MARC REPORT 2013

MODEL ANIMAL RESEARCH CENTER OF NANJING UNIVERSITY
MOE KEY LABORATORY OF MODEL ANIMAL FOR DISEASE STUDY
NANJING BIOMEDICAL RESEARCH INSTITUTE OF NANJING UNIVERSITY
NATIONAL RESOURCE CENTER FOR MUTANT MICE

Director's Words

As MARC embarked on its second decade in 2013, we began the new year with a brainstorming session among all the principal investigators, to discuss how best to tremendously and effectively improve our research efforts. We are committed to engage in high quality research that will lead to innovative discovery with significant impact. This challenge was put forth to all members of MARC. Along this journey, we were met with many difficulties and we have overcome them and grew in the process. This year MARC is proud to have several publications in high profile journals such as J. Exp. Med, Gastroenterology and PNAS.

With the addition of six new research laboratories, we now have a total of 22 labs and a graduate community of approximately 150 students. To enable our principal investigators to lead effectively and efficiently and better utilize talents and resources, we have streamlined our research direction into four core research groups, each with its own central focus. These four focus areas are: 1. Development Biology and Birth Defects; 2. Physiological Homeostasis and Metabolic Disorders; 3. Neural Biology and Diseases; 4. Cancer and Stem Cell Biology. In addition, we welcome to the MARC team Drs. Huiming Gao, Di Chen, Xin Lou, Yun Shi, Zhenji Gan and Jinzhong Qin. In particular, we congratulate Dr. Yun Shi for becoming a member of the Thousand Youth Talent Plan.

MARC continues to reach out in order to promote its mission in developmental biology and genetics. To train the next generation of young scientists, MARC and RIKEN BioResource Center of Japan jointly organized the 2nd International Short Summer Course, an educational program hosted at MARC, focusing on mouse genetics and related experimental technologies. In additional, MARC and the Chinese Society of Cell Biology (CSCB) successfully organized the 2013 China Developmental Biology Conference in Nanjing.

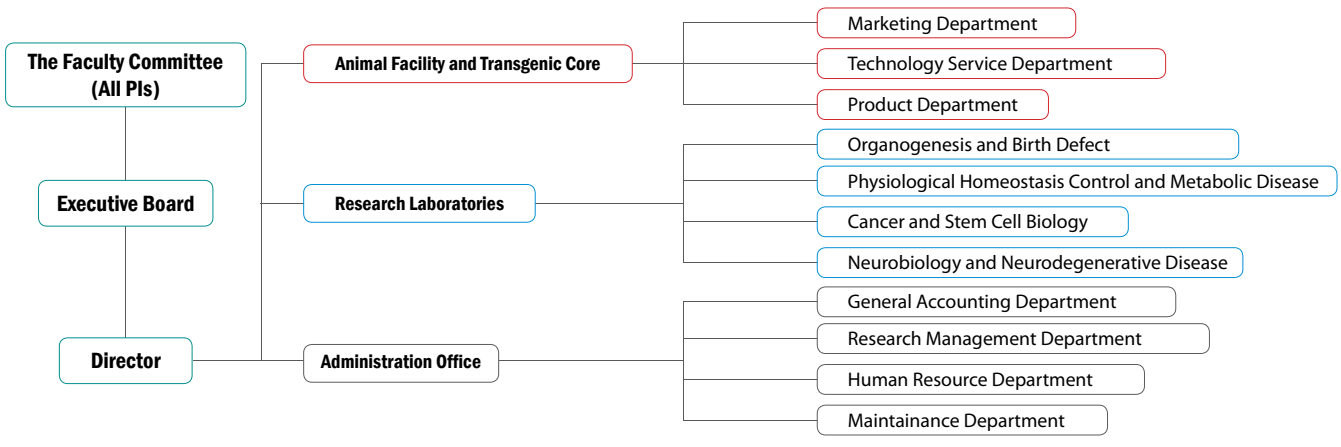
Ambition and resolution constantly drives MARC towards a better future. We look forward to more achievements in the coming year. Finally, I want to thank every member of the mouse facility as well as the administrative team for their support.

Zhongzhou Yang

Director

Contents

Management Structure of Model Animal Research Center (MARC)



Research Highlight in 2013

Childhood mumps and adult infertility (Dr.Chaojun LI and Dr.Xiang GAO's group)

The Journal of Experimental Medicine

Published July 1, 2013

JEM

Altered protein prenylation in Sertoli cells is associated with adult infertility resulting from childhood mumps infection

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Background

Mumps commonly affects children 5-9 year of age, and can lead to permanent adult sterility in certain cases when combined with orchitis. However, the etiology of this long-term effect remains unclear.

Protein prenylation is a critical process for the membrane association of lots of signaling proteins, including small G proteins. Geranylgeranyl diphosphate synthase (GGPPS) catalyzes the formation of geranylgeranyl diphosphate (GGPP) from farnesyl diphosphate (FPP), both of which is used to prenylate proteins with CAAX motif in their carboxyl termini.

Significance

We have observed that the expression of GGPPS was decreased due to elevated promoter methylation in the testes of infertile patients with mumps infection history.

When we deleted GGPPS in mouse Sertoli cells, these cells remained intact, whereas the adjacent spermatogonia began to significantly decrease after the fifth postnatal day, which result in adult infertility. Detailed examination showed that GGPPS-/- Sertoli cells secreted an array of cytokines to stimulate spermatogonia apoptosis, and chemokines to induce macrophage invasion into the seminiferous tubules. Invaded macrophages further blocked spermatogonia development, resulting in a long-term effect through to adulthood. Our results suggest a novel mechanism by which mumps infection during childhood results in adult sterility.

Further molecular mechanism study indicated that the proinflammatory MAPK and NF-kB signaling pathways were constitutively activated in GGPPS-/- Sertoli cells due to the enhanced farnesylation of H-Ras.

Perspective

Notably, the fertility defect could be rescued by FTI administration in GGPPS deleted mice and GGPP administration in EMCV-challenged mice. Thus the protein prenylation pathway is able to serve as a target for pharmacological development of drugs for Mumps related infertility. Another issue we should notice is that the balance of protein geranylgeranylation and farnesylation is critical to cell function. Protein geranylgeranylation and farnesylation could be substituted for one another under certain extreme conditions in which one modification is completely inhibited like GGPPS deletion. FPP accumulation and resulted farnesylation increase after GGPPS deletion may alter the protein prenylation state and disrupt the cellular hemostasis.

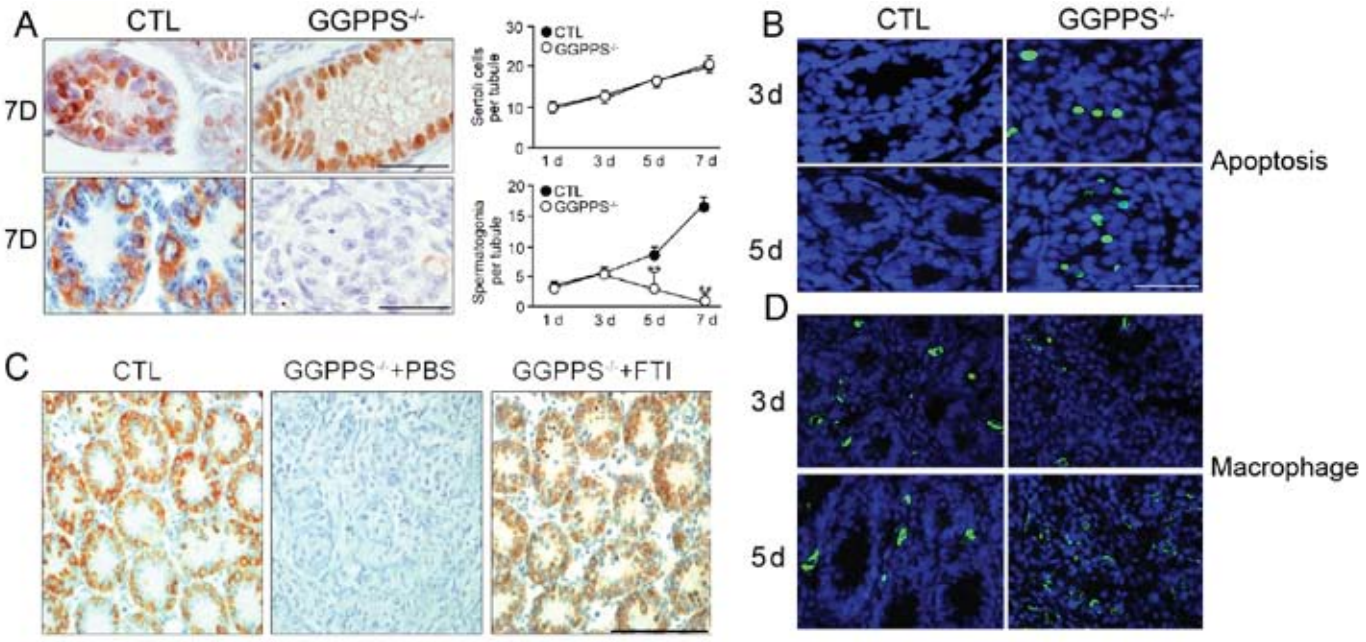

Figure. Mice phenotype of GGPPS-/- deletion in Sertoli cells. A, Sertoli cells remained intact but spermatogonia decreased. B, The apoptosis of spermatogonia increased in KO mice. C, The invasion of macrophage increased in KO mice. D, FTI could rescue the fertility defect of KO mice

Gastroenterology

Altered Contractile Phenotypes of Intestinal Smooth Muscle in Mice Deficient in Myosin Phosphatase Target Subunit 1 (Dr.Minsheng ZHU's group)

Background

The regulatory subunit of myosin light chain phosphatase, MYPT1, has been proposed to control smooth muscle contractility by regulating phosphorylation of the Ca^{2+}-dependent myosin regulatory light chain. We generated mice with a smooth muscle–specific deletion of MYPT1 to investigate its physiologic role in intestinal smooth muscle contraction.

Significance

In this report, we specifically deleted MYPT1 in smooth muscle tissues by crossing *MYPT1*$^{flox/flox}$ mice with smooth muscle alpha-actin *Cre* mice. This is the first report indicates that MYPT1 is not essential for smooth muscle function in mice but regulates the Ca^{2+} sensitivity of force development and contributes to intestinal phasic contractile phenotype. Our results provide strong evidence that MYPT1 plays a critical role in the agonist-induced contraction/relaxation of the smooth muscle. Deletion of MYPT1 results in transition of phasic contraction to tonic like contraction, indicating that MYPT1 is a key molecular factor in smooth muscle. Our results could be applied to artificial sphincter in clinical therapy. Dr. Rattan S wrote a *CORRESPONDENCE* report titled "*Smooth Muscle–Specific Myosin Phosphatase Target Subunit 1 (MYPT1): An Important Piece of the Puzzle*" immediately after this work was published on *Gastroenterology*.

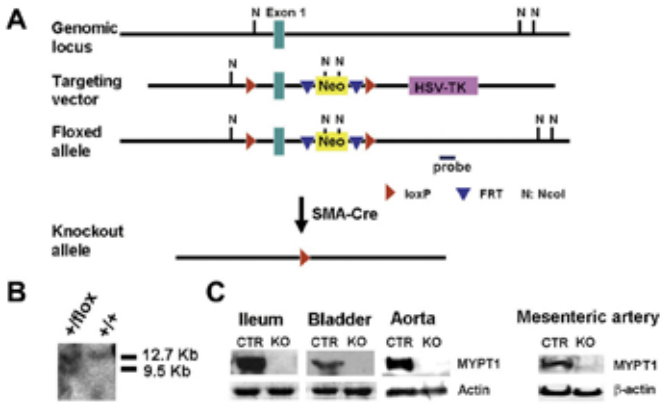

Figure 1. Ablation of the *Mypt1* gene in smooth muscle resulted in the loss of MYPT1 expression.

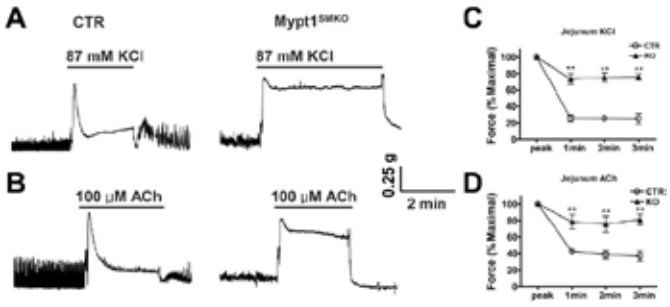

Figure 2. MYPT-deficient intestinal smooth muscles showed altered contractile properties.

BASIC AND TRANSLATIONAL—ALIMENTARY TRACT

Altered Contractile Phenotypes of Intestinal Smooth Muscle in Mice Deficient in Myosin Phosphatase Target Subunit 1

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GASTROENTEROLOGY 2013;■:1–2

CORRESPONDENCE

Smooth Muscle–Specific Myosin Phosphatase Target Subunit 1 (MYPT1):

An Important Piece of the Puzzle

SATISH RATTAN

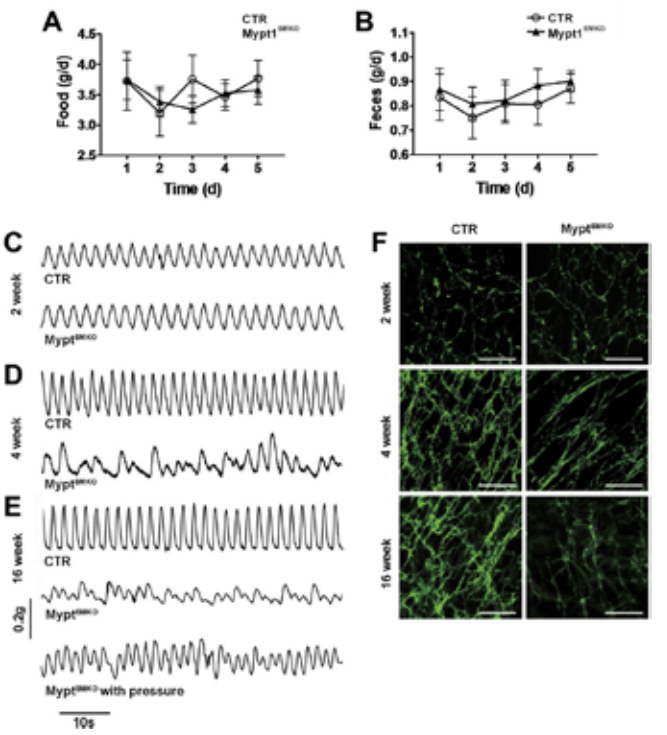

Figure 3. Normal food movement but progressive impairment of rhythmic contraction, ICC networks, and myoelectrical activity in Mypt1^{SMKO} intestine.

Dual roles of FBXL3 in the mammalian circadian feedback loops are important for period determination and robustness of the clock (Dr.Ying XU's group)

Summary

Circadian clocks allow organisms orchestrate the daily rhythms in physiology and behaviors, and disruption of circadian rhythmicity can profoundly influence human health. Deletion of *Fbxl3*, a component of a SKP1– CUL1–F-box (SCF) E3 ubiquitin ligase complex, results in ~27-hr long-period in mice, indicating that FBXL3 plays an important role in circadian period determination. Through a genetic interaction screen, we found that the deletion of *Rev-erba* in *Fbxl3* deficient mice rescued its long-period phenotype. Our studies revealed that FBXL3 not only promotes the degradation of CRYs to regulate E-box activity, as reported by other groups, but also modulates the RORE-mediated transcription in the auxiliary loop that provides a clear explanation for Fbxl3-/- mice with an extremely long period. Our results provided genetic evidence that: (i) deletion of Fbxl3 impairs the amplitudes of E-box-driven gene transcription; (ii) the abnormal retention of HDAC3 further contributes to a phase delay in the RRE-mediated transcriptional expression. We thus proposed that suppression at both E-boxes and RREs in the Fbxl3-/-mice contributes to extremely long period. This work suggests that the proper control and balance of transcriptional activity at the E-boxes of *Pers/Crys* and RREs of *Bma11/Crys* contribute significantly to the period determination of the clock in mammals. A rhythmic orchestration between RORE and E-box activity of two loops is dominant in setting the period of the circadian clock, as opposed to other factors that accelerate or decelerate the clock through the E-box-driven loop itself.

