

Our current result showed the serum pro-inflammatory cytokine, including IFN-γ and IL-1β, got significant decreased in the undifferentiated hWJ-MSCs treatment group than saline treatment group. The serum anti-inflammatory cytokine, including IL-4 and TGF-β, presented significant increase in both the undifferentiated hWJ-MSCs treatment group and differentiated IPCs treatment group in comparison with the saline treatment group. Both undifferentiated hWJ-MSCs and differentiated IPCs treatment could have the anti-inflammatory effect while the undifferentiated MSCs treatment could also dramatically diminish the inflammatory response in STZ induced diabetic rats. **The function of restoration of immune balance was more prominent in the undifferentiated hWJ-MSCs treatment group. We are continuing to study more detail about the mechanisms of decreased inflammation by hWJ-MSCs treatment.**

3. How many hWJ-MSCs and IPCs transplanted via portal vein were remained in the liver? The transplanted IPCs into liver might be functional to produce insulin and improve DM.

Response: Thanks for the Reviewer's comment.
Consisted with our previous study, the estimated survival rate of the undifferentiated hWJ-MSCs and differentiat IPCs eight weeks after transplantation therapy with 5×10^6 cells into the portal vein, was about 1 % in the liver.

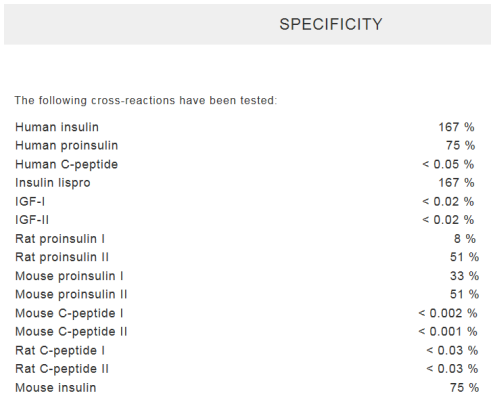
- Tsai PJ, Wang HS, Shyr YM, Weng ZC, Tai LC, Shyu JF, Chen TH. Transplantation of insulin-producing cells from umbilical cord mesenchymal stem cells for the treatment of streptozotocin-induced diabetic rats. J Biomed Sci 2012; 19: 47 [PMID: 22545626 DOI: 10.1186/1423-0127-19-47]
- Tsai PJ, Wang HS, Lin GJ, Chou SC, Chu TH, Chuan WT, Lu YJ, Weng ZC, Su CH, Hsieh PS, Sytwu HK, Lin CH, Chen TH, Shyu JF. Undifferentiated Wharton's Jelly Mesenchymal Stem Cell Transplantation Induces Insulin-Producing Cell Differentiation and Suppression of T-Cell-Mediated Autoimmunity in Nonobese Diabetic Mice. Cell Transplant 2015; 24: 1555-1570 [PMID: 25198179 DOI: 10.3727/096368914X683016]

Minor points

1. In Fig.3B, the undifferentiated hWJ-MSCs produced insulin but not C-peptide. The authors can explain the mechanisms.

   Response: Thanks for the Reviewer's comment.
   The commercial insulin ELISA kit cannot clearly distinguish between human and rat insulin. The level of serum insulin that we measured was the total insulin that was secreted by both the transplanted human cells and the original rat islets. On the other hand, the commercial C-peptide ELISA kit that we used was specific for the human C-peptide. Thus, the level of serum C-peptide that we measured represented only the C-peptide released by the transplanted human cells.
   Since the serum C-peptide level in the undifferentiated hWJ-MSCs treatment rats was little measured by our C-peptide ELISA kit, the serum insulin determined in the undifferentiated hWJ-MSCs treatment rats in Fig.3B was mainly the insulin produced by rat islets.

   The Mercodia Ultrasensitive Rat Insulin ELISA (10-1251-01) commercial kit: The cross-reactivity to human insulin is 167%.
   https://www.mercodia.se/mercodia-ultrasensitive-rat-insulin-elisa



SPECIFICITY

The following cross-reactions have been tested:

Human insulin	167 %
Human proinsulin	75 %
Human C-peptide	< 0.05 %
Insulin lispro	167 %
IGF-I	< 0.02 %
IGF-II	< 0.02 %
Rat proinsulin I	8 %
Rat proinsulin II	51 %
Mouse proinsulin I	33 %
Mouse proinsulin II	51 %
Mouse C-peptide I	< 0.002 %
Mouse C-peptide II	< 0.001 %
Rat C-peptide I	< 0.03 %
Rat C-peptide II	< 0.03 %
Mouse insulin	75 %

