

# Nanjing Medical University Experimental Animal Department

## Application for Ethical Approval for Research Involving Animals

No:

code: 1401005

App. Date(YY-MM-DD):2014-3

Name of Principal Investigator	Lihua cao wei de	Contact Person	Lihua cao wei de	
Department	Department of Biochemistry, Nanjing Medical University	Contact Tel. No.	13705174462 025-86862728	
Project Title	NBC1 Is Expressed on $\beta$ and $\alpha$ Cells During Rat Pancreatic Development			
Funding Source & Number	Development of Medical Science and Technology Project of Jiangsu Province (Grant No. YKK13205).			
Additional Research Personnel	Name	Title	Animal User Permit No.	Tel.No.
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Description of Project (Please describe study objectives and experimental design; specify all procedures performed on animals, post procedure animal care, and the fate of animals including euthanasia criteria and method.)

### Study objectives and experimental design:

We expected to use high-density microarray(Affymetrix)to analyze the expression profiles of pancreas development. We generated transcriptional profiles of pancreatic tissue isolated from five biologically significant stages of development: embryonic day(E)12. 5, E 15. 5, E 18. 5, newborn and adult pancreas. These analyses implicate many genes related to pancreatic function are enriched in the later gestation when islet architecture and function are gradually forming. Our results demonstrated the presence of NBC1 in the plasma membrane of  $\beta$  and  $\alpha$  cells as well as in the basolateral membrane of acinar cells in rat developing pancreas. The data highly suggests that the NBC1 variants are diversely expressed in pancreas in different developmental stage, where they may display their functions in pancreatic development through  $\text{HCO}_3^-$  transport and pH regulation. Combined with the literature and experiments need to select the SD rats as experimental animals.

Sprague Dawley (SD) rats were purchased from Animal Center of Nanjing Medical University (Nanjing, China). Male and female SD rats were mated overnight. At noon of the next day, if the vaginal plug was discovered it was considered as Day 0.5 of gestation (E0.5). The embryos were removed from the uterus of pregnant rats which had been sacrificed by CO<sub>2</sub> asphyxiation. Pancreas from E15.5 and E18.5 rat embryos was isolated under a stereomicroscope. Rat pancreas from postnatal (P) days 0, 7, 14, 21 and adult were directly isolated by the unaided eye after CO<sub>2</sub> asphyxiation. The harvested tissues were immediately rinsed three times with PBS to remove serum proteins, and then fixed with 4% paraformaldehyde in PBS overnight for histology or frozen in liquid nitrogen for RNA and protein isolation.

#### References:

- [1]. George Seki, Hideomi Yamada, Shoko Horita, et al, Activation and Inactivation Mechanisms of Na-HCO<sub>3</sub> Cotransporter NBC1, *Journal of Epithelial Biology and Pharmacology*, 1(2008)35-39.
- [2]. Yi-Fen Lo, Sung-Sun Yang, George Seki, et al, Severe metabolic acidosis causes early lethality in NBC1 W516X knock-in mice as a model of human isolated proximal renal tubular acidosis. *Kidney International*. 79(2011)730-741.
- [3]. Hong C. Li, Joel H. Collier, Ali Shawki, et al, Sequence- or Position-Specific Mutations in the Carboxyl-Terminal FL Motif of the Kidney Sodium Bicarbonate Cotransporter (NBC1) Disrupt Its Basolateral Targeting and  $\alpha$ -Helical Structure, *Journal of Membrane Biology*. 228(2009) 111-124.
- [4]. Amirabbas Monazzami, Hamid Rajabi, Kobra Omidfar, Ali Mostafaie, ENDURANCE TRAINING INCREASES SKELETAL MUSCLE NA/H+ EXCHANGER1 (NHE1) AND NA/HCO<sub>3</sub> CO-TRANSPORTER1 (NBC1) GENE EXPRESSIONS IN TYPE2 DIABETIC RAT, *Iranian journal of diabetes and metabolism*. 13(5)(2014) 400-412.
- [5]. Shmuel Muallem S, Peggy A. Loessberg, Intracellular pH-regulatory Mechanisms in Pancreatic Acinar Cells, *The Journal of Biological Chemistry*. 265(1990)12806-12812.

#### All procedures performed on animals:

CO<sub>2</sub> asphyxiation, extraction of the pancreas.

#### Post procedure animal care:

CO<sub>2</sub> asphyxiation, reduce the pain and fear.

#### The fate of animals including euthanasia criteria and method:

All rats were sacrifice, CO<sub>2</sub> asphyxiation.

Animal Requirements	Animal Source Nanjing medical university experimental animal department		
	Species or Strains rat	Grade SD	Specifications 5g-250g
	Quantity 90 (♀ 30 ♂ 60)		
	Proposed Date of Commencement 2014-3		Proposed Date of Completion 2016-12

Principal Investigator's Declaration	<p>I confirm that I will ensure that the requirements for the treatment of the animals as detailed in this application and as approved by the Animal Ethics Committee will be met during the course of the project.</p> <p>Signature: <i>Lihua Cao</i> <i>Xuehui He</i> <i>Lianghui Yan</i> Date(YY-MM-DD): 2014-3</p>
Ethical Considerations	<p>1. The significances, necessity and the expected scientific benefits of the proposal work.</p> <p>2. The species, strains, grade, specification and number of the animals to be used should be justified.</p> <p>3. Rational for animal use should be justified, including the alternatives to animal use, a refined study design to replace or reduce animal number to be used. .</p> <p>( Appropriate animal care and handling throughout the experiment, including a scientific sound endpoint; anesthetics, analgesics, sedatives or tranquilizers that are to be used; explanation for any procedure cause unrelieved pain or distress; disposition of animals at end of study; and euthanasia criteria and method.</p> <p>5. Are the materials to be used harmful or toxic? Are there any radioactive agents, infectious agents, genetic modified agents, and the genetic manipulation to be used in the experiment? If yes, the safety measures should be specified.</p> <p>6. Are genetic modified animals to be used in the experiments? IF yes, the source should be valid and the animals should be quarantined.</p>

Comments, Provisos or Reservations of Animal Ethics Committee

Signature: *Xia Long*  
 Name of Ethics Committee Representative  
 Institutional Animal Care and Use Committee (IACUC)

Date(YY-MM-DD): 2014.3.10