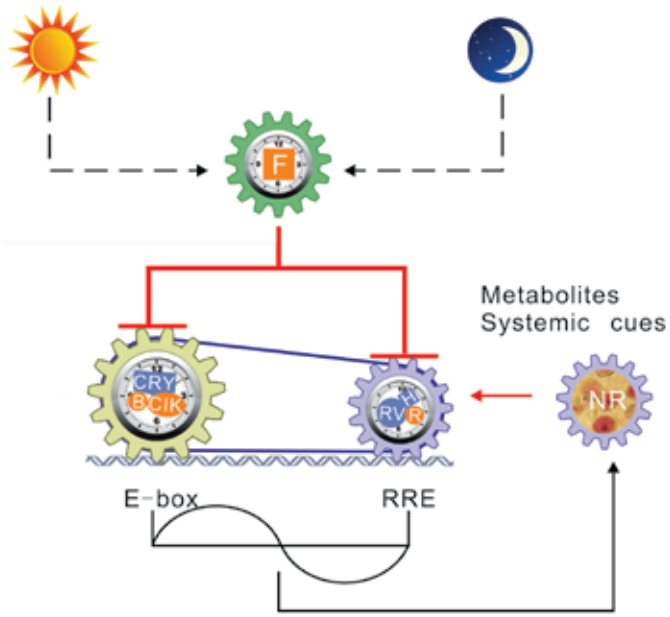

Fig. FBXL3 regulates Rev-Erbα:HDAC3 complex-mediated suppression.

Generation of gene-modified mice via Cas9/RNA-mediated gene targeting (Dr.Xingxu HUNAG's group)

Cell Research (2013) :1-4.

www.nature.com/cr

LETTER TO THE EDITOR

Generation of gene-modified mice via Cas9/RNA-mediated gene targeting

Cell Research advance online publication 2 April 2013; doi:10.1038/cr.2013.46

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems carry out the bacterial adaptive immune responses against virus by using RNAs to guide site-specific DNA cleavage. One type of these systems uses an RNA-guided nuclease (Cas9) to destroy invading DNA. We adapted this system in higher eukaryotes and demonstrated that it could site-specifically cut eukaryote DNA in zebrafish embryos. We further characterized the function of Cas9/RNA was improved by pre-annealing of chimeric RNA. Meanwhile, we added a NLS-flag-linker fragment to N terminal of Cas9, which enhanced Cas9/RNA activity and successfully localized Cas9 to nuclei of mammalian cells. Via embryo microinjection of this modified Cas9 mRNA and pre-annealed chimeric RNA, we successfully achieved endogenous gene knockout of mice for the first time. Our results demonstrated Cas9/RNA is a RNA-based new class of genome engineering approach which works well in mouse.

Publlications in 2013

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2	Ye S, Dongyang C, Zhihong X, Dongquan S, Jin D, Jianghui Q, Jizhen Q, Pu Y, Huacheng H, Wei S, Qing J (2013) The incidence of deep venous thrombosis after arthroscopically assisted anterior cruciate ligament reconstruction. Arthroscopy 29: 742-747.
3	Qin J, Xu Z, Shi D, Chen D, Dai J, Teng H, Jiang Q (2013) Deep vein thrombosis after total hip arthroplasty and total knee arthroplasty in patients with previous ischemic stroke. Int J Low Extrem Wounds 12: 316-319.
4	Xu Z, Shi D, Zhang C, Chen D, Dai J, Teng H, Jiang Q (2013) Postoperative plasma D-dimer value for predicting deep venous thrombosis following hip arthroplasty with nadroparin prophylaxis. Hip Int 23: 411-416.
5	Qin J, Dai J, Xu Z, Chen D, Shi D, Teng H, Jiang Q (2013) Genetic polymorphism of NOS3 with susceptibility to deep vein thrombosis after orthopedic surgery: a case-control study in Chinese Han population. PLoS One 8: e70033.
6	Wang C, Yang F, Xu Z, Shi D, Chen D, Dai J, Gu N, Jiang Q (2013) Intravenous release of NO from lipidic microbubbles accelerates deep vein thrombosis resolution in a rat model. Thromb Res 131: e31-38.
7	Wu X, Wang B, Dong Z, Zhou S, Liu Z, Shi G, Cao Y, Xu Y (2013) A NANOS3 mutation linked to protein degradation causes premature ovarian insufficiency. Cell Death Dis 4: e825.
8	Shi G, Xing L, Liu Z, Qu Z, Wu X, Dong Z, Wang X, Gao X, Huang M, Yan J, Yang L, Liu Y, Ptacek LJ, Xu Y (2013) Dual roles of FBXL3 in the mammalian circadian feedback loops are important for period determination and robustness of the clock. Proc Natl Acad Sci U S A 110: 4750-4755.
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21	Wang XH, Ha TZ, Liu L, Zou JH, Zhang X, Kalbfleisch J, Gao X, Williams D, Li CF (2013) Increased expression of microRNA-146a decreases myocardial ischaemia/reperfusion injury. Cardiovas Res. 97:432-42.
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35	Zhu Y, Ma A, Zhang H, Li C (2013) PPARgamma activation attenuates glycated-serum induced pancreatic beta-cell dysfunction through enhancing Pdx1 and Mafa protein stability. PLoS One 8: e56386.
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Seminar

	Date	Speaker	Title	Unit
1	2013-12-17	Dong Kong	Genetic and Optic Dissection of Neural Circuits Controlling	Havard Medical School
2	2013-12-17	Jianming Jiang	Delineation of molecular mechanisms of heart diseases using an adeno-associated virus mediated delivery system	Havard Medical School
3	2013-12-17	Sui Wang	Dissection of gene regulatory networks that control retinal cell fate specification	Havard Medical School
4	2013-12-17	Zhe Ji	Genome-wide analysis of alternative polyadenylation regulation during cell programming	Havard Medical School
5	2013-12-17	Guoji Guo	Studying stem cell differentiation by single cell gene expression analysis	Havard Medical School
6	2013-12-17	Huafeng Xie	Roles of polycomb repressive complex 2 (PRC2) in normal and malignant hematopoiesis	Havard Medical School
7	2013-12-17	Yuexiang Wang	Decoding Cancer Metastasis in the Genomic Era	Havard Medical School
8	2013-12-17	Jinyan Liu	Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys	Havard Medical School
9	2013-12-17	Hui Yang	One-Step Generation of Genetically Modified Mice by CRISPR/Cas-Mediated Genome Engineering	Havard Medical School
10	2013-12-17	Wei Song	Activin signaling mediates muscle-to-adipose communication in a mitochondria dysfunction-mediated obesity model Activin	Havard Medical School
11	2013-11-18	Wenyuan Wang	epigenetics regulation and genome instability in neurodegenerative disorders	MIT
12	2013-11-8	Wengong Wang	let-7 and cell longevity	Peking University
13	2013-11-8	Jane Y Wu	Neuronal Guidance, Cell migration & Cancer Metastasis	North-western Univeristy
14	2013-10-13	Xinchen Teng	Knockout-driven genome evolution and new insights into animo acid signalling	Johns Hopkins University
15	2013-9-25	Zhengping Jia	Role of actin-reorganization in synapse plasticity especially LTP.	University of Toronto
16	2013-9-3	Jie Du	Inflammation and cardiovascular remolding	Peking University
17	2013-9-2	Chongde Xiao	Zebra fish model of Hutchinson-Gilford Syndrome	Chung Yuan Christian University
18	2013-7-23	Chuan He	Reversible Methylation of DNA and RNA in Biological Regulation	University of Chicago
19	2013-7-25	Fahrenkrug Scott	Accelerated precision cross breeding of livestock animals	University of Minnesota
20	2013-7-8	Yinming Liang	Identifying novel genes involved in T-cell mediated inflammatory disease	Centre d'Immunologie de Marseille-Luminy

21	2013-7-4	ShaoKwan Chen	Hoxb8 knockout: A mouse model linking hematopoietic defects to behavioral abnormallities	Nanjing Institute of Gelolgy and Palaeonotology,Chinese Academy of Sciences
22	2013-7-2	Junyuan Chen	Acquired immunity in evolution	Nanjing Institute of Gelolgy and Palaeonotology,Chinese Academy of Sciences
23	2013-6-24	Jilong Liu	Cytoophidia, CTP synthase and Cancer	University of Oxford
24	2013-6-21	Liang Zhang	microRNA in skin Stem cell	Rockefeller University
25	2013-5-30	Dangsheng Li	How to publish in high profile journals	Institute of Biochem&Cell CAS
26	2013-5-30	Xiaolong Wei	Controlling Stem cell fate for Ovarian cancer therapy	MD Anderson Cancer Center
27	2013-5-27	Ho Yi Mak	Regulation of cellular fat storage at the ER-lipid droplet interface	Hong Kong University of Science and Technology
28	2013-5-25	Liqin Cao	Mitochondrial DNA inheritance in animals and subcellular intravital imaging in mice	RIKEN Japan
29	2013-5-16	Hans Bueler	Mitochondrial and signaling defects in PINK1-deficient mice reveal mechanism of Parkinson's disease pathogenesis and a link to diabetes	University of Kentucky
30	2013-5-16	Minrong Ai	Distinct olfactory circuits mediate attraction and avoidance behaviors in Drosophila	New York University
31	2013-4-25	Zhenji Gan	A nuclear receptor-microRNA circuit links control of muscle fiber type to energy metabolism	Sanford-Burnham Medical Research Institute
32	2013-4-25	Lijian Hui	Change of Cell Identity: Tumorigenesis and Lineage Conversion	Institute of Biochem&Cell CAS
33	2013-4-16	Jinzhong Qin	L3MBTL2:a new Player in Early Development and Pluripotent Stem	Harvard Stem Cell Institute,Harvard Medical School
34	2013-3-28	Duanqing Pei	Cell fate decision and reprogramming	Guangzhou Institue of Biomed&Health, CAS
35	2013-3-23	Feng Shao	Biochemical dissection of bacterial virulence and macrophage innate immunity	National Institute of Biological Science
36	2013-3-23	Minmin Luo	Functional Dissection of Neural Circuits	National Institute of Biological Science
37	2013-3-13	Yang Hong	Polarity Protein Dynamics and Hypoxia in Drosophila	University of Pittsburgh School of Medicine
38	2013-3-12	Ziqiang Yang	The role of Gab/PAR complexes in cell polarity and EMT	Ontario Cancer Institute, Toronto Medical Discovery Tower, MaRS Centre
39	2013-2-21	Axton Myles	Publishing your Work in Nature Journals	Nature Genetics
40	2013-1-30	Weijie Shu	Bioinformatics in cross genome studay	Military medical insitute,Beijing

Ying Cao, Ph.D.

Cao Ying made the PhD study at the University of Essen (now University of Duisburg-Essen), Germany, from 1998 to 2002. During the period he performed a screening of novel genes involved in the early embryogenesis of Xenopus laevis and identified a few new genes that play essential roles in Xenopus embryonic development. In 2002, he earned PhD degree and graduated summa cum laude. Afterwards during the years from 2002 to 2008 he joined the Institute of Biochemistry, University of Ulm, Germany, and continued the study on Xenopus development, especially on the molecular mechanisms underlying germ layer formation. From October 2008, he was offered the professor at MARC and set up the laboratory for Xenopus developmental biology.

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Xenopus early embryonic development

Wnt/β-catenin signaling mediates numerous aspects of cellular functions, and therefore plays essential roles not only in developmental biology, but in stem cell biology and cancer biology as well. Although the regulation of Wnt/β-catenin signaling has been investigated extensively, how β-catenin within the nucleus is regulated remains dubious. In our research we have found that the protein demethylase Jhdm1a inhibits the Wnt-responsive reporter Topflash, suggesting that the enzyme may regulate Wnt/β-catenin signaling. We have found that Jhdm1a affects the stability of β-catenin in a ubiquitin/proteosome pathway dependent fashion, raising the possibility that Jhdm1a might mediate Wnt/β-catenin signaling via the regulation the turnover of β-catenin (Figure 1). Further investigation reveals that Jhdm1a specifically induces the degradation of the nonphosphorylated form of β-catenin within nucleus (Figure 2). Mechanistic study shows that nucleus b-catenin can be methylated, and the methylation can be reversed in the presence of Jhdm1a. Deletion of the region with potential methylation or mutation of the potentially methylated lysine residues leads to failure of methylation and subsequently degradation of β-catenin. We hence put forward a novel model for the regulation of nucleus -catenin turnover, in which methylated β-catenin is demethylated by Jhdm1a, and afterwards is degraded via ubiquitination. We also analyzed the function of Jhdm1a during Xenopus embryogenesis. Jhdm1a gene is transcribed ubiquitously during early stages of development of embryogenesis. Loss of Jhdm1a function in Xenopus embryos leads to disruption of dorsoventral axis formation (Figure 3) and transcription of Wnt/ -catenin target genes. These findings elucidated the mechanism how Wnt/β-catenin signaling is controlled when it is functioning or at the end of Wnt function.

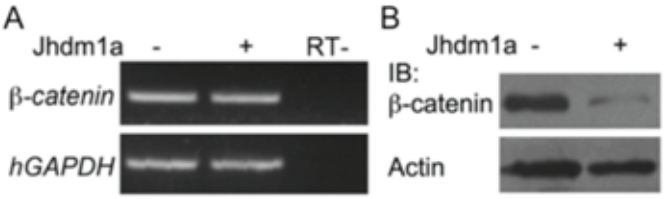

Figure 1. Overexpression of Jhdm1a in cells does not affect the transcription of β-catenin (A) but reduces the level of protein (B).

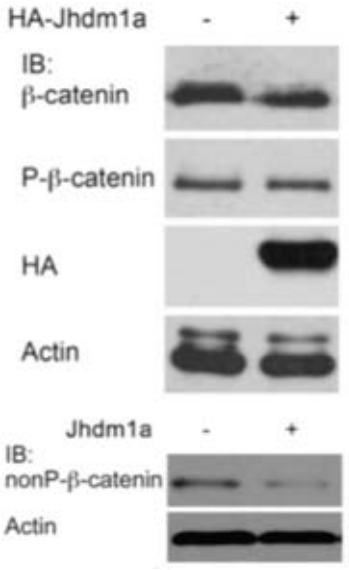

Figure 2. Jhdm1a induces degradation of total β-catenin (β-catenin) and nonphosphorylated form of β-catenin (nonP-β-catenin), but not the phosphorylated form of β-catenin (P-β-catenin).

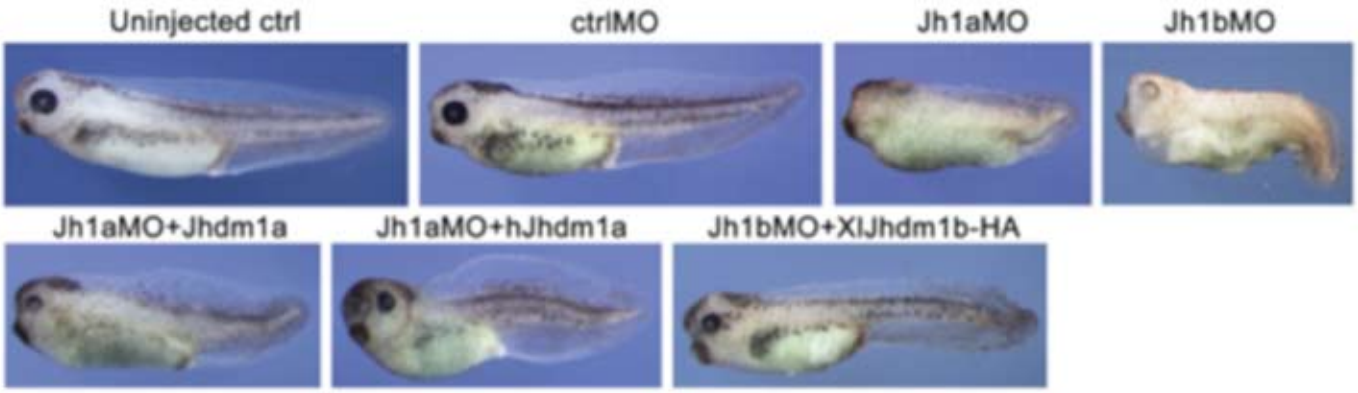

Figure 3. Knockdown of Jhdm1a or its close homologue Jhdm1b with antisense morpholino oligos (Jh1aMO and Jh1bMO) leads to malformation of body axis in Xenopus embryos, while the uninjected embryos or embryos injected with a control morpholino shows normal axis development. The morphants can be rescued nicely with microinjection of their respective mRNAs.

Selected publications

1. Lu L, Gao Y, Zhang Z, Cao Q, Zhang XN, Zou JH, Cao Y*. (2013). The lysine demethylases Jhdm1a/b regulate axis patterning during embryogenesis via mediating the stability of nuclear β-catenin. Dev. Cell (Under revision).
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4. Cao Y, Oswald F, Wacker SA, Bundschu K, Knochel W. (2010) Reversal of Xenopus Oct25 function by disruption of the POU domain structure. J Biol Chem. 285:8408-8421
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6. Cao Y, Siegel D, Donow C, Knochel S, Yuan L, Knochel W. (2007) POU-V factors antagonize maternal VegT activity and beta-Catenin signaling in Xenopus embryos. EMBO J 26: 2942-2954.