The Mercodia Ultrasensitive C-peptide ELISA (10-1141-01) commercial human C-peptide ELISA kit：The cross-reactivity to rat C-peptide is ≦0.07%.

https://www.mercodia.se/mercodia-ultrasensitive-c-peptite-elisa

Mercodia
Immunoassays & Services

Uppsala, October 31, 2019

Regarding Mercodia Ultrasensitive C-peptide ELISA (10-1141-01)

Dear Mercodia Customer,

The Mercodia Ultrasensitive C-peptide ELISA ELISA is an immunoassay for sensitive and specific quantitative determination of human C-peptide in urine, serum or plasma. Investigations show that the cross-reactivity to rat C-peptide is ≤0,07% in this ELISA. Levels up to 137000 pM were tested.

Sincerely,

Pelle Jadeborg
Managing Director, Quality, Mercodia AB

Address Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden
Phone +46 18 57 00 70 | **Fax** +46 18 57 00 80 | **Vat No** SE-556157510001
E-mail info@mercodia.com | **Website** www.mercodia.com

2. In Fig.3B, the undifferentiated hWJ-MSCs could be produce insulin, however, the immunnostaining could not identify insulin in the cells in Fig.5B. What is the explanation for this?

Response: Thanks for the Reviewer's comment.
Since the serum C-peptide level in the undifferentiated hWJ-MSCs treatment rats was little measured by our C-peptide ELISA kit, the serum insulin determined in the

undifferentiated hWJ-MSCs treatment rats in Fig.3B was mainly the insulin produced by rat islets.

According to the Reviewer's comment, we have revised the description in RESULT as follow：

In page 15, paragraph 2, line 8:

in the pancreas. In the pancreas of undifferentiated hWJ-MSCs treatment rats, we found cells that were only with red (human nucleus) fluorescence without green fluorescence as well as a few cells were marked with red (human nucleus) fluorescence and very weak green fluorescence. These meant that the undifferentiated hWJ-MSCs transplanted via the portal vein could also travel to and survive in the pancreas. And a few of them could be differentiated into IPCs in rat pancreatic tissues (Figure 5).

Responses to the Reviewer 2:

Major questions

1. Several times the authors discuss an absence of a cure for diabetes mellitus. The authors should either specify that they are referring to Type 1 diabetes, or remove these statements (since there is ample evidence with regards to weight loss as a treatment option for obese individuals with Type 2 diabetes).

   Response: According to the Reviewer's suggestion, we have revised the Abstract and DISCUSSION as follow：

   In page 4, line 4:

   it is difficult to cure diabetes mellitus (DM), especially type 1 DM.

   In page 16, DISCUSSION paragraph, line 6:

   exist to cure type 1 diabetes

2. There are no p values presented in the Abstract and there is a lack of p values presented in the Results Section. Authors, please include p values.

   Response: According to the Reviewer's suggestion, we have revised the RESULT as follow：

   In page 12, RESULT paragraph 2, line 7:

   Importantly, the differentiated IPCs secreted more C-peptide (High glucose versus Low glucose = 30.79 ± 2.5 versus 6.1 ± 1.0 pmol/L, p <0.001) and insulin (29.8 ± 2.8 versus 9.7 ± 1.7 mU/L, p <0.001) in response to the higher glucose levels in the environment (Figure 2).

   In page 13, line 5:

   s seven days after the transplantation (NS versus IPC = 435.6 ± 32.0 versus 250.3 ± 27.0 mg/dl, p <0.001). Although hyperglycemia diminished gradually from the second week to the eighth week (NS versus IPC = 511.6 ± 43.5 versus 349.1 ± 39.4 mg/dl, p =0.018) after IPC transplantation, the blood glucose level

was still significantly lower every week than that in the saline treatment group. In the rats from the undifferentiated hWJ-MSCs group, the decrease in blood glucose levels after transplantation was lower than in the IPC treatment group (1wk： NS versus MSC = 435.6 ± 32.0 versus 361.6 ± 30.7 mg/dl, p <0.001; MSC versus IPC = 361.6 ± 30.7 versus 250.3 ± 27.0 mg/dl, p <0.001. 8wk： NS versus MSC = 511.6 ± 43.5 versus 439.6 ± 32.8 mg/dl, p =0.026; MSC versus IPC = 439.6 ± 32.8 versus 349.1 ± 39.4 mg/dl, p =0.001), and showed a relative stability until the fifth week (Figure 3A).

