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Title: Stem Cell Quiescence and Its Clinical Relevance

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Dear Editors and Reviewers,

Enclosed please find our revised manuscript, entitled "Stem Cell Quiescence and Its Clinical Relevance" by Luo et al. We appreciate reviewers' comments and have revised the manuscript according to reviewers' suggestions.

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated.

2 Revision has been made according to the suggestions of the reviewer and Editorial Office.

Reviewer1

(1) Reviewers' point: *Cellular quiescence is a conserved mechanism occurring in somatic stem cells, in which they can also rapidly activated, proliferate and differentiate to replace the cells lost to contribute to regeneration in homeostasis and response to tissue injury. Previous studies identified that quiescent CSCs were more resistant to chemotherapy and could retain the capacity to proliferate after chemotherapy withdrawal. In this manuscript, authors reviewed stem cell quiescence and its clinical relevance and discussed the current advances in how stem cells and CSCs maintain and regulate quiescence and potential target therapy to quiescent cancer stem cells. This review highlighted the following aspects 1) Under normal conditions, quiescence protects normal adult SCs from exhaustion and senescence, thus preserving their multipotency, regenerative potential, and ability to maintain tissue homeostasis. 2) Elucidating environmental factors that induce or maintain quiescence in SCs is critical for exploiting their clinical potential. 3) In malignant disease, quiescent CSCs exhibit resistance to conventional treatments and are responsible for relapse. 4) Significant progress has been made in our understanding of molecular mechanisms governing quiescence in CSCs, thus expanding the scope of potential strategies for the treatment of specific types of cancer. This review paper provide a new cellular narrative a for stem cell quiescence and its clinical relevance. I believe that this submission will be very useful in future study of quiescent stem cell. Therefore, as key targets in clinical treatment for a wide range of cancers, activating cancer stem cell may enable their eradication by subsequent treatments with standard chemoradiotherapy. This manuscript can be considered for publication without revise.*

Reply: We appreciate this comment and agree the reviewer's viewpoints as well. In this review, we reviewed stem cell quiescence and its clinical relevance and discussed the current advances in how stem cells and cancer stem cells maintain and regulate quiescence and potential target therapy to quiescent cancer stem cells. Here, we outlined the recent advances and controversies in adult SCs quiescence and CSCs quiescence, hoping to make deeper understanding of the SCs quiescence.

Reviewer2

(1) Reviewers' point: *"Abstract: The stem cells (SCs) concept was proposed for decades, and states that adult SCs maintain tissue homeostasis and repair tissues when injured." Here, the authors talk about adult stem cells, so they need to specify adult stem cells or somatic stem cells. The terminology should be followed: either the concept of stem cells or the stem-cell concept is preferred.*

Reply: We appreciate this comment and have revised language in abstract, and the term 'stem cells' also has also been revised as 'adult stem cells' in the full text. The revised sentence is as followings: Quiescent state has been observed in stem cells (SCs), including in adult stem cells and in cancer stem cells (CSCs).(ABSTRACT-sentence 1)

(2) Reviewers' point: *"Cumulative evidence suggests that part of SCs and CSCs reside in the quiescent state, which not only contributes to self-renew and to avoid unnecessary exhaustion in SCs pool but also conduces to averting death from harsh external stimuli in CSCs, such as chemotherapy and radiotherapy." The sentence is not logical.*

Reply: We appreciate this comment and have revised this sentence to clarify our meanings. The revised sentence is as followings: Quiescent state has been observed in stem cells (SCs), including in adult stem cells and in cancer stem cells (CSCs). Quiescent status of SCs contributes to SCs self-renew and conduces to averting SCs death from harsh external stimuli. (ABSTRACT, sentence 1 and 2)

(3) Reviewers' point: *Both Abstract and Core tips were written like an introduction.*

Reply: We appreciate this comment and have revised the abstract and core tips. The revised abstract is as followings: Quiescent state has been observed in stem cells (SCs), including in adult stem cells and in cancer stem cells (CSCs). Quiescent status of SCs contributes to SCs self-renew and conduces to averting SCs death from harsh external stimuli. In this review, we provide an overview of intrinsic mechanisms and extrinsic factors that regulating adult SC quiescence. The intrinsic mechanisms discussed here include cell cycle, mitogenic signaling, Notch signaling, epigenetic modification, metabolism and transcriptional regulation, while the extrinsic factors summarized here include microenvironment cells, extracellular factors, immune response and inflammation in microenvironment. Quiescent state of CSCs has been known to contribute immensely to therapeutic resistance in multiple cancers. The characteristics and the regulation mechanisms of quiescent CSCs are discussed in detail. Importantly, we also outline the recent advances and controversies in therapeutic strategies targeting CSCs quiescence. (ABSTRACT)