Lab members

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- **Graduate students**
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- **Technicians**
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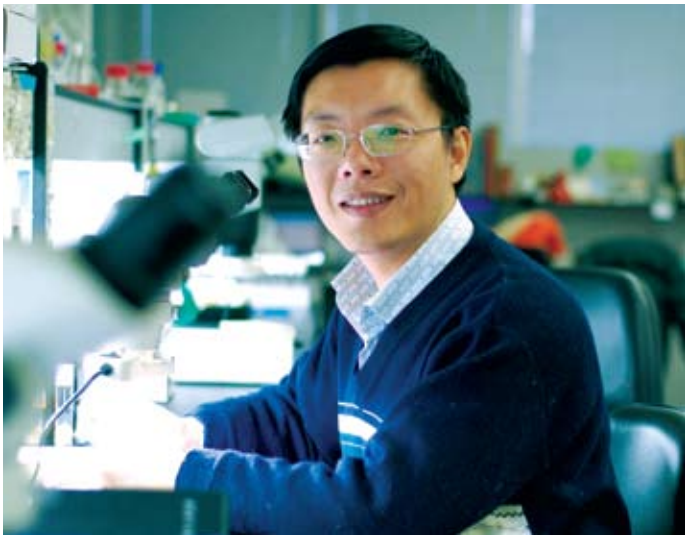

Jiong Chen, Ph.D.

Jiong Chen received his Bachelor in Biochemistry (1995) and his Ph.D. in Molecular, Cell and Developmental Biology (2002), both from University of California, Los Angeles (UCLA). His Ph.D. thesis was carried out in Frank Laski's lab and it was focused on the genetics and developmental studies of cell movement processes in the Drosophila ovary. From 2002 to 2004, Jiong did his postdoctoral research in Drosophila eye development under the guidance of Utpal Banerjee at UCLA, and it was combined with an undergraduate teaching experience that was funded by a HHMI teaching/ research grant. He joined the Faculty of Model Animal Research Center (MARC), Nanjing University full time early 2005. He is now a professor of genetics and developmental biology and a principle investigator in MARC.

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Understanding the driving forces behind morphogenesis

My lab is mainly interested in how morphogenetic processes such as cell migration and epithelial morphogenesis are regulated during development. My lab has employed a mainly genetic and functional genomics approach, using the model animal Drosophila melanogaster and cell biological techniques to conduct most of the experiments. And there are three model systems that we mainly use in the lab: migrating border cells in the Drosophila ovary and the epithelia of larval eye disc and the ovarian follicles (Fig. 1-3).

Border cell migration is an excellent in vivo and genetically tractable system to study molecular mechanism underlying guided migration or chemotaxis, and the tumor-like invasive migration of border cells through large germline tissues can also be used as a model to identify novel genes essential for cell migration in development as well as tumor metastasis in cancer (Fig. 1). In addition, since 6-10 border cells always migrate as a coherent cluster, it has been recently used as a model system to study collective cell migration, which is prevalent in morphogenesis, cancer and regeneration. Currently, we are interested in the following three questions. 1. How extracellular factors (gradients) guide the cluster of border cells and generate asymmetry within cluster? 2. How signaling pathways affect actin cytoskeleton? And through what actin dynamics regulators? 3. Are there novel regulatory mechanisms that link other important cellular process with cell migration? Below is a list of three projects (1-3) ongoing in the lab to address these questions.

The single layer of developing follicle epithelium (during oogenesis) is a system that we used to probe the mechanism of apical-basal polarity generation and maintenance. We are currently studying the role of Dlg5 in apical polarity formation and maintenance (Project 3 below, Fig. 3). We are also using AIP1 conditional knockout mouse to address if AIP1's roles in epithelial morphogenesis of eye disc are conserved in mammals (Projects 4 and 5, in collaboration with Dr. Zhongzhou Yang's lab and Dr. Xingxu Huang's lab).

Below is a list of projects currently going on in the lab.

1. Mechanism of RTK's asymmetry generation during collective migration of border cells in Drosophila ovary. (Fig. 1, 2)

2.Mechanism of coupling other cellular processes with migratory machinery during border cell migration.

3. The roles of Dlg5 in regulation of apical polarity formation and maintenance in follicle epithelial cells. (Fig. 3)

4. Adherence junction (AJ) remodeling during epithelial morphogenesis in Drosophila eye development.

5. Establish various mouse models with AIP1 conditional knockout.

6. Cofilin and AIP1's roles in bristle morphogenesis.

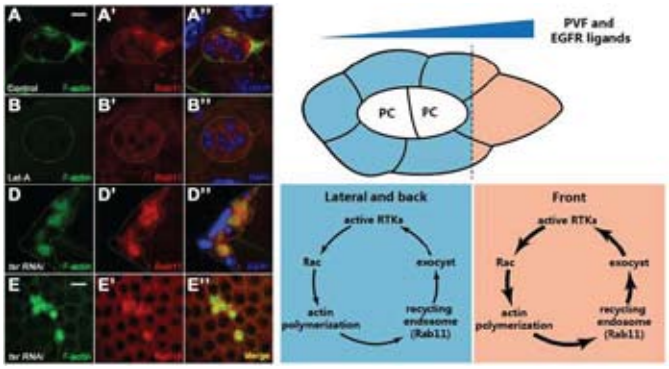

Figure 1. A model showing a positive-feedback loop promoting guided collective migration of border cells. (Taken from Wan et al, 2013)

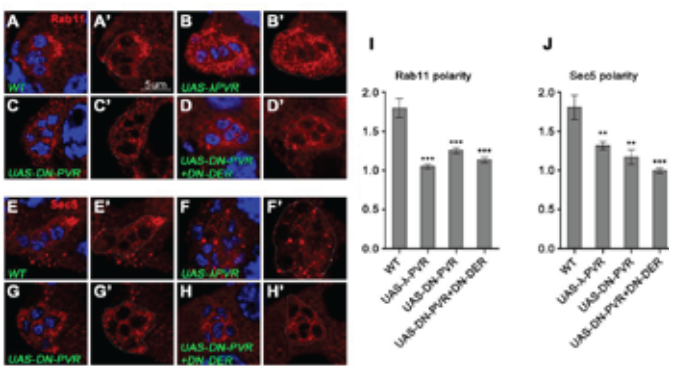

Figure 2. The guidance receptors PVR and EGFR mediate front-back asymmetry of Rab 11 (recycling endosome) and Sec 5 (exocyst) within a border cell cluster. (Taken from Wan et al, 2013)

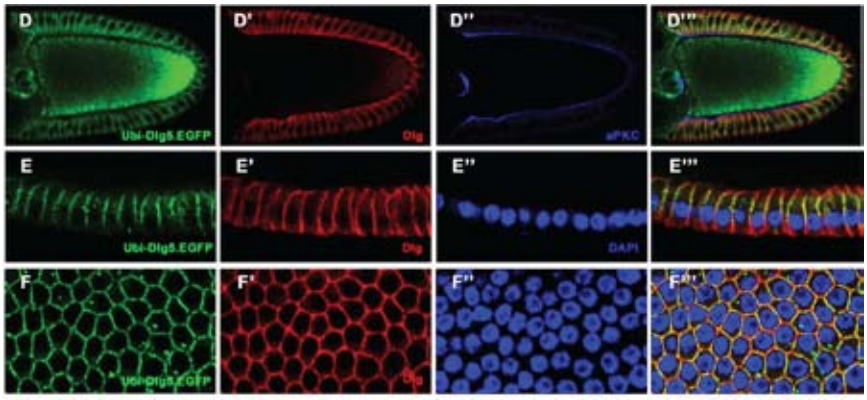

Figure 3. Dlg5 is involved in the formation and maintenance of apical polarity of the follicle epithelium in Drosophila ovary

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7. Couderc, J., Godt, D., Zollman, S., Chen, J., Tiong, S., Li, M., Cramton, S., Sahut-Barnola, I., and Laski, F. The bric à brac locus consists of two paralogous genes encoding BTB/POZ domain proteins, and acts as a homeotic and morphogenetic regulater of imaginal development in Drosophila. Development 129(10): 2419-33 (2002).
8. Chen, Jiong, Dorothea Godt, Kris Gunsalus, Istvan Kiss, Michael Goldberg and Frank A. Laski. Cofilin/ADF is required for cell motility during Drosophila ovary development and oogenesis. Nature Cell Biology 3(2), 204-209 (2001).

Lab members

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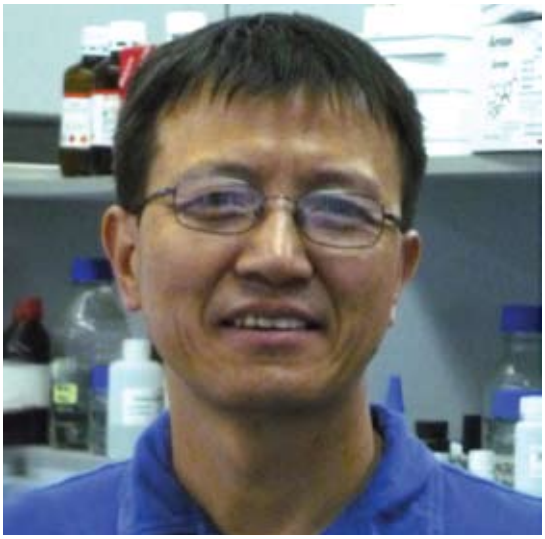
- **Former graduate students**
  Lijun Zhang (Ph.D), Ping Wan (Ph.D), Dandan Chu (Ph.D), Juntao Zuo (M.S.)



Xingxu Huang, Ph.D.

Xingxu Huang received his Ph.D. degree from Nanfang Medical University (Guangzhou, China) in 1998. From 2001 to 2008, Xingxu did his postdoctoral research on roles of cell cycle regulator in development and diseases under the guidance of Dr. Pumin Zhang in Baylor College of Medicine (Houston, Texas, USA). He joined the Faculty of Model Animal Research Center (MARC), Nanjing University in 2008. He is now a professor of genetics and developmental biology and a Principal Investigator in MARC.

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Development of SSCs & modeling of infertility-causing mutations or SNPs

The germ cell lineage is the only lineage that is responsible for the transmission of the genetic information across the generations. To keep genome stability, the chromosome segregation of the PGCs (primordial germ cells), the founders of germ cells, should be strictly regulated. Meanwhile, the spermatogonial stem cells (SSCs) are the only stem cell population that contributes genetic information to subsequent generations, suggesting the specificity of SSC pluripotency. To reveal how SSC regulates its pluripotency, we have been focusing our current efforts on.

1. Investigating the regulation of quiescence and activation of SSCs

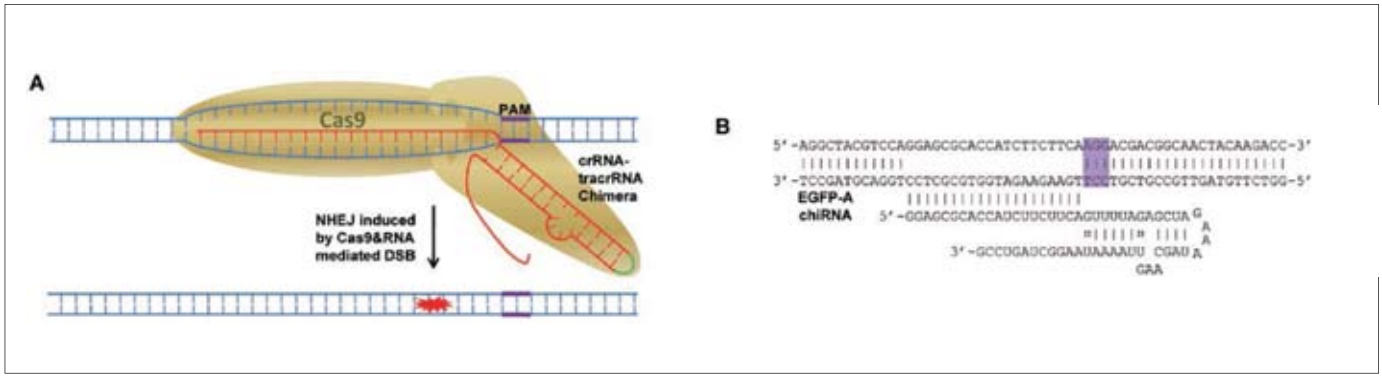
Quiescence is critical for the maintenance of pluripotency of stem cells. The molecular mechanisms underlying stem cell quiescence and activation remains unclear because the absence of study model. It has been demonstrated recently, P57, one of the CDK inhibitors which regulate G1/S transition, is required for hematopoietic stem cells (HSCs) quiescence. P57 binds to Npat (nuclear protein, ataxia-telangiectasia locus), which is involved in histone synthesis. Employing SSC neonatal development as a study model, we have been studying stem cell quiescence by exploring Npat function in gonocyte, the progenitor of SSC, quiescence and activation. Our results showed Npat is critical for gonocyte quiescence by organizing heterochromtin structure, then keep transcriptional silencing.

2. Dissecting dependence of the niche accessibility of SSCs on F-actin dynamics

Under the navigation of multiple guidance factors, stem cells migrate, access and reside in niche, which provides signals to control the pluripotency of the stem cells. The molecular mechanism underlying the niche accessibility remains unclear. The SSCs relocation from the lumen to the basal compartment of the seminiferous tubule, the niches of SSCs, provides an ideal model to study stem cell niche accessibility. Actin-interacting protein 1 (Aip1) plays an important role in cell migration via promoting rapid turnover and reassembly of actin filaments. By generating germ cell and Sertoli cell specific Aip1 knockout mouse model then examining the SSC relocation, we have been studying the mechanisms of niche accessibility of stem cells. Our preliminary data showed Aip1 deletion resulted in F-actin dissembled abnormally, leading to the spermatogenesis failure caused by SSC unaccessible to their niche, then being degenerated.

3. Developing genome manipulation technologies

Successful gene manipulations have been achieved by reprogrammable endonucleases, including ZFN and TALEN. Adoption and use of ZFN and TALEN have been hindered by lack of an efficient strategy for engineering ZFN and TALEN pairs. We have developed a novel ZFN design and test strategy, and a TALEN assembly library. Meanwhile, we successfully applied a RNA-programmed CRISPR-Cas9 for and genome targeting (Fig. 1). Now we are improving this system by using Cas9-assisted homologous recombination to generate conditional knockout rat model, optimizing dual sgRNA and Cas9 nickase to prevent off-target mutation, developing a high through-put strategy to express multiple sgRNAs, etc.



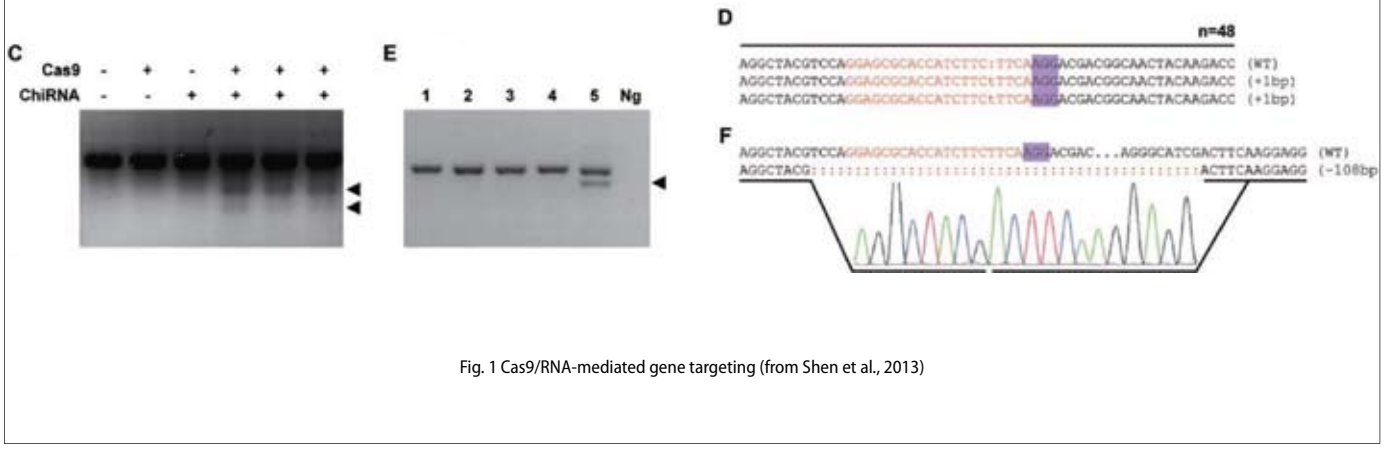


Fig. 1 Cas9/RNA-mediated gene targeting (from Shen et al., 2013)

Selected publications

1. Ma Y, Zhang X, Shen B, Lu Y, Chen W, Ma J, Bai L, Huang X*, Zhang L*. Generating rats with conditional alleles using CRISPR/Cas9. Cell Research. (In press)
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3. Hou J, Xia Y, Jiang R, Chen D, Xu J, Deng L, Huang X*, Wang X, Sun B. PTPRO plays a dual role in hepatic ischemia reperfusion injury through feedback activation of NF-κB. J Hepatol. 2013 Oct 12. doi:pii: S0168-8278(13)00694-6. 10.1016/j.jhep.2013.09.028. [Epub ahead of print
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  Jianying Wang
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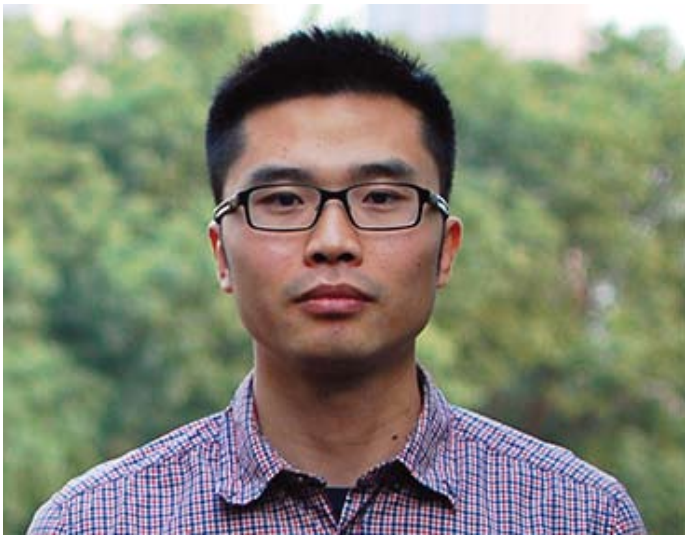
- **Trainee in collaboration**
  Ke Ma
  Hongya Wu



Xin Lou, Ph.D.