In page 13, paragraph 2, line 4:

the saline treatment group (192.2 ± 25.9 versus 53.7 ± 14.2 mU/L, p <0.001), and it was stable for eight weeks (92.2 ± 18.2 versus 50.7 ± 9.3 mU/L, p <0.001) after transplantation. It was worth noting that the serum insulin level in rats from the IPC group decreased progressively between week 1 and week 8. As compared to the undifferentiated hWJ-MSCs group, the insulin level of rats in the IPC group was significantly higher only at the first week after transplantation (192.2 ± 25.9 versus 112.6 ± 15.6 mU/L, p <0.001). When the

In page 14, line 1:

was also significantly higher (1wk： 112.6 ± 15.6 versus 53.7 ± 14.2 mU/L, p =0.001) until the eighth week after transplantation (99.1 ± 14.4 versus 50.7 ± 9.3 mU/L, p <0.001), though the weekly decline in serum insulin levels was not volatile (Figure 3B). We also measured the serum C-peptide level using the ELISA kit, which could detect human C-peptide levels. The changes of serum C-peptide levels in the IPC treatment group was consistent with the changes seen for insulin (1wk： 64.5 ± 23.9 versus 26.4 ± 0.7 pmol/L, p =0.001). However, the serum C-peptide level of rats in the undifferentiated hWJ-MSCs treatment group had a smaller change than the one seen in the saline group (1wk： 28.4 ±

1.2 versus 26.4 ± 0.7 pmol/L, p =0.002), a finding that was more consistent with the results of blood glucose changes (Figure 3B).

In page 14, paragraph 2, line 13:
at the first week (IPC versus NS：p =0.002; IPC versus MSC：p =0.03), but the improvement

In page 15, line 5:

The serum IFN-γ (23.0 ± 1.6 versus 68.9 ± 5.5 pg/ml, p <0.001) and IL-1β (7.8 ± 0.8 versus 15.7 ± 1.7 pg/ml, p <0.001) level were significant decreased

In page 15, line 10:

undifferentiated hWJ-MSCs treatment group. (IL4： MSC versus NS = 322.6 ± 42.0 versus 149.2 ± 12.7 pg/ml, p <0.001. TGF-β： MSC versus NS = 97.1 ± 10.1 versus 57.2 ± 4.5 pg/ml, p <0.001)

In page 16, paragraph 2, line 4:
hWJ-MSCs treatment group (p=0.004). Only 18% of the islets from the hWJ-MSCs -treated rats showed severe insulitis compared with 43% of the saline-treated animals (p=0.003, Figure 6G).

3. The very first sentence in RESULTS is the validation for the method being used. This sentence must be within the first 2 paragraphs of MATERIALS AND METHODS.

Response: According to the Reviewer's suggestion, we have revised the MATERIALS AND NETHODS as follow：
In page 8, paragraph 2, line 2:
The differentiation protocol followed the steps described in our published article [11]. Briefly, at the fourth passage,

4. Methods, "Immunofluorescence … pancreas in rats": degree of the insulitis was

scored. Authors: either provide a reference for the scoring, or define/validate your scoring method. For example, did the authors count in a set number of islets or in a set number of sections?

Response: According to the Reviewer's suggestion, we have revised the METERIAL AND METHODS as follow：

In page 11, line 8:

Assessment of insulitis

Pancreatic tissue obtained from the rats 8 weeks after transplantation was fixed in formalin, embedded in paraffin, serial sectioned at 5 μm thickness, stained with hematoxylin and eosin, and examined for inflammation. The degree of insulitis in the pancreas was evaluated by scoring 100 pancreas serial sections/rat in a blinded fashion using the following criteria: 0, normal islet; 1, peri-insulitis (mononuclear cell infiltration < 25% of the islet); 2, intra-insulitis (mononuclear cell infiltration 25–50% of the islet); 3, severe insulitis (mononuclear cell infiltration > 50% of the islet); as previously described [12, 14]. Investigators were blind to the identity of the section.