The revised core tips are as followings: The quiescent state is very important for both adult stem cells and cancer stem cells. Quiescence of adult stem cells is regulated by multiple intrinsic mechanisms and extrinsic factors. Quiescence of cancer stem cells contributes immensely to therapeutic resistance in multiple cancers. Targeting the quiescence of cancer stem cells may be a novel strategy in clinic. (Core tip)

(4) Reviewers' point: *"Adult SCs can be classified into normal SCs and cancer (C)SCs[4]." That is a misleading statement, as the standard somatic stem cells are classified by organs.*

Reply: We appreciate this comment and have revised this misleading statement. The revised sentence is as followings: Besides, the growth of tumors is promoted by a few cells, termed as cancer (C)SCs, which also possess self-renewal ability like the adult normal tissue stem cells. (INTRODUCTION, paragraph 1, sentence 5)

(5) Reviewers' point: *"it has been described in multiple SC types including hematopoietic (H)SCs, muscle (Mu)SCs, neural (N)SCs, hair follicle (HF)SCs, and intestinal SCs." Citations should be given - ideally, a table should be provided.*

Reply: We appreciate this comment. This sentence has been revised to clarify the definition of quiescence phenomenon in adult SCs more clearly, and new table has been designed to classify different types of stem cells and their regulatory factors. The revised the sentence and the given citation are as followings:

The quiescent state (G0) is defined as reversible cell cycle arrest characterized by reduced metabolic activity. And SCs can exit quiescence and re-enter the cell cycle in response to various types of stress or changes in the microenvironment. The phenomenon of quiescence has been found in multiple adult SCs, including hematopoietic (H)SCs[6], muscle (Mu)SCs[7], neural (N)SCs[8], and hair follicle (HF)SCs[9].(INTRODUCTION, paragraph 2, sentence 5)

[Reference]

6 He L, Beghi F, Baral V, Depond M, Zhang Y, Joulin V, Rueda BR, Gonin P, Foudi A, Wittner M, Louache F. Cables1 deficiency impairs quiescence and stress responses of hematopoietic stem cells in intrinsic and extrinsic manners. Stem Cell Reports 2019; 13: 274-290 [PMID: 31327733 DOI: 10.1016/j.stemcr.2019.06.002]

7 Kitajima Y, Suzuki N, Nunomiya A, Osana S, Yoshioka K, Tashiro Y, Takahashi R, Ono Y, Aoki M, Nagatomi R. The ubiquitin-proteasome system is indispensable for the maintenance of muscle stem cells. Stem Cell Reports 2018; 11: 1523-1538 [PMID: 30416048 DOI: 10.1016/j.stemcr.2018.10.009]

8 Engler A, Rolando C, Giachino C, Saotome I, Erni A, Brien C, Zhang R, Zimber-Strobl U, Radtke F, Artavanis-Tsakonas S, Louvi A, Taylor V. Notch2 signaling maintains nsc quiescence in the murine ventricular-subventricular zone. Cell Rep 2018; 22: 992-1002 [PMID: 29386140 DOI: 10.1016/j.celrep.2017.12.094]

9 Yan H, Gao Y, Ding Q, Liu J, Li Y, Jin M, Xu H, Ma S, Wang X, Zeng W, Chen Y. Exosomal micro rnas derived from dermal papilla cells mediate hair follicle stem cell proliferation and differentiation. Int J Biol Sci 2019; 15: 1368-1382 [PMID: 31337968 DOI: 10.7150/ijbs.33233]

The new Table is as followings:

Table 1 Regulation of quiescent stem cells

Stem cells	Regulatory Factors
Adipose-derived stem cells	CDKI1C [14]
Airway club progenitor cells	P53 [19]
hepatic stellate cells	Laminin 521 [96]
hair follicle stem cells	MiR-22-5p [9], Acer1 [43,44], Mpc1 [49], OSM [83], Nlrc5 [91]
Hematopoietic stem/progenitor cells	CDKI1 [6], Asri [20], RB [22], Tet1 [35], BMI1 [36], SIN3 [37], MMP [42], NRF2 [45], RISP [47], SRC-3 [48], Autophagy [51], NR4A1 and NR4A3 [53], $CD150^{high}$ $FOXP3^{+}$ regulatory T cells [63,71], Ebf1 [64], Eosinophils [66], ICAM-1 [69], lineage-committed Hdc+ myeloid cells [72], $NG2^{+}$ cells [73], NPY [74], ANG [77], SHP-1 [78], TGF-β [80], luteinizing hormone [89], Jak1 [84], PTPN21[97]
Mammary Stem cells	BCL11b [16], FOXP1
Muscle satellite (stem) cell	Rpt3 [7], miR-708 [27], Notch3 [29,29], PTEN [30,31], ZEB1 [31,55,56], miR-31 [39], AMPK [46], N-cadherin and M-cadherin [68], Wnt4 [75], OSM [76], Nlrc5 [91], Col5a1 [95]
Neural stem/progenitor cells	Notch2 [8], Lfng [23], ID4 [24], Notch3 [25], Cpt1a [41], ASCL1 [57], Huwe1 [58], VCAM-1 [67], MFGE8 [86]

(6) Reviewers' point: *Table 1 should be clustered by cancer types in column 1, with related biomarkers in column 2. The same arrangement should be used for Table 2, which should be expanded to include more cancer types.*

Reply: We appreciate this comment and have revised Table1 and Table2 according to suggestions. And the related research of quiescent stem cells has not proposed specific biomarkers that can be used to identify quiescent stem cells until now. It may be a misunderstanding that the factors in previous table 1 mainly refer to the factors that regulate quiescent stem cells, not refer to the biomarkers that can be used to distinguish quiescent stem cells. In addition, I'm sorry that previous Table 2 contains nearly all the literature about targeting therapy on quiescent CSCs in the past five years, and it is difficult to find more research in this field.

The revised Tables are as followings:

(Due to the addition of Table 1, previous Table 1 and Table 2 have been revised as Table 2 and Table 3.)

Table 2 Regulation of quiescent cancer stem cells

Type of cancer	Regulatory Factor	Regulatory Mechanism
Ovarian cancer	Autophagy	Knockdown of ATG5 inhibits autophagy and arrests ovarian cancer cells in G0/G1 state through upregulating production of ROS.[115]
Breast cancer	SETD4	SETD4 regulates breast CSCs quiescence by facilitating the formation of heterochromatin via H4K20me3 catalysis.[11]
Breast cancer	LIFR	Loss of LIFR in dormant breast cancer cells reduced the expression of quiescence and cancer stem cell-associated genes, such as TGF-β2 and Notch1.[131]
Breast cancer	mitochondrial DNA	CAF-derived EVs, containing mitochondrial DNA, promote estrogen receptor-independent oxidative phosphorylation and facilitate an exit from quiescence in HT-naive breast cancer stem-like cells.[133]
Breast cancer	Macrophages	Macrophages with M1 phenotype secreted exosomes to activate NFκB pathways, and thus reversed breast CSCs (BCSCs) quiescence; Macrophages exhibiting an M2 phenotype causes quiescence and lessened proliferation via gap junctional intercellular communication. [134]
Breast cancer	NOTCH4	NOTCH4 transcriptionally activates GAS1 to sustain quiescence in BCSCs. [139]
Colorectal cancer	ZEB2	ZEB2 upregulates cell cycle-related factors including HDAC9, Cyclin A1, Cyclin D1, HDAC5 and TGFβ2, to keep stem cells quiescent.[121]
Colorectal cancer	SPDEF	SPDEF breaks binding of β-catenin to TCF1 and TCF3, and regulate cell cycle-associated genes, such as CCND1, HDAC4, CDK6, MYC, and AXIN2, to induce a quiescent state .[122]
Liver cancer	Tyrosine metabolism	Targeting Tyrosine metabolism impairs quiescence accelerating degradation of Forkhead box D3 (Foxd3).[125]