Xin Lou got his Ph.D. in Shanghai Institute of Biochemistry and Cell Biology, CAS in 2008. He was supervised by Prof. Xiaoyan Ding to study body axis patterning in vertebrate. He did post-doctoral training in Dr. Ian Scott's lab at the Hospital for Sick Children, Toronto, where he studied the molecular mechanisms of cardiomyocyte differentiation. He joined the Model Animal Research Center (MARC), Nanjing University as a Principle Investigator in 2013.

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Cardiovascular system development and regeneration

Congenital defects and adult-onset cardiovascular disease are among the most critical health problems throughout the world. A greater understanding of the process of cardiogenesis will ultimately be essential for developing new approaches for curing and diagnosing heart defects. Zebrafish is an ideal model to study cardiovascular development and regeneration; researchers are working with this tiny fresh water fish to illustrate the delicate molecular mechanisms regulating these processes.

Currently, our research focuses on the following aspects:

REVEAL THE DYNAMIC CHANGE AND ROLE OF EPIGENETIC REGULATION IN HEART REGENERATION

The mammalian heart is incapable of significant regeneration following injury such as an acute myocardial infarction. Unlike the mammalian heart, the injured zebrafish heart normally undergoes minimal scarring and in 30 days the transient fibrin clot is replaced with new contractile muscle. Epigenetic regulation involves all stages of cellular processes in cardiac regeneration: stress-response, re-entry into mitotic cell cycles, "de-differentiation" and re- establishment of mature cell types. We applied transcription array and proteomics approaches to profile the dynamic change of epigenetic regulators in heart regeneration, the results showed the components of PRC2 complex (a chromatin remodeling complexes) may play important roles in this event and detail function analyz are ongoing.

IDENTIFICATION OF NOVEL REGULATORS OF CARDIOVASCULAR DEVELOPMENT AND REGENERATION

Zebrafish is widely used model organism for investigating organogenesis. The rapid external development, optical clarity, and large number of embryos laid allows scientist observe early developmental events lively and applied a wide range of method to understood organ formation. Recently the zebrafish molecular genetic toolbox has expanded to include sophisticated approaches including the Cre-loxP system, transposon-mediated transgenesis and gene modification via use of nucleases. We optimized a "gene-breaker" transposon system, which both recapitulates endogenous gene expression and disrupts gene function to generate a null allele of the trapped gene. By using this system, 35 trapping fish line have been established and we are trying to identify new heart development/regeneration genes and analyze their biological function.

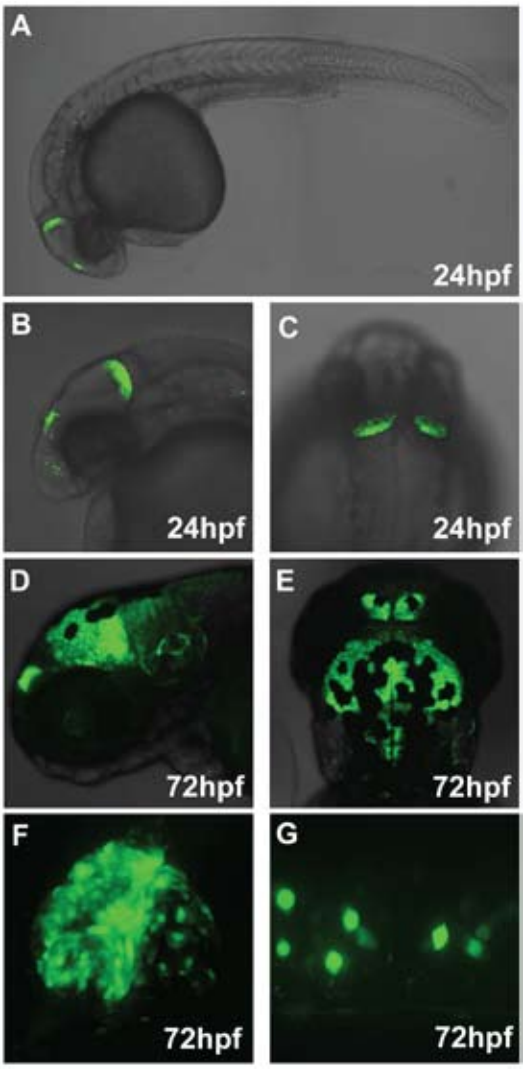

Fig1. GFP expression in RP22-589 gene trapping zebrafish embryos.

A-C: GFP expression starts from the 24hpf and is restricted to midbrain-hindbrain boundary and a subset of forebrain neurons. D-G: The GFP expression expands to whole brain (D and E) at 72hpf, heart (F) and motor neuron (G).

Selected publications

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  Jinrong Ma
- **Lab manager**
  Peipei Yin
- **Intern students**
  Yu Zhou
- **Administrative assistant**
  Yijun Huai

Zhongzhou Yang, Ph.D.

Zhongzhou Yang was trained in the Department of Biochemistry & Molecular Biology at the Beijing Medical University during the time 1994-97 and was awarded a Master's degree. In between 1998 and 1999, he worked in the University of Pennsylvania as a visiting scholar. From 1999 till 2005, he pursued PhD and postdoctoral training in the Friedrich Miescher Institute for Biomedical Research (Novartis Research Foundation) /University of Basel, Switzerland on mouse genetics. He was appointed professor in the Model Animal Research Center in 2005.

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Heart development & regeneration

In the mouse, gastrulation starts at embryonic day 6.5 (E6.5). Formation of the anterior visceral endoderm (AVE) determines the antero-posterior (A-P) axis and the primitive streak (PS) is induced in the posterior epiblast. Cells in the epiblast epithelium with proximity to the PS proliferate, delaminate and migrate anterior and lateral to specify into cardiac mesoderm, which initiates early heart development. A hierarchy consisting of signaling molecules and transcription factors regulate cardiac mesoderm specification and development. We are aimed to elucidate the cellular and molecular mechanisms underlying early heart developmental processes, such as cardiac mesoderm specification, proliferation and differentiation as well as migration of the cardiac progenitors in mouse models.

Wntless regulates cardiac mesodermal formation through Nodal

At E6.5, the transcription factor Eomesodermin (Eomes) can directly activates the expression of Mesp1. As the cardiac master regulator, Mesp1 regulates the expression of a panel of key transcription factors in heart development including Hand2, Nkx2.5, Gata4 and Mef2c. Therefore, Eomes-Mesp1 controls cardiac mesodermal development. However, the relationship between Eomes-Mesp1 and the developmental signals such as Wnt and BMP is elusive.

Wntless (Wls) regulates germ layer development with unknown mechanisms. We deleted Wls in the epiblast using Sox2-Cre and observed significant reduction of expression levels or absent expression of Mesp1 and Mlc2a in E7.5 embryos (Fig. 1). These results suggest of defects in cardiac mesodermal development.

Furthermore, we analyzed the expression of Eomes and Mesp1 in E6.5 epiblast and found profoundly reduced expression levels in Wls deletion mice compared to control (Fig. 1). We then investigated the expression of Bmp4 and Nodal and detected substantial reduction of Nodal expression levels (Fig. 1).

Study of the second heart field (SHF) progenitor development

By E8.5, cardiac mesoderm develops into linear heart tube that contains cardiamyocytes and endothelial cells. The ardiac progenitors located in the splanchnic mesoderm (SM) and pharyngeal mesoderm (PM) migrate to the heart tube, contributing to the development of the right ventricle and outflow tract (OFT). These cardiac progenitors are called the second heart field (SHF) progenitors.

The properties of SHF progenitors are continuous proliferation and differentiation delay. BMP, Wnt and FGF signaling regulate the SHF development. We investigated the role of Akt signaling, Hand2 transcription factor and regulator of actin dynamics in SHF development.

Akt signaling regulates SHF progenitor proliferation

Either enhancing or decreasing Akt activity in the SHF progenitors impairs heart development. We found that Akt signaling promotes SHF progenitor proliferation (Fig. 2).

Hand2 transcription factor promotes SHF progenitor proliferation

Deletion of Hand2 in mice causes defects in the right ventricle and OFT without clear mechanisms. We deleted Hand2 in the SHF progenitors and observed impeded SHF progenitor proliferation. The expression levels of Fgf ligands was greatly reduced in the SHF upon Hand2 deletion, suggesting that FGF-ERK signling may function downstream of Hand2 in the SHF (Fig. 3).

Wdr1 may regulate SHF progenitor migration

Wdr1 is involved in the regulation of actin dynamics that regulates cell motility and migration. We deleted Wdr1 in the SHF progenitors and found SHF developmental defects (Fig. 4).

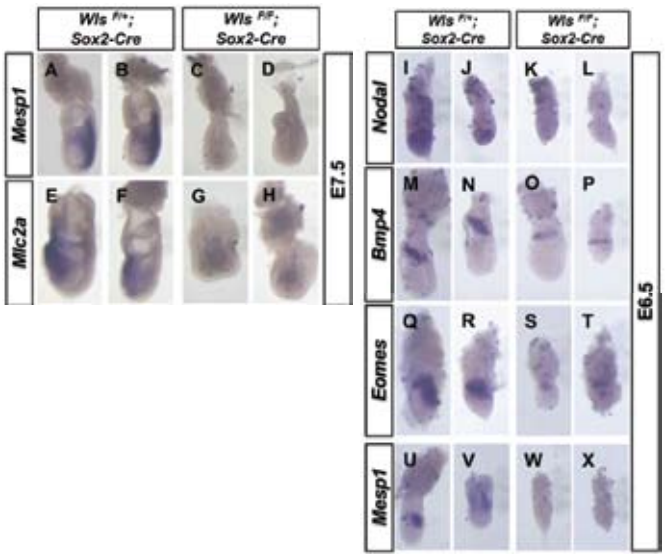

Fig. 1. Gene expression analysis in early mouse embryos.

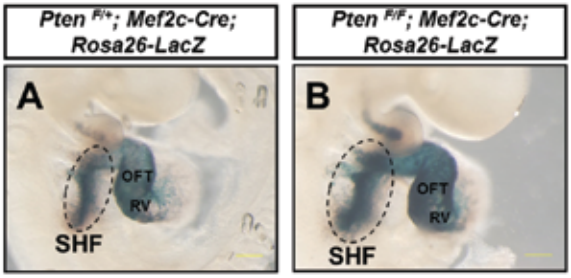

Fig. 2. Lac Z staining to display the OFT and RV.

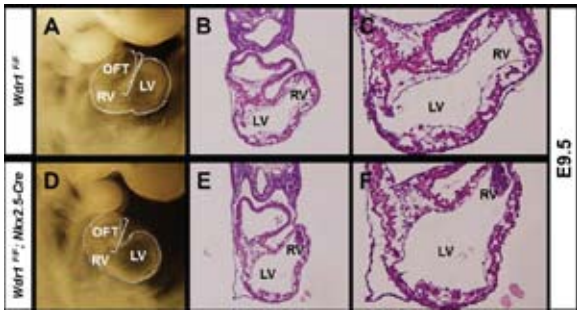

Fig. 4. Cardiac progenitor-deletion of *Wdr1*.

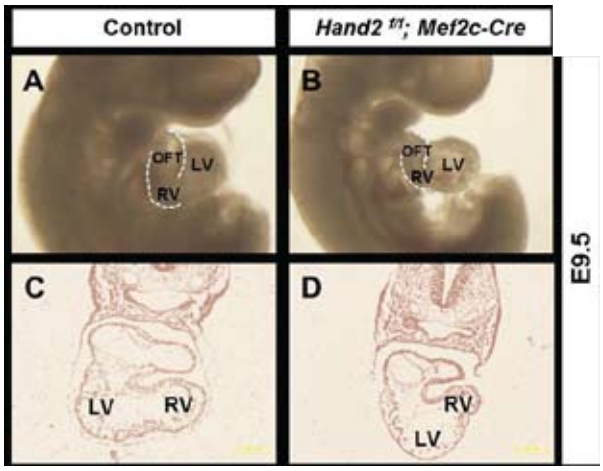

Fig. 3. SHF progenitor-deletion of Hand2.

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1. Shuilong Guo, Hui Ye, Yan Teng, Youliang Wang, Guan Yang, Xiubin Li, Chong Zhang, Xue Yang, Zhongzhou Yang and Xiao Yang. (2013) Akt-p53-miR-365-cyclin D1/cdc25A axis contributes to gastric tumorigenesis induced by PTEN deficiency. *Nat. Commun*. 4:2544.
2. Xiangqi Wu, Yunshan Cao, Junwei Nie, Hailang Liu, Shuangshuang Lu, Xiaoshan Hu, Jingai Zhu, Xia Zhao, Jiandong Chen, Xiaohu Chen, Zhongzhou Yang* and Xinli Li*. (2013) Genetic and pharmacological inhibition of Rheb1-mTORC1 signaling exerts cardioprotection against adverse cardiac remodeling in mice. ***Am. J. Pathol.*** 182: 2005-2014. (*co-corresponding author)
3. Jing-wen Yin, Yan Liang, Ji Yeon Park, Dongrui Chen, Xiao Yao, Qi Xiao, Zhen Liu, Bo Jiang, Yu Fu, Menghan Bao, Yan Huang, Yuting Liu, Jun Yan, Min-sheng Zhu, Zhongzhou Yang, Pingjin Gao, Bin Tian, Dangsheng Li and Gang Wang. (2012) Mediator MED23 plays opposing roles in directing smooth muscle cell and adipocyte differentiation. *Genes Dev.*
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5. Ruomin Di, Xiangqi Wu, Zai Chang, Xia Zhao, Qiuting Feng, Shuangshuang Lu, Qing Luan, Brian A. Hemmings, Xinli Li and Zhongzhou Yang. (2012) S6K inhibition renders cardiac protection against myocardial infarction through PDK1 phosphorylation of Akt. *Biochem. J.* 441:199-207.
6. Zai Chang, Qin Zhang, Qiuting Feng, Jie Xu, Teng Teng, Qing Luan, Congjia Shan, Yali Hu, Brian A Hemmings, Xiang Gao and Zhongzhou Yang. (2010) Deletion of Akt1 causes heart defects and abnormal cardiomyocyte proliferation. *Dev. Biol.* 347: 384-391.
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Qing Zhang, Ph.D.

Qing Zhang received his Ph.D in Microbiology from Fudan University in 2002. Afterwards, he had had his postdoctoral training in Department of Developmental Biology of UT Southwestern Medical Center at Dallas for six years. In 2009, he joined the Model Animal Research Center of Nanjing University as a professor and principle investigator.

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Regulation of hedgehog signaling

Research in my lab is mainly focused on two fields: one is the regulation of Hedgehog signaling, the other is oogenesis of Drosophila ovary.

Hedgehog signaling plays critical role in embryonic development and adult tissue homeostasis in species ranging from insects to human. Aberrant Hh signaling activity is associated with many human disorders including birth defects and cancers.

In Drosophila, Hh tansduces signal through binding its receptor, a 12-transmembrane protein Patched (Ptc), that alleviates suppression of Ptc on Smoothened (Smo),a GPCR-like seven-transmembrane protein. Active Smo as the Hh signal transducer triggers a largely conserved signaling cascade that culminates at the activation of latent transcription factors Cubitus interruptus (Ci) which controls Hh targets decapentaplegic (dpp), ptc and engrailed (en) expression .

Based on Hh pathway is conserved among species, we take advantage of Drosophila as a model to investigate the mechanism of Hh signaling. Our work mainly focuses on getting the whole picture of around 7000 conserved genes on Hh signaling regulation and trying to answer the long-standing questions, for example, how does Ptc, the membrane receptor of Hh, inhibit Smo activity? how is hyperactive Ci degraded? and so on.