Reference

12. Tsai PJ, Wang HS, Lin GJ, Chou SC, Chu TH, Chuan WT, Lu YJ, Weng ZC, Su CH, Hsieh PS, Sytwu HK, Lin CH, Chen TH, Shyu JF. Undifferentiated Wharton's Jelly Mesenchymal Stem Cell Transplantation Induces Insulin-Producing Cell Differentiation and Suppression of T-Cell-Mediated Autoimmunity in Nonobese Diabetic Mice. Cell Transplant 2015; 24: 1555-1570 [PMID: 25198179 DOI: 10.3727/096368914X683016]
14. Verdaguer J, Schmidt D, Amrani A, Anderson B, Averill N, Santamaria P. Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. J Exp Med 1997; 186(10): 1663-1676 [PMID: 9362527 DOI: 10.1084/jem.186.10.1663]

5. Insulin ELISA kit "detect not only human insulin but also rat insulin". Authors: either provide a reference or demonstrate validation of this statement.

Response: Thanks for the Reviewer's comment, reference and validation of this statement is as follow：

The Mercodia Ultrasensitive Rat Insulin ELISA (10-1251-01) commercial kit: The cross-reactivity to human insulin is 167%.

https://www.mercodia.se/mercodia-ultrasensitive-rat-insulin-elisa

SPECIFICITY

The following cross-reactions have been tested:

Human insulin	167 %
Human proinsulin	75 %
Human C-peptide	< 0.05 %
Insulin lispro	167 %
IGF-I	< 0.02 %
IGF-II	< 0.02 %
Rat proinsulin I	8 %
Rat proinsulin II	51 %
Mouse proinsulin I	33 %
Mouse proinsulin II	51 %
Mouse C-peptide I	< 0.002 %
Mouse C-peptide II	< 0.001 %
Rat C-peptide I	< 0.03 %
Rat C-peptide II	< 0.03 %
Mouse insulin	75 %

6. Discussion; Line 10: “hyperglycemic state in diabetic rats”. Authors: please provide a reference for this statement.

Response: According to the Reviewer’s suggestion, we have revised the discussion as follow：

In page 17, line 3:

we found that insulin-secreting cells that are induced by human Wharton's jelly mesenchymal stem cells can treat the hyperglycemic state in diabetic rats [8].

7. Page 16, line 2: “Other scholars believe”. I believe the authors mean “Other investigators believe”.

Response: According to the Reviewer’s suggestion, we have revised the discussion as follow：

In page 17, paragraph 2, line 4:

Other investigators believe that

Page 16, Line 6: “could indeed home to the pancreas of”; I do not know what the word “home” means in this sentence.

Response: According to the Reviewer’s suggestion, we have revised the discussion as follow：

In page 17, paragraph 2, line 8:

undifferentiated hWJ-MSCs could indeed exist in the pancreas of STZ-induced

Page 16, Line 7: “diabetic rats and repair the insulitis”. I believe that the authors need a different work for “repair” such as “reduce” or “suppress”.

Response: According to the Reviewer's suggestion, we have revised the discussion as follow：

In page 17, paragraph 2 , line 9:

diabetic rats and reduce the insulitis.

Page 16, lines 12-13: "the effects declined gradually afterwards". Authors: was this change caused by inflammatory damage?

Response: Thanks for the Reviewer's comment.

We now do not have strong evidence to conclude about why the treatment effects of the IPCs declined gradually afterwards. Inflammatory damage may be the possible cause of the change. We are now continuing to study more detail about it.

Page 16, last sentence: "C-peptide ELISA kit … was specific for the (can omit the) human C-peptide". Authors: please either include a reference for this statement or results of validation experiments should be included in the RESULTS.

Response: According to the Reviewer's suggestion, we have revised the DISCUSSION as follow：

In page 18, line 10:

C-peptide ELISA kit that we used was specific for human C-peptide.

The reference and validation of this statement is as follow：

The Mercodia Ultrasensitive C-peptide ELISA (10-1141-01) commercial human C-peptide ELISA kit： The cross-reactivity to rat C-peptide is ≦0.07%.

https://www.mercodia.se/mercodia-ultrasensitive-c-peptite-elisa

Mercodia
Immunoassays & Services

Uppsala, October 31, 2019

Regarding Mercodia Ultrasensitive C-peptide ELISA (10-1141-01)

Dear Mercodia Customer,

The Mercodia Ultrasensitive C-peptide ELISA ELISA is an immunoassay for sensitive and specific quantitative determination of human C-peptide in urine, serum or plasma. Investigations show that the cross-reactivity to rat C-peptide is ≤0,07% in this ELISA. Levels up to 137000 pM were tested.

