Author’s response to reviews

Title: Notch1 signaling induces Epithelial-Mesenchymal Transition in lens epithelium cells during hypoxia

Authors:

Lei Liu (leilei_315@hotmail.com)
Wei Xiao (estherllsy@hotmail.com)

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Author’s response to reviews:

Dear editors,

We are sorry to reply late.

Here are our response to the major concerns;

1. The author should explain EMT and migration of LECs in the anterior capsule, which located in high oxygen tension in anterior chamber before migration into posterior capsule.

It’s well known that the anterior portion of lens absorbs the nutrition including oxygen from the anterior chamber, aqueous humor, while the oxygen supply of the posterior portion of the lens is maintained by the vitreous [1]. The oxygen tension in aqueous humor is much higher than that in the vitreous[2-4], which creates an oxygen-rich environment in the anterior portion of the lens but an oxygen-poor environment in the posterior part. The lens epithelium is physiologically located only in the oxygen-rich anterior portion of the lens. After cataract surgery, the LEC moves to the posterior portion of the lens to a hypoxic microenvironment, where it undergo EMT. Hypoxia environment is a stimulation factor for EMT. We just make a hypothesis that LEC cells may have EMT in the new microenvironment. We think, it’s an original idea, and we report some primary researches on it. Definitely, there will be many works to continue.

2. In their methods, more detailed descriptions are needed, esp, CoCl2 prep and treatment, verification of transfection, and type of needles for wound healing.

We supplement some detailed instructions about CoCl2 prep and treatment, and the type of needles for wound healing. The efficiency of transfection was verified by western blot. The effect was verified by WB(Fig3 A-B). The expression of Notch1 was enhanced after transfection comparing to the control group.

3. It is helpful to add immunohistochemical assay for E-cadherin, keratin, fibronectin, and vimentin in Fig-1.

It’s a good advice for us. Immunocytochemical assay for Keratin and Fibronectin was provided.

4. How many samples are statistically analyzed in Fig 1-3?

Every assay in this study repeated three times and three samples were used in each time. In wound healing assay, the scratch width was measured in five randomly selected high power fields under microscope. Data presented are repeated three times.

5. CoCl2 helps stabilize HIF1 transcription factor and it only mimics HIF1 activation and many other effects of hypoxia that are not mimicked by CoCl2 treatments. They need to compare hypoxic condition and CoCl2 treatment on LECs.

Yes, This is an another good advice for us. CoCl2 is only a mimic factor to activate HIF-1. It can’t mimic the whole effect of hypoxia environment. In fact, it was in our plan. But the sensors on the incubators in our lab are out of work and the oxygen concentration couldn’t be controlled exactly. These works will be continued as soon as the machines were repaired.

Figure 1. It is necessary to describe why the cellular morphology looked more mesenchymal. What were the characteristics the authors considered when judging cellular morphology. In addition, the phase contrast microscopic pictures have poor resolution. It is necessary to provide both low resolution and high resolution images. If the authors can provide the pictures with immune-cytochemical staining, it would be far better to compare the groups. What was the expression level of keratin and fibronectin at 12 and 24 hour of CoCl2 exposure? Specify how many times the experiments were repeated to get the graphs provided.

Every assay in this study repeated three times and three samples were used in each time.

Immunocytochemical assay for Keratin and Fibronectin was provided.
Figure 2. Again, please specify the experimental repeat times to get the final graphs. It is necessary to provide the western blot results from 12 and 24 hour of CoCl2 exposure.

Every assay in this study repeated three times and three samples were used in each time. In wound healing assay, the scratch width was measured in five randomly selected high power fields under microscope. Data presented are repeated three times. And the western blot results from 12 an 24 hour of CoCl2 exposure are provided.

And we rewrite the Declarations section.

Don't hesitate to contact us if you have any question.

Thank you!

Have a good day.

Wei Xiao and Lei Liu