

## Itemized List of Responses to Reviewer' Comments

(Manuscript No: **25362**, by Lihua Cao, Chengcai Xia, zhaochun Shi, Ning Wang, zhenghua Gu, lizhi Yu, Qi Wan ,Wei De.Title:  $\text{Na}^+/\text{HCO}_3^-$  cotransporter (NBC1) Is Expressed on  $\beta$  and  $\alpha$  Cells During Rat Pancreatic Development)

We thank the referee for the comments, which are very helpful in improving the quality of the manuscript. Critical points have been revised and suggestions by referee are made in the revision. Following are our responses to the comments.

### Comments:

*1.  $\text{Na}^+/\text{HCO}_3^-$  cotransporter instead of NBC1 in the title can be written.*

**Response to comment:** We thank for the reviewer to point out our inappropriate expression and we apologize for it.

**Response:** As suggested, we have rewritten the title in a more precise way in the revised manuscript, which is shown as follows:

$\text{Na}^+/\text{HCO}_3^-$  cotransporter (NBC1) Is Expressed on  $\beta$  and  $\alpha$  Cells During Rat Pancreatic Development

*2. E14.5, E18.5 and E19 can be expressed more clearly.*

**Response to comment:** We thank for the reviewer to make us more aware of the preciseness of scientific analysis. However, combining with the characteristics of embryonic pancreas development, the reasons of E15.5 and E18.5 are selected as research stages as follows:

### Response:

Rat pancreas begins to develop in the embryonic day 8.5 (E8.5); By E14.5, pancreatic ductal epithelial cells differentiate into acinar cells, endocrine  $\alpha$ 、 $\beta$ 、 $\delta$  cells mature, but only a small amount insulin and glucagon can be secreted; About E15.5, the differentiation and maturation of endocrine cells begin to break through the basement membrane to migrate the surrounding mesenchymal cells, endocrine cells gathering each other,  $\alpha$ 、 $\delta$ 、PP cells surrounding  $\beta$  cells form the islet structures. In E18.5, the structures of acinar cells gradually become clear, and the typical islet structure is basically formed.<sup>[a, b]</sup> Therefore, combining with the characteristics of embryonic pancreas development, E15.5 and E18.5 are selected as research stages.

a. Kim SK, Hebrok M. Intercellular signals regulating pancreas development and function. *Genes Dev*,2001;15:111-127.

b. Habener JF, Kemp DM, Thomas MK. Transcriptional regulation in pancreatic development. *Endocrinology*,2005;146(3):1025-34.

3. *Why was donkey anti-rabbit IgG used? Rat or mouse IgG may be more good.*

**Response to comment:** We thank for the reviewer to make us more concern about the preciseness of experimental process and the importances of antibodies selective.

**Response:** As suggested, we study again the Double fluorescence immunohistochemical analysis and the rule of the antibodies selective, meanwhile ,we have provided some evidence by citing others' study. which is shown as follows:

\* **P.8** “ Double fluorescence immunohistochemical analysis

For NBC1 and amylase, insulin, glucagon double immunofluorescence, the ***rabbit anti-NBC1 primary polyclonal antibodies*** were applied and revealed using the FITC-labeled **donkey anti-rabbit IgG**. ***Mouse anti-amylase primary polyclonal antibody***, was then applied and revealed by CY3-labeled **donkey anti-mouse IgG** . ”

In immunofluorescent antibody test, the primary polyclonal antibodies need to choose the different kinds of antibodies which belong to different species,such as the rabbit anti-NBC1 primary polyclonal antibodies and the mouse anti-insulin polyclonal antibodies,etc.in this experiment.Then, in order to avoid the cross-reactivity of antibodies and improve the specificity, the second polyclonal antibodies have to select the anti-rabbit and anti-mouse antibodies belonging to different species according to the primary polyclonal antibodies.

And these antibodies and their using method were successfully certified by Lars J. Jensen et al.<sup>[28]</sup>

[28] Lars J. Jensen, Bernhard M. Schmitt, Urs V. Berger, Localization of Sodium Bicarbonate Cotransporter (NBC) Protein and Messenger Ribonucleic Acid in Rat Epididymis, *Biology of Reproduction*. 1999;60:573-579.

4. *The differences between the present study with study titled “Hiroaki Satoh, Nobuo Moriyama, Chiaki Hara, et al, Localization of Na<sup>+</sup>-HCO<sub>3</sub><sup>-</sup>cotransporter (NBC-1)*

*variants in rat and human pancreas, Am J Physiol Cell Physiol. 2003;284:729-737”*  
*can be written to the discussion part as comparative.*

**Response to comment:** We thank the reviewer for the kindly suggestion, which are very helpful in improving the quality of the manuscript.

**Response:**

It have been written to in the discussion part as comparative in the manuscript .which is shown as follows:

“Overall our results are at odds with the previous report that the anti-pNBC1 and anti-kNBC1 antibodies did not label the islet cells in rat pancreas<sup>[29]</sup>. In their study they used two polyclonal antibodies against variant-specific regions in the NH<sub>2</sub>-terminal of the rat kNBC1 (amino acids 4-16) and the rat pNBC1 (amino acids 2-12). On the other hand, in our study two affinity purified rabbit polyclonal antibodies raised against the common region of the two NBC1 isoforms at the COOH-terminal amino acids 928-1035 or NH<sub>2</sub>-terminal amino acids 338-391 were used in the immunohistochemical analysis. ”

29 Hiroaki Satoh, Nobuo Moriyama, Chiaki Hara, et al, Localization of Na<sup>+</sup>-HCO<sub>3</sub><sup>-</sup> cotransporter (NBC-1) variants in rat and human pancreas, Am J Physiol Cell Physiol. 2003;284:729-737.