Sincerely,

Pelle Jadeborg
Managing Director, Quality, Mercodia AB

Address Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden
Phone +46 18 57 00 70 | **Fax** +46 18 57 00 80 | **Vat No** SE-556157510001
E-mail info@mercodia.com | **Website** www.mercodia.com

8. Page 18, paragraph 2: “the insulin produced by the rat pancreatic B cells had a higher resistance”. I do not know what is meant by a “higher resistance”. Page 18, paragraph 3: “studies are needed to improve the survival of cells”. Authors: do you mean “studies are needed to develop methods to improve the survival of cells”?

Response: According to the Reviewer’s suggestion, we have revised the DISCUSSION as follow：

In page 19, paragraph 3, line 8:

the insulin produced by the rat pancreatic β cells failed to achieve a substantial hypoglycemic effect.

In page 19, paragraph 4, line 2:

more *in vivo* studies are needed to develop methods to improve the survival of cells and to effectively extend the period of euglycemia after treatment.

9. Figure 6: “severe insulitis” should either be defined in the text in MATERIALS AND METHODS, or a reference should be provided. Since there is no definition, it is less likely that another research group could replicate the authors’ research study. Figure 6 Legend: p values are provided, but the method of quantitation used to obtain the percentages being tested is not well defined; it is not clear what statistical testing was utilized to obtain these p values.

   Response: According to the Reviewer’s suggestion, we have revised the METERIAL AND METHODS as follow：

   In page 11, line 8:

   Assessment of insulitis

   Pancreatic tissue obtained from the rats 8 weeks after transplantation was fixed in formalin, embedded in paraffin, serial sectioned at 5 μm thickness, stained with hematoxylin and eosin, and examined for inflammation. The degree of insulitis in the pancreas was evaluated by scoring 100 pancreas serial sections/rat in a blinded fashion using the following criteria: 0, normal islet; 1, peri-insulitis (mononuclear cell infiltration < 25% of the islet); 2, intra-insulitis (mononuclear cell infiltration 25–50% of the islet); 3, severe insulitis (mononuclear cell infiltration > 50% of the islet); as previously described [12, 14]. Investigators were blind to the identity of the section.

   Reference

   12. Tsai PJ, Wang HS, Lin GJ, Chou SC, Chu TH, Chuan WT, Lu YJ, Weng ZC, Su CH, Hsieh PS, Sytwu HK, Lin CH, Chen TH, Shyu JF. Undifferentiated Wharton's Jelly Mesenchymal Stem Cell Transplantation Induces Insulin-Producing Cell Differentiation and Suppression of T-Cell-Mediated Autoimmunity in Nonobese Diabetic Mice. Cell Transplant 2015; 24: 1555-1570 [PMID: 25198179 DOI: 10.3727/096368914X683016]
   14. Verdaguer J, Schmidt D, Amrani A, Anderson B, Averill N, Santamaria P. Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. J Exp Med 1997; 186(10): 1663-1676 [PMID: 9362527 DOI: 10.1084/jem.186.10.1663]

Minor Issues

1. The authors use the phrase “got significantly” when I believe that they mean “are significantly”.

   Response: According to the Reviewer’s suggestion, we have revised the RESULT and DISCUSSION as follow：
   In page 15, line 6:
   level were significant decreased in the

   In page 19, paragraph 2, line 3:
   pro-inflammatory cytokine, including IFN-γ and IL-1β, were significant decreased in

2. Figure 2 Legend: Authors: please remove the “coulds”.

   Response: According to the Reviewer’s suggestion, we have revised the Figure 2 legend as follow
   In page 26, line 5:
   Differentiated IPCs secrete significant amounts of C-peptide and insulin, whereas the undifferentiated hWJ-MSCs secrete lower amounts.

Responses to the Reviewer 3:

1. Very few data is provided for characteristics, recognition and function of hWJ-MSCs

   Response: According to the Reviewer's suggestion, we have revised the INTRODUCTION as follow:
   In page 7, line 6:
   cause teratomas *in vivo* [5,6]. Our research team had well studied the characteristics, recognition and function of hWJ-MSCs and published article at Stem Cells in 2004 [5]. In addition to their ability to improve cardiac function in animal models of acute myocardial infarction [7], treat carbon tetrachloride induced liver failure rat [8], reverse pulmonary fibrosis in bleomycin-induced pulmonary fibrosis rat [9] and ameliorate mouse spinocerebellar ataxia type 1 [10], our research team found that hWJ-MSCs can also restore