We also investigate the mechanism of Drosophila oogenesis and try to get some clues for helping understand the underlying mechanism of human Premature ovarian failure (POF). POF which is the loss of function of the ovaries before age 40 affects 1-4% of the population. POF is caused by genetic disorders, autoimmune damage, chemotherapy and infection, the mechanism underlying POF is elusive. The evidence showed Drosophila ovary aging dramatically reduced the egg production, so we use the numbers of eggs and hatched flies as judge criteria to select genes which may affect Drosophila ovary aging. Through the work, we hope we can uncover some genes' function in both Drosophila and human ovary aging.

We search the genes expessed in gonad and identify an interesting candidate gene, dpl, which plays an essential role in oogenesis of Drosophila. We find that the homozygous hypomoth mutant females cannot lay any eggs, the most of the egg chambers can only develop into the stage 12. and the apoptosis of nurse cells is delayed. There are also a few stage 13 egg chambers which have defects in formation of dorsal appendages. dpl is mainly expressed in follicle cells from stage 5 to stage 14 egg chambers, implying it may function in somatic cells rather than germ cells. In the future work, we will focus on uncovering the signal sent by the somatic cells which affects germ cell development.

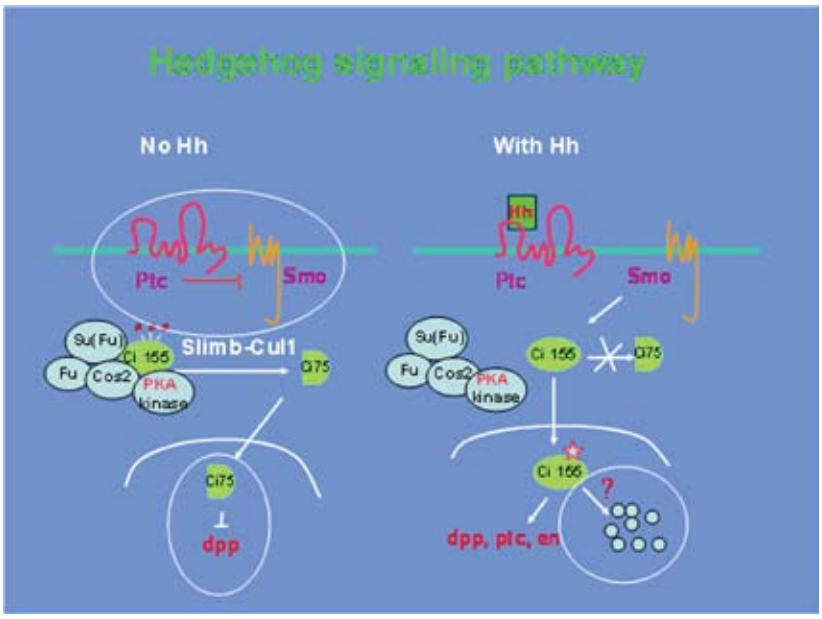

Fig1. Hedgehog signaling pathway in Drosophila.

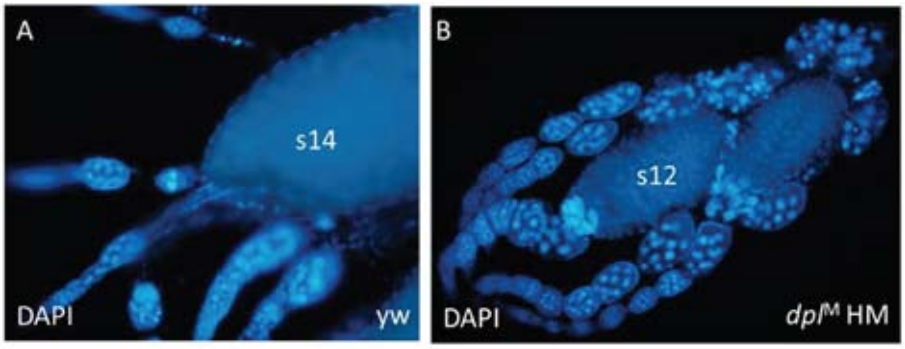

Fig.2. Oogenesis defect in dpl mutant homozygotes.

(A) In yw flies, oogenesis is normal.

(B) In dpl mutant homozygotes, adult females cannot lay any eggs. The egg chambers show persistent nurse cell nuclei and the development process arrests in stage 12. The egg chambers also have defect in dorsal appendage formation.

Selected publications

1. Zhang Q, Shi Q, Chen YB, Yue T, Li S, Wang B, and Jiang J (2009) Multiple Ser/Thr rich degrons mediate the degradation of Ci/Gli by the Cul3-HIB/SPOP E3 ubiquitin ligase. PNAS 106, 21191-21196..
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3. Zhang Q, Zhang L, Wang B, Ou CY, Chien CT, Jiang J (2006) A Hedgehog-induced BTB protein modulates Hedgehog signaling responses by degrading Ci/Gli transcription factor. Developmental Cell 10, 719-729.
4. Jia J, Zhang L, Zhang Q*, Tong C, Wang B, Hou F, Amanai K, Jiang J (2005) Phosphorylation of Cubitus interruptus by Double-time/CKI and CKI targets it for Slimb/ -TRCP mediated proteolytic processing. Developmental Cell 9, 819-830. (*Co-first author)

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  Wan Gao, Ping Chen, Xia Yao

Qingshun Zhao, Ph.D.

Qingshun Zhao obtained his B.S. degree in 1987 and M.S. degree in 1990 from Nanjing University (Nanjing, Jiangsu, China), and received his Ph.D. degree in 2001 from Purdue University (West Lafayette, Indiana, USA). His Ph.D. dissertation was carried out in Dr. Paul Collodi's Lab and it was focused on identification and characterization of fibronectin isoforms during zebrafish development. From 2001 to 2003, Qingshun did his postdoctoral research on roles of retinoid signaling in zebrafish hindbrain development under the guidance of Dr. Elwood Linney in Duke University Medical Center (Durham, North Carolina, USA). In 2003, he became an associate professor and a principal investigator in Model Animal Research Center, Nanjing University. In 2006, Qingshun was promoted full professor of Nanjing University.

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Zebrafish development lab

The research interests of the lab focus on investigating the roles of retinoic acid (RA) signaling in vertebrate early development, such as embryonic patterning and germ layer differentiation, using zebrafish as a model animal.

RA plays essential roles in vertebrate embryogenesis through heterodimers of retinoic acid receptors (RAR) and retinoid-X receptors (RXR) that bind to RA response elements (RAREs) in the regulatory region of target genes. In the absence of RA, the DNA-bound receptors recruit co-repressors to actively repress expression of target genes. Upon binding of RA to the receptors, however; the co-repressors are released from DNA-associated heterodimers and co-activators are recruited, leading to activation of target genes. Therefore, the spatial and temporal presence of RA is crucial for vertebrate embryos to develop.

In vertebrates, RA homeostasis is determined by the presence of Aldh1A (aldehyde dehydrogenase 1 family, member A) that produces RA and Cyp26 (cytochrome P450, family 26) that metabolizes RA into bio-inactive metabolites. Unlike mammals, zebrafish only have aldh1a2, aldh1a3 and aldh8a1 but not aldh1a1 that are responsible for RA production. Because both aldh1a3 and aldh8a1 are expressed in late organogenesis, it is strongly suggest that aldh1a2 is the major gene that is responsible for RA synthesis in zebrafish early development (Liang et al, 2008). Like mammals, zebrafish possesses a third cyp26 gene (cyp26c1) (Gu et al., 2005) in addition to cyp26a1 and cyp26b1. The cyp26c1 metabolizes RA but not retinol or retinal in a similar way to cyp26a1, the major gene that is responsible for RA metabolism in zebrafish early development (Gu et al., 2006). Like cyp26a1, proper expression of cyp26c1 at early developmental stage is essential for the development of anterior–posterior axis and left–right symmetry in zebrafish embryos (Gu et al., 2005; Gu et al., 2006). Analyzing the promoter of cyp26a1, we reveal that zebrafish cyp26a1 possesses three conserved RAREs in response to RA signal and therefore maintains RA homeostasis in a feedback way (Hu et al., 2008; Li et al., 2012). Other than Cyp26s that can limit RA signaling, we show that N-CoR is essential for patterning the anterior–posterior axis of zebrafish hindbrain by actively repressing retinoid signaling (Xu et al., 2009).

Aside from its global role in embryonic patterning, proper RA signaling is essential to vertebrate mesoderm differentiation. Administrating zebrafish embryos with exogenous RA, we demonstrate that excessive RA inhibits the specification of ventral mesoderm cells into anterior hemangioblasts through acting downstream of gata4/5/6, upstream of or parallel to cloche, and upstream to scl in a dose dependent manner (Liang et al., 2012). Moreover, zebrafish embryos exposed with excessive RA from 11 hpf or 14 hpf but not from 16 hpf displayed cardiac edema at 108 hpf (Figure 1 A-F, Yue YY, 2013, Unpublished data). Histological analyses reveled that the developmental defect is due to abnormal formation of cardiac valves. Consistent with the cardiac development defects, whole amount in situ hybridization showed that nppa, normally expressed in cardiac chamber, was ectopically expressed in the atrial-ventricle canal (AVC) (Figure 1 G-M, Yue YY, 2013, Unpublished data). Further analyses demonstrated that the expression patterns of AVC markers including notch1b, bmp4, tbx2b, versican, has2, and klf2a were all disrupted (Figure 1 N-Y, Yue YY, 2013, Unpublished data). On the other hand, embryos lack of RA signaling either by knocking down aldh1a2 or treating with DEAB, the inhibitor of Aldh1a (DEAB), exhibited abnormal heart development with obvious pericardial edema (Figure 2A-E, Li JB, 2013, Unpublished data). Histological and hemodynamics analyses revealed that the defective heart phenotype of embryos with insufficient RA signaling was a result of defective formation of primitive valve leaflets. Interestingly, expressions of notch1b but not versican, cardiac valve marker genes, were greatly reduced in the RA signaling depleted embryos (Figure 2G-O, Li JB, 2013, Unpublished data). Time window assays revealed that the effect of RA signaling deficiency on defective valulogenesis occurred during 16-26 hpf (Figure 2P-Y, Li JB, 2013, Unpublished data). Taken together, the results suggest that proper RA signaling is crucial for the fate determination of endocardial cells at AVC that are derived from anterior hemangioblasts.

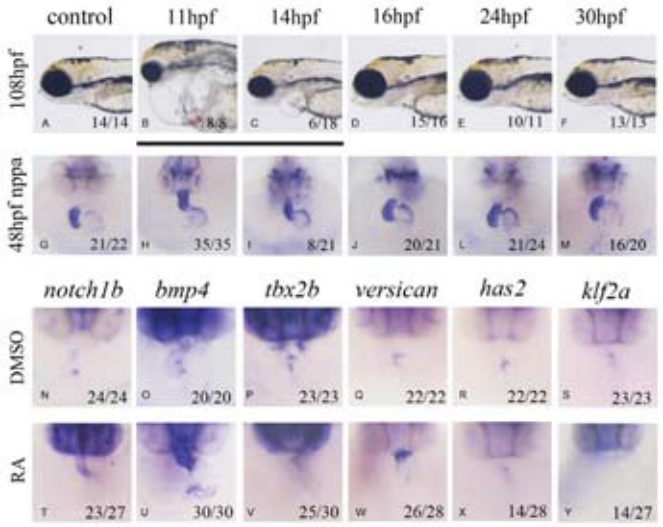

Figure 1. Excessive RA signaling affects zebrafish valulogenesis. Zebrafish embryos were exposed with excessive RA from 11 hpf (B), 14 hpf (C), 16 hpf (D), 24 hpf (E) and 30 hpf (F) and embryonic phenotypes were observed at 108 hpf together with control embryos (A). Cardiac edema was found in the embryos exposed with excessive RA from 11 hpf (B) or 14 hpf (C) but not from 16 hpf (D) or later (E-F). Consistent with the cardiac development defects, whole amount in situ hybridization showed that nppa, normally expressed in cardiac chamber (G), was ectopically expressed in the atrial-ventricle canal (AVC) of the embryos exposed with excessive RA from 11 hpf (H) and 14 hpf (I), but relatively normal in the embryos exposed with excessive RA from 16 hpf (J), 24 hpf (L) and 30 hpf (M), respectively. Moreover, the expression patterns of AVC markers including notch1b, bmp4, tbx2b, versican, has2, and klf2a were all disrupted in the embryos exposed with excessive RA from 11 hpf (N-Y)

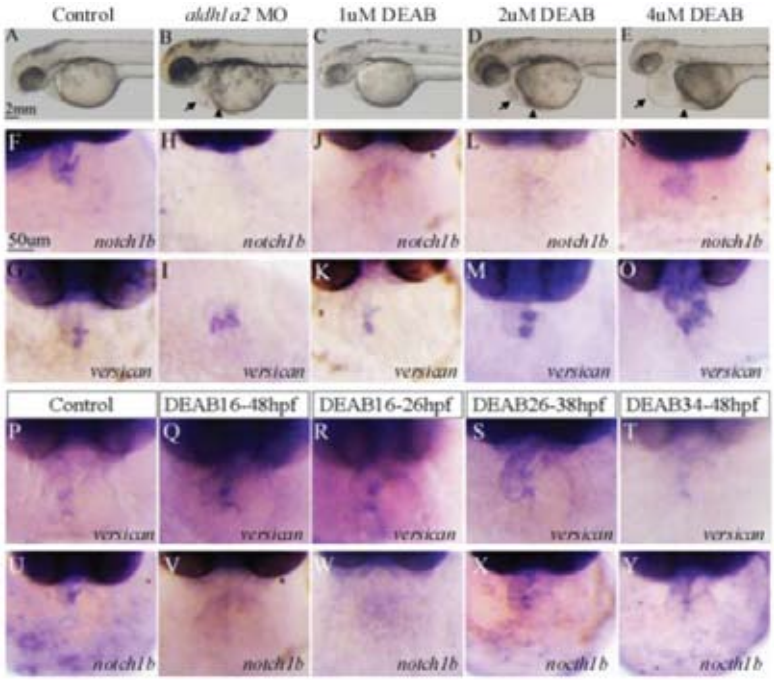

Figure 2. Insufficiency of RA signaling in a defined developmental stages leads to defective valve leaflet formation in zebrafish embryos. Compared to control embryos (A), embryos with aldh1a2 being partially knocked down exhibited abnormal heart development at 50 hpf (B). In contrast, embryos treated with 1uM DEAB, the inhibitor of Aldh1a, did not show obvious cardiac edema (C). But the embryos displayed a similar edema phenotype to aldh1a2 morphants when being treated with 2 uM DEAB (D). When treated with 4 uM DEAB, the embryos exhibited more seriously heart defect (E). Consistent with the phenotype, expressions of notch1b and versican, the marker genes of AVC, were affected differently in aldh1a2 morphants (H-I), 1uM DEAB treated embryos (J-K), 2uM DEAB treated embryos (L-M), and 4uM DEAB treated embryos (N-O) respectively when compared to in control embryos (F-G). (P-Y) Changes of expression patterns of versican and notch1b in the embryos treated with DEAB during different developmental stages revealed that 16-26 hpf are the key time window when sufficient RA signaling is essential for AVC to develop normally. Black arrow: edema; Black arrowhead: blood accumulation.

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1. Dong Liang, Wenshuang Jia, Jingyun Li, Kui Li, Qingshun Zhao *. 2012. Retinoic acid signaling plays a restrictive role in zebrafish primitive myelopoiesis. PLoS ONE, 7(2): e30865.
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4. Fang Xu, Kui Li, Miao Tian, Ping Hu, Wei Song, Jiong Chen, Xiang Gao, Qingshun Zhao*. 2009. N-CoR is required for patterning the anterior–posterior axis of zebrafish hindbrain by actively repressing retinoid signaling. Mechanism of Development, 126:771-780.
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6. Dong Liang, Mei Zhang, Jie Bao, Luqing Zhang, Xiaofeng Xu, Xiang Gao, Qingshun Zhao*, 2008. Expressions of Raldh3 and Raldh4 during zebrafish early development. Gene Expression Patterns, 8(4):248-253.
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8. Xingxing Gu, Fang Xu, Xiaolin Wang, Xiang Gao, Qingshun Zhao*, 2005. Molecular cloning and expression of a novel CYP26 gene (cyp26d1) during zebrafish early development. Gene Expression Patterns, 5(6):733-739.

