

Dear Editorial Office,

Thank you for your careful and detailed consideration of our manuscript "MULTIPOTENT STROMAL CELLS STIMULATE LIVER REGENERATION BY INFLUENCING THE MACROPHAGE POLARIZATION" by Andrey Elchaninov , Timur Fatkhudinov, Natalia Usman, Irina Arutyunyan, Andrey Makarov, Anastasia Lokhonina, Irina Eremina , Viktor Surovtsev, Dmitry Goldshtein, Galina Bolshakova, Valeria Glinkina, Gennady Sukhikh.

We appreciate all comments made by the Reviewers and are grateful for the opportunity to submit a revision based on these comments.

Since the submitted manuscript is an animal study using rats, it is better to modify the title as "MULTIPOTENT STROMAL CELLS STIMULATE LIVER REGENERATION BY INFLUENCING THE MACROPHAGE POLARIZATION IN RAT" in order to reflect the content more accurately.

The title was corrected.

This is an interesting manuscript that proposes that multipotent stromal cells (MSC) enhance regeneration of solid organs specifically liver regeneration. The authors use valid methodologies to address the mechanism by which MSC enhance liver regeneration. Unfortunately, it is clear if this manuscript provides new information. It is known that MSC exert anti-inflammatory actions, is it known that this is mediated by class shifting of macrophages?

MSCs are capable of inducing macrophage polarization – this has been demonstrated *in vitro* and also in the aftermath of critical ischemic damage to skeletal muscles *in vivo*. Our study is the first to demonstrate similar effects in a post-resection liver repair model.

1. Kang WC, Oh PC, Lee K, Ahn T, Byun K. Increasing injection frequency enhances the survival of injected bone marrow derived mesenchymal stem cells in a critical limb ischemia animal model. Korean J Physiol Pharmacol. 2016;20(6):657-667.

2. Gao WH, Yu JY, Li HM, Guan Y, Li SZ, Huang PP. The Immunomodulatory Effects of Umbilical Cord Mesenchymal Stem Cell in Critical Limb Ischemia Patients. J Stem Cell Res Ther. 2016;6:349. doi:10.4172/2157-7633.1000349
3. Dayan V, Yannarelli G, Billia F, Filomeno P, Wang XH, Davies JE, Keating A.
4. Mesenchymal stromal cells mediate a switch to alternatively activated monocytes/macrophages after acute myocardial infarction. Basic Res Cardiol. 2011;106(6):1299-310. doi: 10.1007/s00395-011-0221-9.
5. Shohara R, Yamamoto A, Takikawa S, Iwase A, Hibi H, Kikkawa F, Ueda M. Mesenchymal stromal cells of human umbilical cord Wharton's jelly accelerate wound healing by paracrine mechanisms. Cytotherapy. 2012;14(10):1171-81. doi: 10.3109/14653249.2012.706705.

Additionally, although in general macrophages can be thought of as M1 or M2 this is an arbitrary designation with gradations. The authors state that in previous studies 90% of cells transplanted to the spleen are destroyed by association with CD68+ cells. If this is case what cells are mediating this presumed paracrine effect?

The paracrine effect is mediated by the transplanted MSCs. However, the paracrine effect provided by MSCs is dose-dependent and typically short-term, and apparently difficult to study in vivo. Although MSCs show the ability to synthesize a rich set of biologically active molecules, several other studies demonstrating the positive effect of MSCs on liver regeneration show that repeated administration of MSCs, as well as an "in advance" transplantation of the cells before damage, gives a stronger effect, which may have a stronger paracrine component.

Commas are used in the figures were periods should be used.

The legends were corrected

The legends refer to asterisk yet none are included.

The legends were corrected

The manuscript is confusing in many places and needs to be carefully proofread.

The text was corrected

Figure 4 is not convincing. It looks like many of the cells positive for Ck18 are positive for PKH26. Also, why are not the majority of the cells positive for Ck18?

CK18 is a structural protein of the cytoskeleton. It is found throughout the volume of a cell – unlike PKH26, which is membrane bound. Yellow glow produced by combination of these two signals in one cell, as well as characteristic hepatocyte-like morphology of the cell emitting the glow, is considered as a sign of MSC switch to hepatocyte/hepatocyte-like differentiation. However, most of the PKH26-positive cells in the relevant microscopic images show no signs of CK18 presence, and they are definitely not hepatocytes by their morphology. Moreover, their majority belong to connective tissue streaks rather than to the parenchyma, and they are surrounded by similar CK18-negative cells. Solitary cases of the double-positiveness, represented by individual cells, were not counted as convincing signs of the hepatocyte or hepatocyte-like differentiation.

In Figure 2, it appears that more cells are positive in the SR and saline at 10 day than in the SR and MSC images. Additionally, it looks like more cells are positive in the SR and saline at day 10 than day 7.

More representative microphotographs are provided in the revision

What is the x-axis?

Horizontal axis represents time elapsed after the surgery

How can you have relative numbers in animal survival?

Animal survival was calculated as [the total number of operated animals minus the number of spontaneous deaths] divided by the total number of operated animals.

How were the images quantified?

Mitotic index of hepatocytes was calculated for each animal individually as a number of mitoses per 6×10^3 hepatocytes, expressed in promille (‰)

The Ki67 proliferation index was calculated for each animal individually as a number of Ki67-positive hepatocytes per 3×10^3 hepatocytes.

The CD68+ and CD206+ cells were counted in microscopic images and related to the total cell counts to obtain corresponding indexes of macrophage content (min. 3×10^3 cells for each animal).

MSCs differentiation assessment was carried out using microscopic images for 1×10^3 PKH26+cells for each animal

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Yours faithfully,
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