Liver cancer	CXCL1	The CXCL1 induces quiescence in hepatocellular carcinoma stem cells by activation of the mTORC1 kinase.[128]
Multiple myeloma	TRIM44	TRIM44 deubiquitinates HIF-1α to stabilizes HIF-1α expression and HIF-1α contributes to MM stem cell quiescence.[120]
Glioblastoma	Ca^{2+}	Inhibition of store-operated channels increases capacity of mitochondria to capture Ca2+ in GSLCs, and thus impels proliferous GSLCs to turn to quiescence.[9]
Glioblastoma	PSF1	Defect of PSF1 suppressed reactivation of quiescent CSCs after serum supplement or reoxygenation.[135]
Melanoma	GILZ	Deficiency of GILZ expression in vivo arrested these cells in the G0 phase, and induced quiescence.[127]
pancreatic cancer	lncRNA GAS5	GAS5 restrain the cell cycle to suppress proliferation by inhibiting glucocorticoid receptors (GR) mediated cell cycle regulation.[138]
Lung cancer	Fbxw7, Skp2	Knockdown of Fbxw7 upregulated c-myc and knockdown of Skp2 increased the expression of p27, and then transforms cells into quiescence.[136]
AML	FOXM1	FOXM1 binds to β-catenin and decreases degradation of β-catenin protein, thus activates the Wnt/β-catenin signaling pathways, and preserves leukemia stem cell(LSC) quiescence.[123]
AML	lncRNA DANCR	Knock-down of DANCR in LSCs caused reduced stem-cell renewal and quiescence.[137]
AML	EVI-1	Evi-1 depresses promotes the quiescence of LSCs possibly through Notch4.[141].
AML	PRC2	PRC2 regulated suppression of Cyclin D to maintain quiescence in LSCs.[145]
CML	Mir-126	Endothelial cells provides miR-126 for CML LSCs to restrain cell cycle progression through targeting PI3K/AKT/mTOR signaling pathway.[8,117]
CML	CXCL12	knockout of CXCL12 in mesenchymal stromal cells promotes leukemic stem cells (LSCs) expansion via downregulation of gene associated with quiescence such as TGF-β and STAT3.[129]
CML	BMP4	BMP4 directly regulates quiescence of CML LSCs through regulating JAK/Stat3 pathway, dependent upon BMPR1B kinase activity.[130]

AML: Acute myeloid leukemia

CML: Chronic myelogenous leukemia

Table 3 Therapeutic strategies against quiescent

Type of cancer	Therapeutic Target	Potential therapy	Therapeutic Mechanism
AML	HDM2	PNC-27	● PNC-27 binds to mHDM2, leads to E-cadherin degradation, and causes membrane injury and cell necrobiosis.[140]
AML	EVI-1	ATRA	● ATRA enhances Evi-1-dependent depression of the maturation and promotes the quiescence[141,142]
AML	c-MPL	AMML2	● AMML2 blocks c-MPL, stimulates entry of quiescent LSCs into the cell cycle, and increases the sensitivity of LSCs to chemotherapy.[143]
AML	EZH1, EZH2	OR-S1, OR-S2	● OR-S1 and OR-S2 inhibit EZH1/2, inactivate PRC2, and then eliminate quiescent LSCs, induces cell differentiation, and turn chemotherapy-resistant LSCs into a chemotherapy-sensitive population.[145]
CML	Autophagy	Lys05, PIK-III	● Lys05 achieves autophagy inhibition in LSCs and promotes differentiation; Lys05 and PIK-III inhibit TKI-induced autophagy and increases the sensitivity of LSCs to TKI.[146]

AML: Acute myeloid leukemia
CML: Chronic myelogenous leukemia

(7) *Reviewers' point:* *"Figure 1 Schematic representation of various factors that lead to the promoting or exit of quiescence in SCs. The intrinsic elements are in the left boxes, whereas the extrinsic elements are in the right boxes." The current scheme did not differentiate the promoting or exit of quiescence in SCs – which should be regripped to show such different effects, hitting the home run for the review manuscript. Neither did they get the point crossed with "Figure 2 Schematic presentation of main factors that regulate quiescent CSCs in intrinsic and extrinsic aspects."*

Reply: We appreciate this comment and have revised the figures to differentiate the promoting or exit of quiescence in SCs and CSCs. The revised figures are as followings:

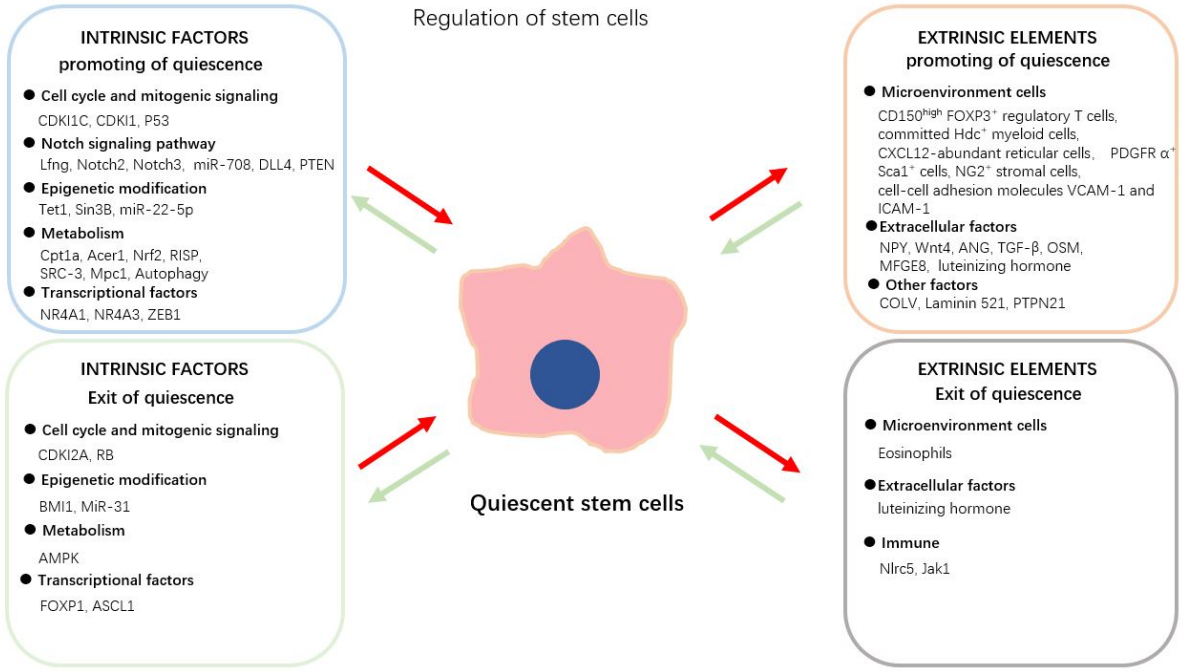

Figure 1 Schematic representation of various factors that lead to promoting or exit of quiescence in SCs. The intrinsic elements are in the left boxes whereas the extrinsic elements are in the right boxes.