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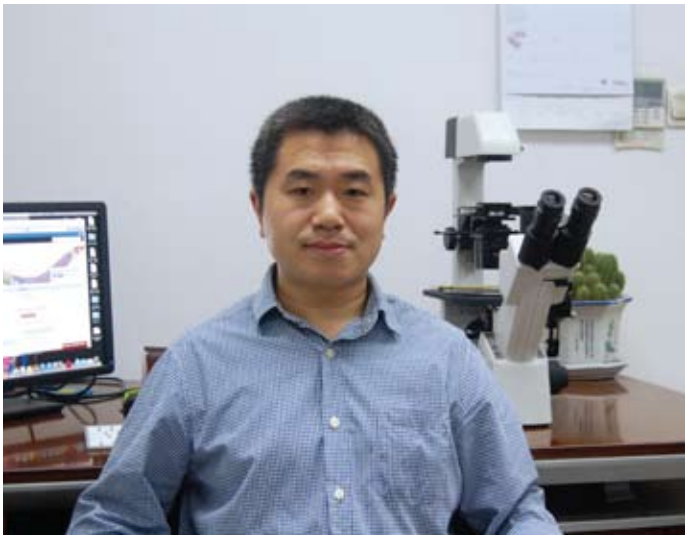

Di Chen, Ph.D.

Di Chen got his Ph.D. in Genetics from University of Missouri-Columbia, USA in 2004. He was supervised by Dr. Donald L. Riddle to study how the nematode *C. elegans* respond to genetic and environmental cues to enter and exit a developmental diapause stage called dauer. He did post-doctoral training in Dr. Pankaj Kapahi's lab at the Buck Institute for Research on Aging, USA, where he studied the molecular mechanisms of aging in *C. elegans*. He joined the Model Animal Research Center (MARC), Nanjing University as a Principle Investigator in 2013.

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C. elegans Aging and metabolism

Aging is a process of gradual function decline accompanied with increased mortality rate. The evolutionary theory of aging proposes that aging takes place because natural selection favors genes that confer benefit early on life at the cost of deterioration later in life (antagonistic pleiotropy). Using model organisms, researchers have demonstrated that aging is modulated by highly conserved signaling pathways, and genetic or environmental modulations can lead to significantly extended lifespan and delayed functional decline. Many exciting discoveries on the molecular mechanisms of aging were initially made in *C. elegans*, which is a great system for biology research because of the ease of genetics analysis and the conservation with higher species.

The highly conserved Insulin/IGF-1 signaling (IIS) and Target of Rapamycin (TOR) pathway play an important role in aging. Inhibition of these pathways leads to prolonged longevity in many species. We found that these pathways function interactively to modulate longevity in a tissue-specific manner (Figure 1).

Dietary restriction (DR), reduced food intake without malnutrition, has been shown to be one of the most robust environmental manipulations that not only extend lifespan but delay age-related pathologies. We demonstrated that the beneficial effects of DR are mediated by the TOR downstream proteins such as the ribosomal S6 Kinase (S6K) and Hypoxia Inducible Factor-1 α (HIF-1α) via the Endoplasmic Reticulum (ER) stress pathway in *C. elegans* (Figure 2).

Currently, our research focuses on the following aspects:

Tissue-specific interactions between Insulin/IGF-1 and TOR pathways;

Molecular mechanisms of dietary restriction;

The roles of fat metabolism in aging.

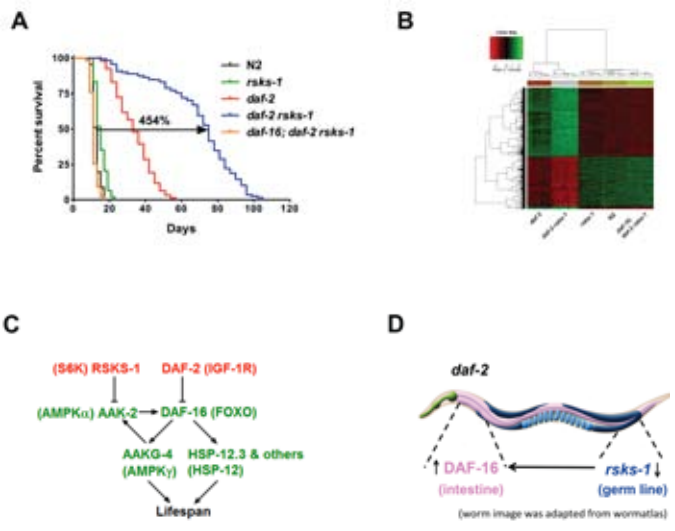

Figure 1. Tissue-specific modulation of the synergistic lifespan extension by the *daf-2 rsks-1* double mutant in *C. elegans*.

(A) Double mutations in DAF-2 (IGF-1 receptor) and RSKS-1 (ribosomal S6 kinase) leads to nearly 5-fold synergistic lifespan extension, which requires the DAF-16 (FOXO) transcription factor.

(B) Identification of genes differentially expressed in the *daf-2 rsks-1* double mutant.

(C) AMPK-mediated positive feedback regulation of DAF-16 in *daf-2 rsks-1*.

(D) Inhibition of *rsks-1* in the germ line results in non-autonomous activation of DAF-16 in the intestine.

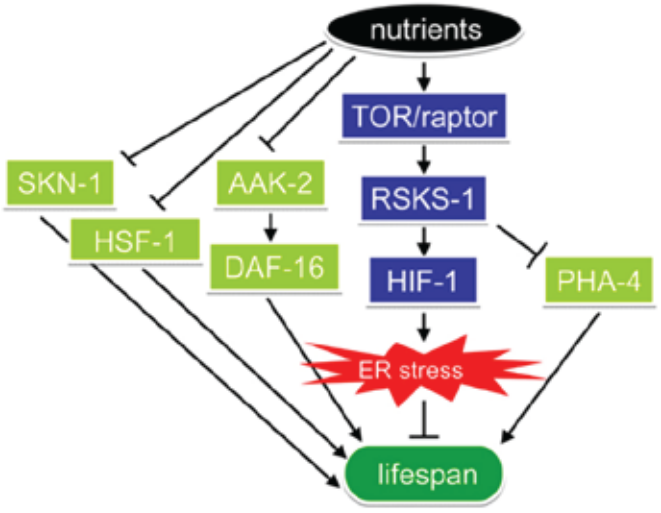

Figure 2. A genetic model depicting the modulation of lifespan by nutrients, HIF-1 and ER stress in *C. elegans*. High nutrients activate HIF-1 through the TOR-S6K pathway, which leads to increased ER stress and shortened lifespan. Other regulators such as PHA-4, SKN-1, AAK-2, DAF-16 and HSF-1 may function in parallel to HIF-1 to modulate DR-induced longevity phenotypes.

Selected publications

1. Chen D*, Li PW, Goldstein B, Cai W, Thomas EJ, Hubbard AE, Melov S and Kapahi P* Germline Signaling Mediates the Synergistically Prolonged Longevity by Double Mutations in *daf-2* and *rsks-1* in *C. elegans* (*Cell Reports*, in press). *, corresponding authors.
2. Rogers AN, Chen D, McColl G, Czerwieniec G, Felkey K, Gibson BW, Hubbard A, , Melov S, Lithgow GJ and Kapahi P. (2011). Life Span Extension via eIF4G Inhibition Is Mediated by Posttranscriptional Remodeling of Stress Response Gene Expression in *C. elegans*. *Cell Metabolism* 14(1): 55-66.
3. Kapahi P, Chen D, Rogers AN, Katewa SD, Li P, Thomas EL and Kockel L (2010). With TOR, Less Is More: A Key Role for the Conserved Nutrient-Sensing TOR Pathway in Aging. *Cell Metabolism* 11(6): 453-465.
4. Chen D, Thomas EL and Kapahi P (2009). HIF-1 Modulates Dietary Restriction-mediated Lifespan Extension via IRE-1 in *C. elegans*. *PLoS Genetics* 5(5): e1000486.
5. Chen D and Riddle DL (2008). Function of the PHA-4/FOXA Transcription Factor During *C. elegans* Post-embryonic Development. *BMC Developmental Biology* 8(1): 26.
6. Chen D, Pan KZ, Palter JE and Kapahi P (2007). Longevity Determined by Developmental Arrest Genes in *Caenorhabditis elegans*. *Aging Cell* 6(4): 525-533.
7. Pan KZ, Palter JE, Rogers AN, Olsen A, Chen D, Lithgow GJ and Kapahi P (2007). Inhibition of mRNA Translation Extends Lifespan in *C. elegans*. *Aging Cell* 6(1): 111-119.
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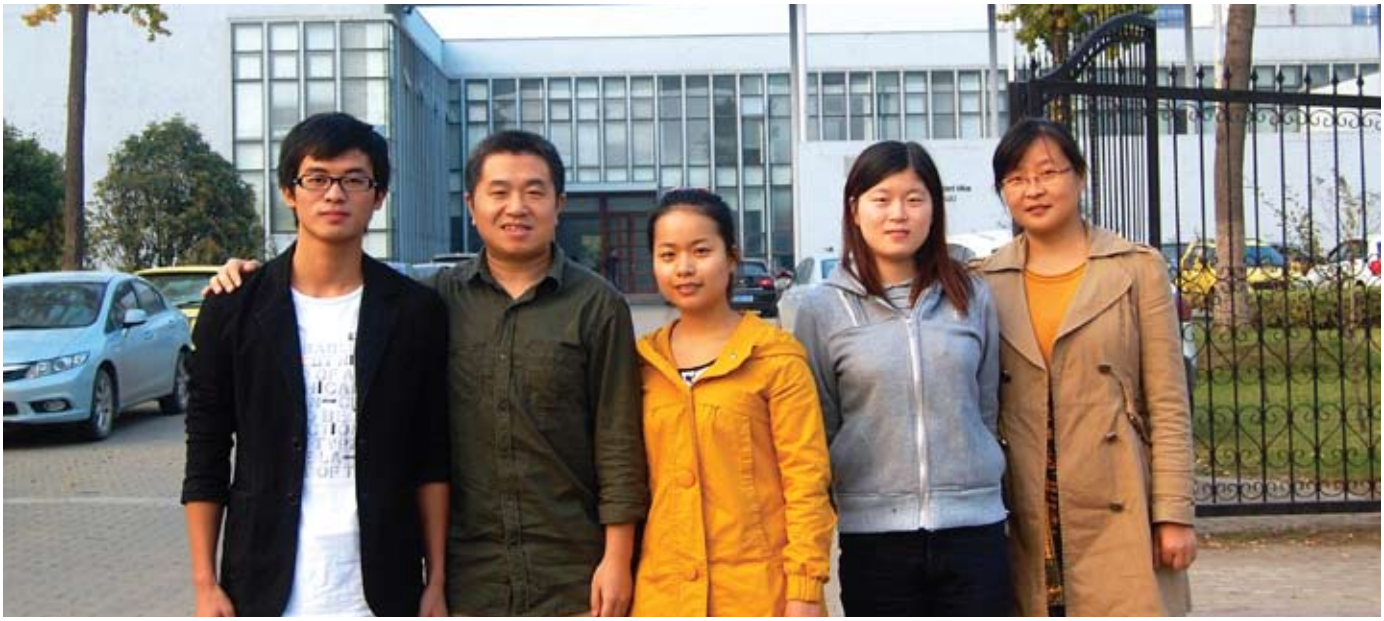

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  Xuan Zhang
- **Lab manager**
  Fen Chen
- **Intern students**
  Samuel Chen
  Yufang Mao
- **Administrative assistant**
  Xing Liu

Shuai Chen, Ph.D.

Shuai Chen received his Ph.D. degree in Plant Molecular Physiology from Martin-Luther University (Germany) in 2005. After his postdoctoral training in the field of cell signaling and molecular physiology at the MRC Protein Phosphorylation Unit (UK) from 2006 to 2011, Dr. Chen joined MARC as a principle investigator in 2012.

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Cell signaling and type II diabetes

Blood sugar lowering effect is one of the major functions of insulin, and insulin sensitivity is most often referred to its ability to regulate glucose homeostasis. Upon binding to its receptor, insulin shifts phospho-proteome in various target organs towards preparation for assimilation of glucose from the bloodstream into muscle and liver glycogen and into fat in adipose, and also towards inhibition of glucose production from the liver. Deregulation of insulin signaling can directly cause type II diabetes that currently affects nearly 100 million people in China. Type II diabetic patients often receive treatments such as exercise and metformin that regulate glucose homeostasis independent of insulin (Fig 1).

Therefore, the goal of my laboratory is to elucidate the signaling pathways that regulate glucose homeostasis in insulin-dependent and –independent manners. Centering on this theme, we employ proteomics, biochemistry, cell biology and transgenics approaches to identify novel signaling components that may be potential therapeutic targets for type II diabetes treatment in the future.

The recent progress of my lab is as follows:

AS160 deficiency causes whole-body insulin resistance via composite effects in multiple tissues

AS160 is a Rab GTPase activating protein implicated in insulin control of GLUT4 trafficking. In humans, a truncation mutation (R363X) in one allele of AS160 decreased the expression of the protein and caused severe postprandial hyperinsulinemia during puberty. To complement the limited studies possible in humans, we generated an AS160 knockout mouse (Fig 2). In wild-type mice, AS160 expression is relatively high in adipose tissue and soleus muscle, low in EDL muscle and detectable in liver only after enrichment. Despite having lower blood glucose levels under both fasted and random-fed conditions, the AS160 knockout mice exhibited insulin resistance in both muscle and liver in a euglycemic clamp study. Consistent with this paradoxical phenotype, basal glucose uptake was higher in AS160 knockout primary adipocytes and normal in isolated soleus muscle, but their insulin-stimulated glucose uptake and overall GLUT4 levels were markedly decreased. In contrast, insulin-stimulated glucose uptake and GLUT4 levels were normal in EDL. The liver also contributes to the AS160-knockout phenotype via hepatic insulin resistance, elevated hepatic expression of phosphoenolpyruvate carboxykinase isoforms and pyruvate intolerance, which are indicative of increased gluconeogenesis. Overall, as well as its catalytic function, AS160 influences expression of other proteins, and its loss deregulates basal and insulin-regulated glucose homeostasis not only in tissues that normally express AS160, but also by influencing liver function.

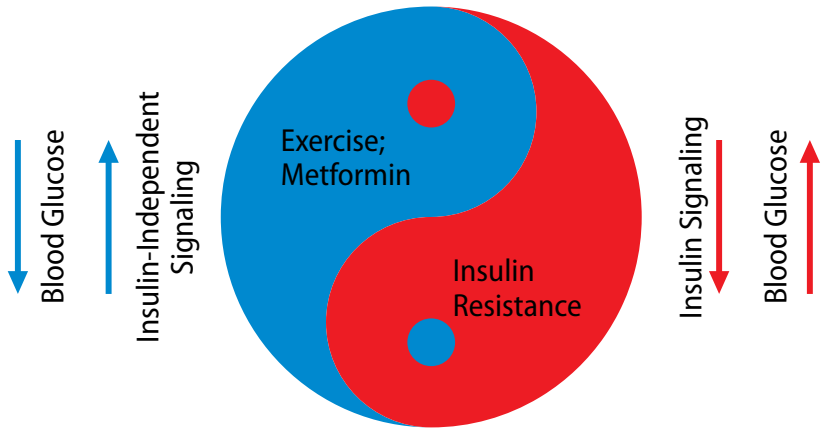

Figure 1 Insulin dependent and independent signaling in the pathogenesis of type II diabetes and its treatment.

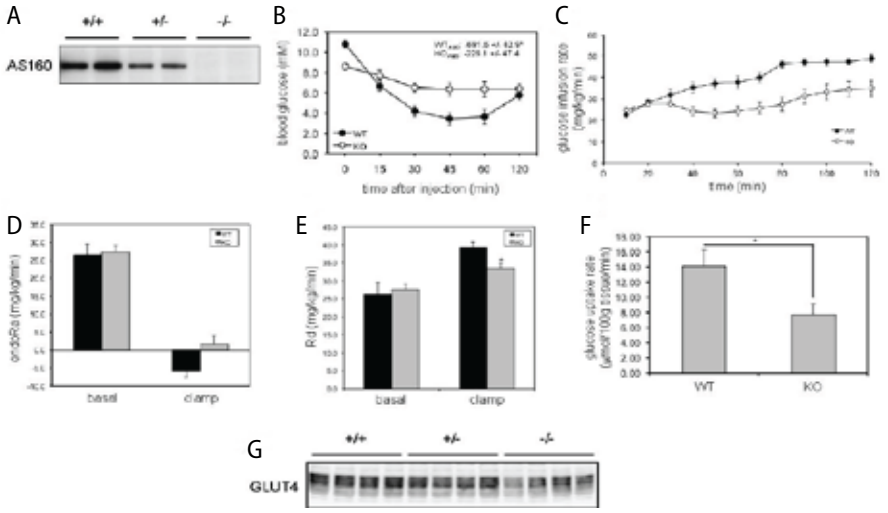

Figure 2 AS160 deficiency and whole body glucose homeostasis.

A, AS160 expression in wild-type and AS160 KO mice;

B, insulin tolerance test;

C, glucose infusion rate during insulin clamp;

D, hepatic glucose production in the insulin clamp study;

E, glucose disposal in the insulin clamp study;

F, glucose uptake in skeletal muscle;

G, GLUT4 expression in skeletal muscle.