   Reference

   8. Kao SY, Shyu JF, Wang HS, Hsiao CY, Su CH, Chen TH, Weng ZC, Tsai PJ. Transplantation of Hepatocyte-like Cells Derived from Umbilical Cord Stromal Mesenchymal Stem Cells to Treat Acute Liver Failure Rat. J Biomedical Sci 2016; 4: 1 [DOI: 10.4172/2254-609X.10002]
   9. Chu KA, Wang SY, Yeh CC, Fu TW, Fu YY, Ko TL, Chiu MM, Chen TH, Tsai PJ, Fu YS. Reversal of bleomycin-induced rat pulmonary fibrosis by a xenograft of human umbilical mesenchymal stem cells from Wharton's jelly. Theranostics 2019; 9(22): 6646-6664 [PMID: 31588241 DOI: 10.7150/thno.33741. eCollection 2019]
   10. Tsai PJ, Yeh CC, Huang WJ, Min MY, Huang TH, Ko TL, Huang PY, Chen TH, Hsu SPC, Soong BW, Fu YS. Xenografting of human umbilical mesenchymal stem cells from Wharton's jelly ameliorates mouse spinocerebellar ataxia type 1. Transl Neurodegener 2019; 8: 29 [PMID: 31508229 DOI: 10.1186/s40035-019-0166-8. eCollection 2019]

2. Cite your experiment especially culture and separation of stem cells to a known and adjusted method

   Response: According to the Reviewer's suggestion, we have revised the MATERIALS AND METHODS As follow:
   In page 7, MATERIALS AND METHODS paragraph, line 3:

the Institutional Review Board. The isolation of hWJ-MSCs was carried out as described by Wang et al [5]. Briefly, with the written informed

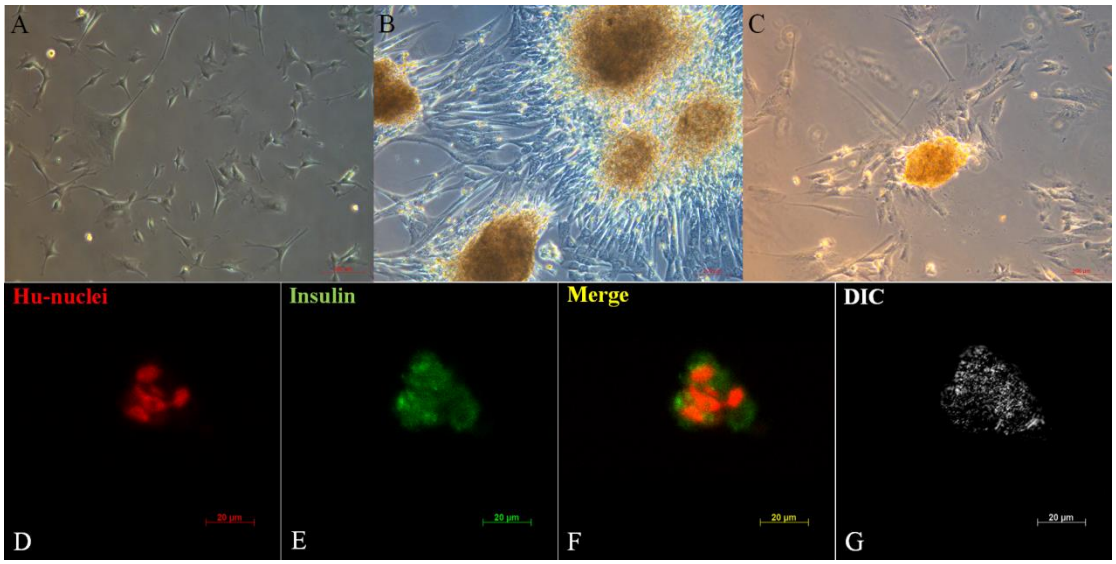
In page 8, line 11:
The differentiation protocol followed the steps described in our published article [8]. Briefly, at the fourth passage,

3. Adjust your dose of injected stem cells into the rat (dose is different from other studies)

Response: Thanks for the Reviewer's comment. We are now continuing to study the treatment effects of different dose of injected stem cells.

4. Quality of figure 1 D-G that demonstrating immunofluorescence staining with anti-insulin antibodies is bad, I think it is not working.

Response: According to the Reviewer's suggestion, we have revised the Figure 1 D-G as follow：



5. Many spelling and grammar mistakes are found that necessitates strong deal with language

Response: Thanks for the Reviewer's suggestion, we have revised and carefully proofread the manuscript to minimize typographical, grammatical, and bibliographical errors.