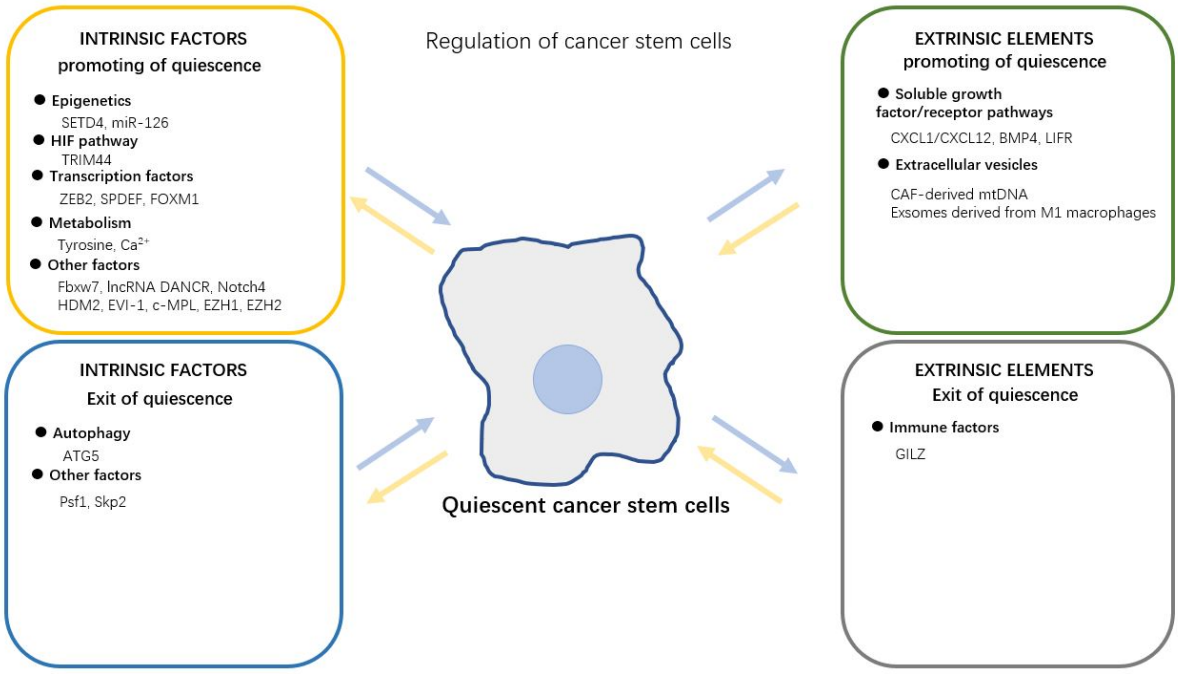

Figure 2 Schematic presentation of main factors that regulate quiescent CSCs in intrinsic and extrinsic aspect.

(8) Reviewers' point: *English language and style are fine tone/minor spell check required for clarity. There are numerous typographical/grammatical errors (also incorrect punctuation with abbreviation) throughout the Manuscript (some examples as marked by […] track, but not an exhaustive presentation. E.g., 1 - "The former have [has] unlimited potential for cell division but maintain[s] totipotency or pluripotency [1] and can differentiate into various cell types, which is regulated by specific transcription factors at each developmental stage[2]." E.g., 2 – "Additional agents targeting different classes of [the] molecule[s] or pathways are needed;" E.g., 3 – "A subtype of AML that accounts for ~10% of AML cases is characterized by high expression of EVI-1 and has [a] very poor outcome." E.g., 4 – "The CSCs showed chemotherapy resistance and slow growth in vivo and [in] vitro;" E.g., 5 – "Using the fluorescent tracer PKH26, quiescent stem-like cancer cells were identified in multiple myeloma (MM) that were present in the osteoblast niche of BM and expressed high levels of tripartite motif containing (TRIM)44[119], an E3 ubiquitin ligase that deubiquitinates and stabilizes the expression of HIF-1α under normoxia and hypoxia."*

Reply: We appreciate this comment and have tried to revised manuscript to reduce language errors. The revised content is as followings:

The revised sentences are as followings:

E.g.1 The former has unlimited potential for cell division but maintains totipotency or pluripotency and can differentiate into various cell types, which is regulated by specific transcription factors at each developmental stage. (INTRODUCTION, paragraph 1, sentence 3)

E.g.2 However, more agents targeting different classes of molecule or pathways are needed. (CONCLUDING REMARKS AND FUTURE DIRECTIONS, paragraph 2, sentence 4)

E.g.3 A subtype of AML, which is characterized by high expression of EVI-1, has very poor outcome, and shows sensitivity to all-trans retinoic acid (ATRA). (PART3, Therapeutic strategies targeting CSC quiescence, paragraph 3, sentence 1)

E.g.4 The CSCs showed chemotherapy resistance and slow growth in vivo and in vitro. (PART3, Regulations of quiescent CSCs, paragraph 5, sentence 3)

E.g.5 Using the fluorescent tracer PKH26, quiescent stem-like cancer cells were identified in multiple myeloma (MM). Those quiescent stem-like cancer cells were present in the osteoblast niche of BM and expressed high levels of tripartite motif containing (TRIM)44, an E3 ubiquitin ligase that deubiquitinates and stabilizes the expression of HIF-1α under normoxia and hypoxia. (PART3, Regulations of quiescent CSCs, paragraph 4, sentence 2 and 3)

The typographical/grammatical errors and incorrect punctuation pointed out by reviewers have been revised. Besides, we carefully checked the entire review again and other more grammatical and spelling errors have also been corrected.

Science Editor

(1)* Editor*'* *comments and suggestions: *Issues raised: (1) The language classification is Grade C. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240; (2) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); and (3) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.*

Reply: We appreciate this comment and have tried to resolve the problems. (1) We have revised the manuscript and polished the language as the reviewer suggested. We have let the professional English language editing companied to edit the language for us, before we submitted the manuscript. If the language still not meet the classification requirement, we would like to polish again. (2) We added the approved grant application form(s) or funding agency copy of any approval document(s) in subsequent uploading process; (3) We revised pictures according to reviewer's suggestion in PowerPoint and uploaded the original pictures.

3 References and typesetting were corrected.

Thank you for reviewing our manuscript and considering it for publication in the *World Journal of Stem Cells.*

Sincerely,

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