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  Co-PI: Hong Yu Wang
- **Research assistant**
  Shuilian Zhou
  Min Li
- **Graduate students**
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  Chao Quan
  Qiaoli Chen
  Liang Chen

Zhenji Gan, Ph.D.

Zhenji received his Ph.D. degree in Biochemistry and Molecular Biology (2003 - 2008) from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. His Ph.D. work was carried out in Dr. Yong Liu's lab focused on metabolic diseases. From 2008 to 2013, Zhenji pursued his post-doctoral training in the areas of nuclear receptor signaling and energy metabolism under the guidance of Dr. Daniel Kelly at Sanford-Burnham Medical Research Institute. In 2013, he started a Principal Investigator position in the Model Animal Research Center (MARC) of Nanjing University.

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Energy metabolism and muscle fitness

Muscle fitness is an important determinant of health and disease. Exercise training enhances muscle endurance and performance by augmenting the capacity of mitochondria to burn fuels and by increasing the proportion of slow oxidative fibers and blood supply. Conversely, reduced physical activity such as occurs with chronic illness, obesity, and aging results in de-trained muscle (Fig 1). Our lab focuses on fundamental mechanisms that control muscle energy metabolism in physiological and disease states. We are particularly interested in exploring the regulatory networks involved in the coordinate control of energy metabolic and structural programs that define muscle fitness and their potential for therapeutic development.

Unraveling the nuclear receptor-miRNA networks and downstream targets involved in the coordinate regulation of muscle metabolic and structural programs.

Our recent work in genetically-modified mice has shown that the nuclear receptor PPAR β/δ and estrogen-related receptor-γ (ERRγ) drive the slow, type I muscle fiber program by activating transcription of Myh7 and Myh7b which in turn drive expression of miR-208b and miR-499, respectively. Surprisingly, the related nuclear receptor PPARα suppresses the activating effects of PPAR β/δ /ERRγ on Myh7/miR-208b and Myh7b/miR-499 (Fig 2).

The activation of the Myh7 and Myh7b gene promoters by ERRγ was shown to occur via conserved ERR binding sites in the promoters of both genes. To further investigate the mechanism whereby ERRγ regulates Myh7/miR-208b and Myh7b/miR-499 transcription, additional bioinformatics analyses were conducted leading to the identification of ERRγ/MEF2 transcriptional network controlling muscle fiber type through co-localized sites on Myh7/Myh7b promoters (Fig 3A). ERRγ induces MEF2A gene transcription via ERR-RE supports a notion of a feed-forward mechanism. PGC-1α and β have been shown to activate the slow-twitch skeletal muscle program and to coactivate ERR to induce expression of target genes. Surprisingly, MHC1 positive staining was significantly increased in the PGC-1αβ KO myocytes (Fig 3B), suggesting that PGC-1αβ deficiency triggers an adaptive myocyte reprogramming. Interestingly, ERRγ/MEF2 were induced in PGC-1αβ KO myocytes, suggesting that the ERRγ/MEF2 circuit is an inducible program that is responsive to a variety of external stimuli, such as energy deficiency induced by PGC-1αβ KO. Currently, we are investigating the upstream regulatory mechanisms.

In addition, we are also interested in dissecting the role of miR-499/miR-208b in the broad control of muscle metabolic and structural programs (mitochondria biogenesis; angiogenesis; and changes in contractile proteins).

Genome-wide chromatin state mapping to identify novel transcriptional components involved in the control of muscle energy metabolism and fitness.

We are trying to conduct genome-wide chromatin state mapping to identify regions with cis-regulatory potential in the genome of muscle cells undergoing beneficial reprogramming. We hypothesize that epigenetic genome-wide chromatin state mapping to find cis-elements exhibiting dynamic changes under beneficial muscle reprogramming (such as endurance exercise training), computational motif finding with these regions will allows us to discovery of novel transcriptional pathway of importance. Then proof-of-concept studies will be conducted using cell-based and mouse genetic approaches by manipulation of the new candidates.

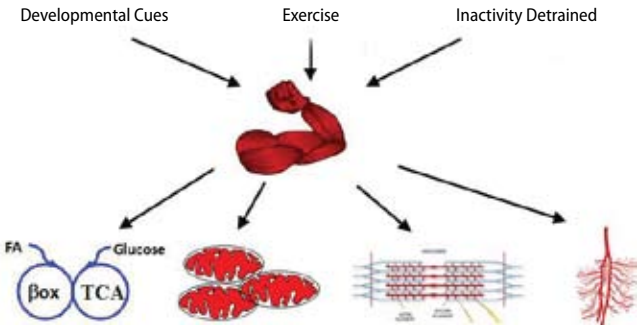

Fig 1. The muscle fitness is determined by developmental as well as physiological inputs. These inputs coordinately control the programs including fuel burning, mitochondrial ATP production, contraction, and angiogenesis.

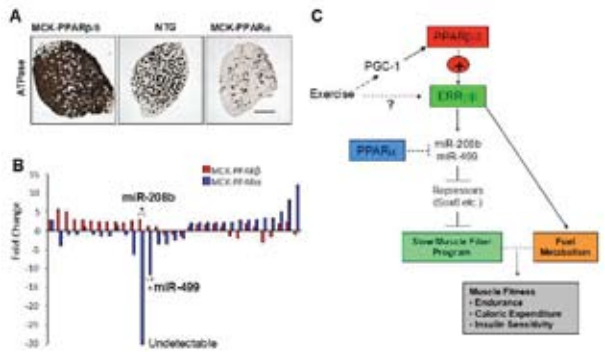

Fig 2. A nuclear receptor-miRNA circuit orchestrates programs controlling energy metabolism and muscle fiber type.

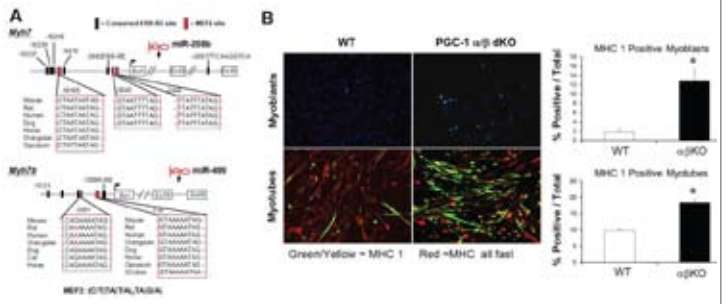

Fig 3. Nuclear receptor ERRγ and MEF2 co-occupy the Myh7/miR-208b and Myh7b/miR-499 promoters; Activation of type I muscle gene program in PGC-1αβ KO myocytes.

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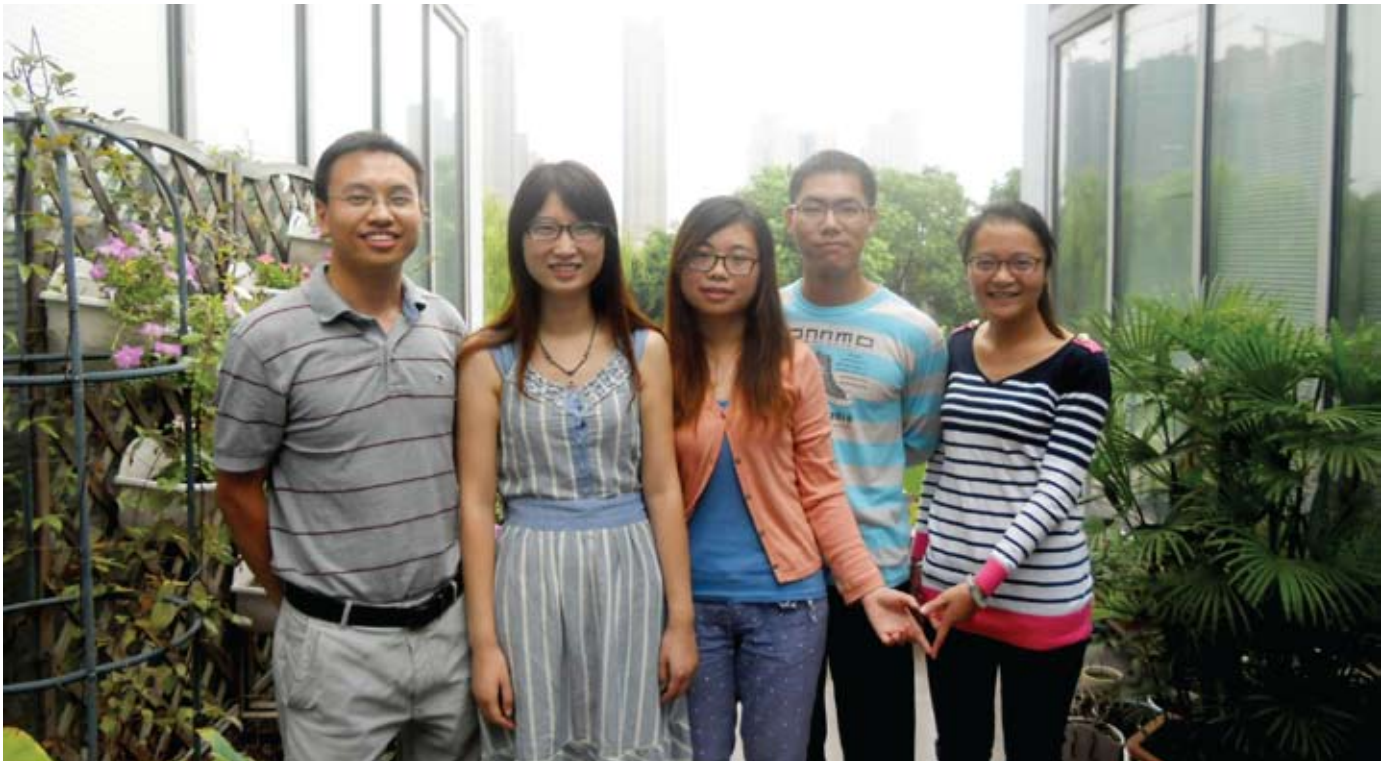

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- **Technical Assistant**
  Qin Wei

Xiang Gao, Ph.D.

Xiang was the founder for both MARC and National Resource Center for Mutant Mice. He is awarded Professor of Cheung Kong Scholars by Ministry of Education in 2002. He is the recipient for the National Science Fund for Distinguished Young Scholars. Xiang currently service as the president of Asian Mouse Mutagenesis and Resource Association (AMMRA) and Director for Nanjing Biomedical Research Institute of Nanjing University (NBRI).

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Genetic manipulation and disease models

My laboratory is fascinated by the tight control of physiological homeostasis. Over the years, we have worked in this field with different organs, including regulation of bone mineral density regulation responded to mechanical force by sost1; maintenance of calcium and phosphate level by PHEX, establishing of skin immune privilege by Gsdma3, and sustain of glucose homeostasis after meal by Pax6-mediatd GLP-1 expression.

Control of one "simple" physiological process often involves many organs and gene functions. For instance, the glucose homeostasis is regulated by brain, muscle, liver, adipose tissue, gut and bones. Now we know the micro flora in gut also participates the metabolic control. And because of that, some of these regulation pathways may only be activated when the organism is challenged under specific environments or physiological stresses. Unfortunately, most of our experiments with live animals are carried out in a SPF facility therefore how to apply the challenge becomes a crucial issue.

Understanding the physiological homeostasis may also be tackled at difference stages, the establishment, maintenance, and reset of a specific physiological status. One of the projects in my lab is try to understand the obesity "memory". It is commonly believed an obese person will obtain a tendency to get fatty again after losing weight by food restriction or excises. We confirmed this phenomenon using mouse models (Figure 1). The mice used to be fat gain body weight faster than the little mate control in both normal diet as well as high fat diet, suggesting a "memory" of old physiological status.

Finally, we should keep in mind of the interaction between different signaling pathways that control physiological regulation. Separated protein domains execute these cross-talks. By analyzing two mouse strains carrying different point mutations in c-Kit gene, we found they share the abnormalities in many cell types but display opposite phenotypes in myeloid tissue. Mutation in the first immunoglobulin-like domain of Kit leads to myeloproliferation, whereas the kinase-dead mutation leads to anemia. Detailed analysis revealed that KIT protein can physically interacts with the beta common (βc) receptor of the gp140 family through first Ig domain. And the mutation in this position of KIT activates the Epo-R and IL-7R in the absence of their ligands, Epo and IL-3, by facilitating the constitutive recruitment of JAK2 to Kit (Figure 2). Considering active mutations of the βc receptor lead to myeloproliferative neoplasms, it would be interesting to determine whether these mutations enhance the interaction with the Kit receptor to induce cytokine- independent proliferation.

The mystery of physiological homeostasis is just beginning to be understood. Careful studies will not only shed the light in the regulatory loops for the beauty of complicated life, but the potential cure for diseases resulting from disruption of these feedback loops.

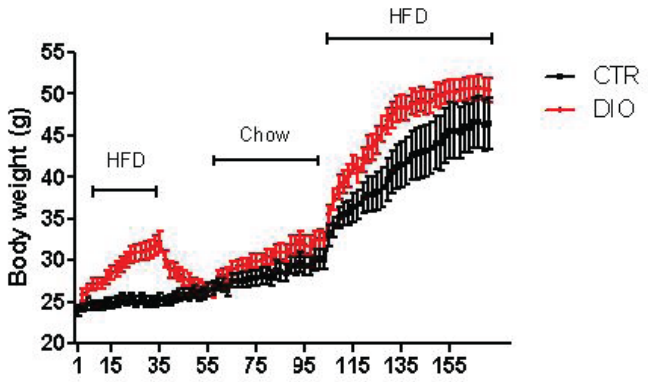

Figure 1. Mice with obese history gain body weight fast than the control mice. Redline, 2 month-old mice were fed with HFD for 5 weeks and followed by calorie restriction to lose weight. Mice then were fed with regular chow or HFD.

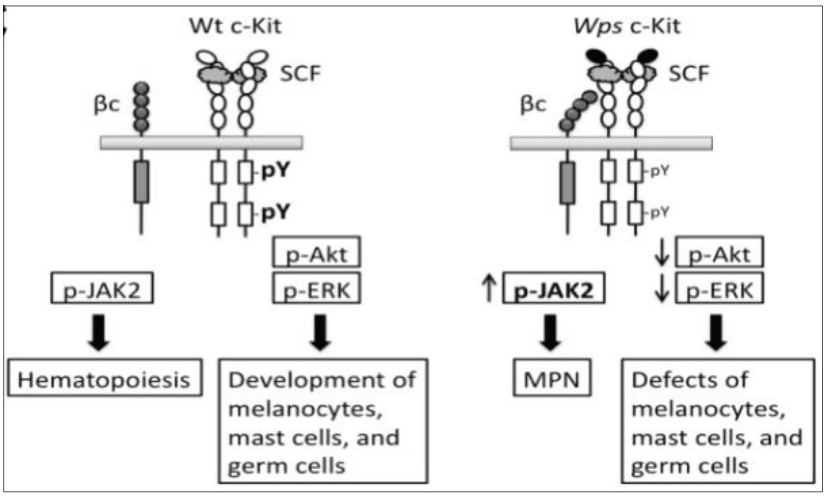

Figure 2. The first Ig domains of Kit interacts with βc receptor. Left, when activated by SCF, wild-type Kit autophosphorylates itself and elicits downstream ERK, Akt and JAK2 activation to mediate its biological functions. Right, carrying mutation at first Ig domain, Wps Kit shows decreased autophosphorylation and ERK and Akt activation, which results in defects in development of melanocytes, mast cells and germ cells. On the other hand, Wps Kit forms heterodimer with βc receptor, and recruits more JAK2 and activates JAK2, which leads to the myeloproliferation in mice.

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ChaoJun Li, Ph.D.

Chao-Jun Li received his Ph.D in Physiology from Nanjing University in 1994. He also did his postdoctoral training at the Hong Kong University of Science and Technology from 1996-1998 and the Medical School of Yale University from 1999-2000. He joined the Faulty of Model Animal Research Center (MARC), Nanjing University in 2008. He is now a professor of Cell Biology and a principle investigate in MARC and the Medical School of Nanjing University

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Protein prenylation and metabolic disorder

Protein prenylation is a critical process for the membrane association of lots of signaling proteins, including small G proteins. Geranylgeranyl diphosphate synthase (GGPPS) catalyzes the formation of geranylgeranyl diphosphate (GGPP) from farnesyl diphosphate, both of which is used to prenylate proteins with CAAX motif in their carboxyl termini. The prenylated proteins can attach to the membrane to initiate their function. We first identified GGPPS as a directly target gene of Egr-1, which can positively feedback to increase Egr-1 accumulation during chronic stress stimulation through enhance Ras prenylation and membrane association. We have constructed GGPPS Flp mice and conditionally deleted GGPPS gene in different tissue to examine its functions involving human diseases. Using these animal models, we further explore the function of protein prenylation in different disease models. We found that the balance of protein farnesylation/geranylgeranylation or FPP/GGPP is critical for disease development.

Farnesyl diphosphorylate to geranylgeranyl diphosphorylate ratio regulates de novo lipogenesis through FXR/SHP pathway

Liver is the central metabolic organ that regulates several key aspects of lipid metabolism in response to nutritional signals. Hepatic GGPPS plays an essential role in regulating lipid homeostasis in mouse. We found that enhanced GGPPS expression is a common feature of obesity-associated hepatic steatosis, as in the liver of ob/ob obese mice, db/db diabetic mice and diet-induced hepatic steatotic mice. Meanwhile, deletion of GGPPS in liver protected mice from diet-induced hepatic lipid accumulation (Figure 1). Further analysis revealed that GGPPS deletion decreased de novo lipogenesis while enhanced TG-VLDL transportation. The underneath molecular mechanism relies on GGPPS tilting the ratio of FPP to GGPP which results in activating FXR/SHP signaling that suppresses SREBP pathway. Our results suggested that GGPPS played an essential role in lipid metabolism under HFD stress.

The constitutive activation of Egr-1/C/EBPa mediates the development of type 2 diabetes mellitus by enhancing hepatic gluconeogenesis

Increased glucagon/insulin ratio is believed to be a major cause of the hyperglycemia seen in type 2 diabetes. We reveal that the early growth response gene Egr-1 can be transiently activated by glucagon in hepatocytes, which mediates glucagon-regulated gluconeogenesis by increasing the expression of gluconeogenesis genes. Blockage of Egr-1 function in the liver of mice leads to lower fasting blood glucose, better pyruvate tolerance and higher hepatic glycogen content. The mechanism analysis demonstrates that Egr-1 can directly bind to the promoter of C/EBPa and regulate gluconeogenesis genes expression in the later phase of glucagon stimulation. Thus, the transient increase of Egr-1 by glucagon can keep the glucose homeostasis after fasting for longer periods of time. Whereas constitutive Egr-1 elevation found in the liver of db/db mice and high serum glucagon level might over-activate the C/EBPa/gluconeogenesis pathway and result in hyperglycemia. Blockage of Egr-1 activation in pre-diabetic db/db mice is able to delay the progression of diabetes (Figure 2). Our results suggest that dysregulation of Egr-1/C/EBPa upon glucagon stimulation may provide an alternative mechanistic explanation for type 2 diabetes.

Protein prenylation is essential for lung branching morphogenesis

We generated SPC-rtTA/Teto-Cre/GGPPSflox/flox mice that specific knockout GGPPS in lung epithelium though tetrocyclin induction. GGPPS deletion during fetal lung development resulted in Neonatal Respiratory Distress Syndrome (NRDS) in mice. The neonatal knockout (KO) mice died at P1 due to respiratory failure with decreased ratio of wet lung weight to body weight. Meanwhile, lungs of KO mice showed compensatory alveolar pneumonectasis, dilated alveolus, pulmonary atelectasis and hyaline membrane. Further analysis revealed that during fetal lung development, GGPPS deletion enhanced apoptosis behavior, decreased proliferation activity in mice that resulted in failure of branching morphogenesis (Figure 3). In the all, our results suggested that GGPPS might play a critical role in fetal lung development, especially the branching morphogenesis.

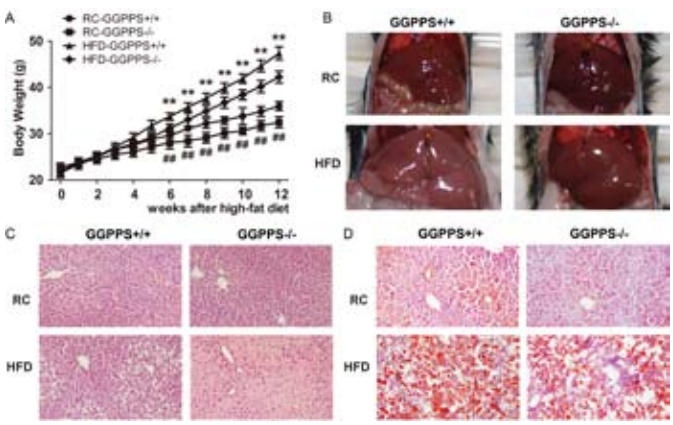

Figure 1.Liver specific deletion of GGPPS protected mice from diet-induced hepatic lipid accumulation.

(A). Body weight of mice during regular chow and high-fat diet feeding. The gross view (B), HE staining(C) and lipid droplets staining with oil-red O (D) showed that liver specific deletion of GGPPS could attenuate lipid accumulation.

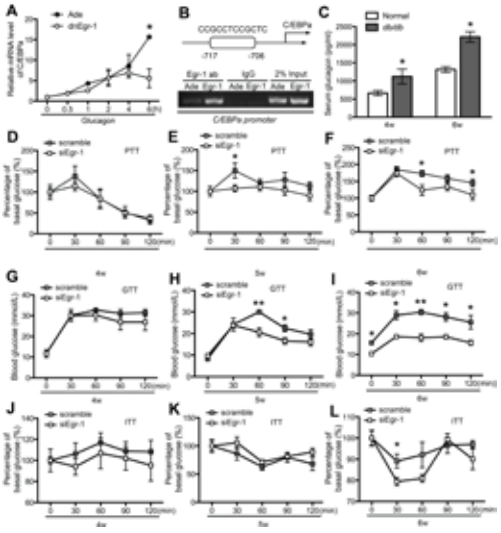

Figure 2.Early intervention through inhibition of hepatic Egr-1 expression alleviated the insulin resistance of db/db mice.

A, Blockage of Egr-1 inhibited glucagon-induced C/EBPa expression; B, Egr-1 can directly bind to C/EBPa promoter; C, Serum glucagon level was increased during the development of type 2 diabetes; D, E, F, Blockage of hepatic Egr-1 in 4w db/db mice showed no change in PTT, GTT and ITT; G, H, I, Blockage of hepatic Egr-1 in 5w db/db mice showed impaired pyruvate tolerance and improved glucose tolerance; J, K, L, Blockage of hepatic Egr-1 in 6w db/db mice showed impaired pyruvate tolerance, improved glucose tolerance and insulin sensitivity.

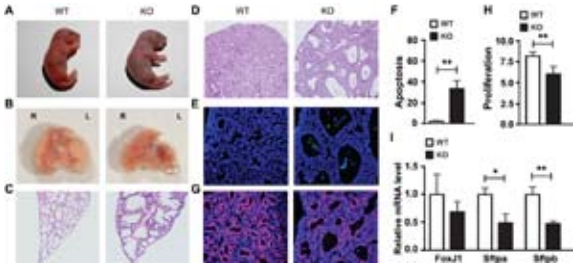

Figure 3.Protein prenylation is essential for lung branching morphogenesis.

A-C. Lung epithelial-specific knockout GGPPS mice died after birth

(A) with lungs showed dilated alveolus

(B) and atelectasis

(C). D-H. At E16.5, GGPPS deletion mice lungs showed failure of branching morphogenesis

(D),enhanced apoptosis behavior

(E and F) and decreased proliferation activity

(G and H). I. Differentiation markers as foxJ1, sft pa, sftpb were decreased after GGPPS deletion.

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Minsheng Zhu, Ph.D.

Minsheng Zhu received his Ph.D. degree from the Shanghai Biochemistry Institute of Academia Sinica in 1995. From 1995 to 1999, he worked in the Huadong Research Institute for Medical Biotechnics. In 1997, he was appointed associate professor in the same institute. Dr. Zhu performed his postdoctoral fellowship in the Department of Physiology of UT Southwestern Medical Center at Dallas, and moved to Model Animal Research Institute of Nanjing University as a professor of Genetics in 2004.

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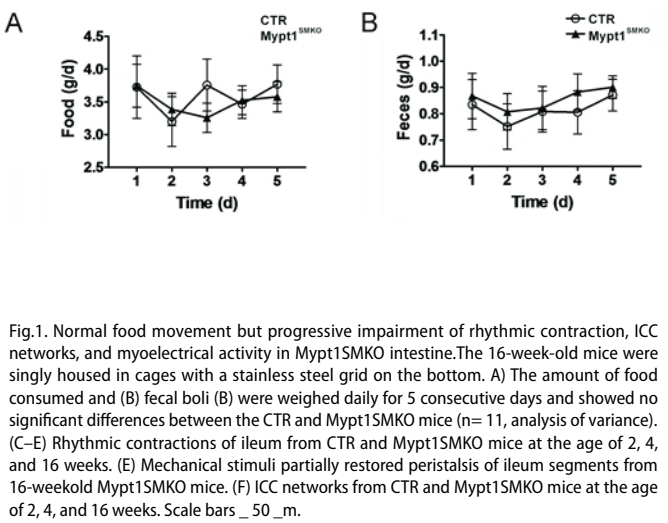
Smooth muscle contraction and diseases

Smooth muscle is essential for maintaining homeostasis for many body functions and provides adaptive responses to stresses imposed by pathological disorders. Abnormal contractile properties of smooth muscles have been implicated in several diseases, such as asthma, hypertension and gut diseases. Zhu's lab focuses on the regulatory mechanism of smooth muscle contraction and smooth muscle-related diseases. Smooth muscle contractility is regulated by a network of signaling pathways centered on the molecular motor myosin as well as membrane properties associated with calcium handling and cell adhesion. Identified cell signaling networks have defined many potential mechanisms for initiating smooth muscle contraction with or without myosin regulatory light chain (RLC) phosphorylation. Despite many years of extensive studies, the regulatory mechanism of smooth muscle contraction is still controversial. To understand of the signaling mechanism of smooth muscle contraction and their functional importance in diseases, we developed a series of smooth muscle-specific knockout mice by Cre/LoxP-mediated mutagenesis with deletion of signal module genes, such as MLCK, zip kinase, MYPT1 and Myl-9. We previously found that Ca2+/CaM-dependent MLCK and its myosin light chain phosphorylation were central to smooth muscle contraction, and MLCK is required for gut motility, asthmatic constriction and blood pressure maintenance. In contrast to force production by such a Ca2+/CaM-dependent mechanism, force maintenance is performed by a calcium sensitization mechanism. By use of MYPT1 knockout mice, we found that ROCK/MYPT1/PP1cδaxis was essential for calcium sensitized contraction both of gut and vascular smooth muscles. The altered sensitization leads to modest attenuation of gut motility and to hypertensive blood pressure (Fig.1, 2). Interestingly, MYPT1 deletion causes phenotypic transition of phasic and tonic smooth muscles. Our result revealed a key determinant of specification of smooth muscle subtypes (Fig.3). To understand the signal pathways converging on MYPT1, we mutated the phosphorylation sites with alanine in vivo, we delineated the role of Thr694 and Thr852 phosphorylation in mice.

We are also interested in the development of cerebellum. Cerebellar development is coordinated with multiple processes of cerebellar morphogenesis and patterning, which are primarily mediated by remarkable cell migration. Our focus is to study the signal conversion of development signal to cell migration signal. We established a Trio conditional knockout mouse line as a migration model of developing cerebellum. The results showed that deficiency of Trio caused severe signs of ataxia and extensive defects of cerebellar development through regulating the neurites morphogenesis via small GTPases during parallel fiber formation. Trio mutant granule cells also appeared to be unresponsive to neurite growth-promoting molecules such as Netrin-1 and Semaphorin 6A. We thus propose that Trio may serve as a common integrator decoding extrinsic signals to cerebellar development processes, and the mission of our future work is to elucidate the mechanistic regulation underlying this process.



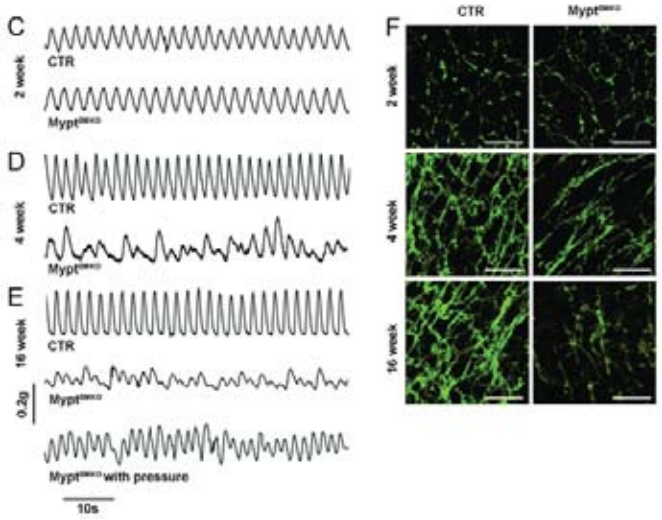


Fig.1. Normal food movement but progressive impairment of rhythmic contraction, ICC networks, and myoelectrical activity in Mypt1SMKO intestine.The 16-week-old mice were singly housed in cages with a stainless steel grid on the bottom. A) The amount of food consumed and (B) fecal boli (B) were weighed daily for 5 consecutive days and showed no significant differences between the CTR and Mypt1SMKO mice (n= 11, analysis of variance). (C–E) Rhythmic contractions of ileum from CTR and Mypt1SMKO mice at the age of 2, 4, and 16 weeks. (E) Mechanical stimuli partially restored peristalsis of ileum segments from 16-weekold Mypt1SMKO mice. (F) ICC networks from CTR and Mypt1SMKO mice at the age of 2, 4, and 16 weeks. Scale bars _ 50 _m.

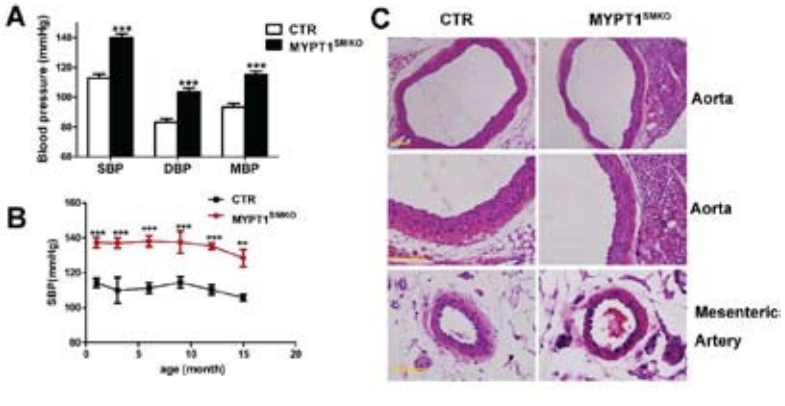


Fig.2. Blood pressure of MYPT1SMKO mice was significantly increased without changes in cardiac function or vascular structure. A, Blood pressure recordings of conscious MYPT1SMKO and control adult mice. Systolic, diastolic and mean blood pressures (SBP, DBP, MBP) were significantly elevated in MYPT1SMKO mice (***: p<0.001, n=10). B, Systolic blood pressure in MYPT1SMKO and CTR mice at different ages. The SBP of MYPT1SMKO mice was increased in one month old mice (KO: 136.98±2.91 mm Hg vs. CTR: 109.88±7.34 mm Hg, ***: P<0.001, n=3) and maintained up to 15 months of age (KO: 128.4 mm Hg vs. CTR: 105.7, **: p<0.01, n=3). C, Histology of aortic and mesenteric artery. Tissue slices of the arteries were stained with hematoxylin and eosin. The results showed normal vascular morphology of the MYPT1SMKO blood vessels compared to CTR vessels. Scale bar: 100 µm.

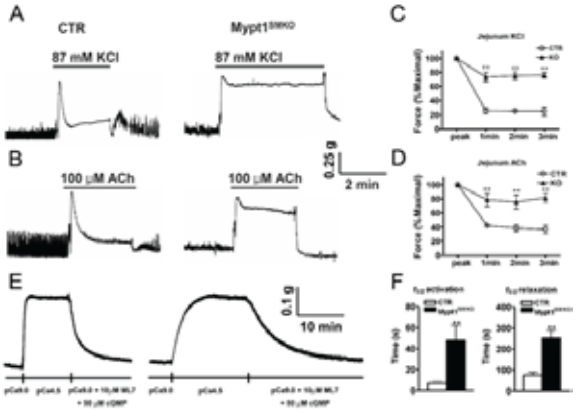


Fig.3. MYPT-deficient intestinal smooth muscles showed altered contractile properties. (A and B) Representative tracings of jejunum from 16-week-old CTR and Mypt1SMKO mice elicited by 87 mmol/L KCl or 100 _mol/L ACh. (C and D) Quantification of the sustained force responses to treatment with KCl or ACh. The values represent 5 independent experiments and are expressed as percent of the peak force. (E) Representative force tracings of permeable ileal strips after Ca2_-mediated force development and relaxation. (F) Quantification of the t1/2 values of force development and relaxation, respectively. N=3, **P< .01.

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1. Gao N, Huang J, He WQ, Zhu MS, Kamm KE and Stull JT. Signaling through Myosin Light Chain Kinase in Smooth Muscles J Biol. Chem. 2013: 288(11):7596-605

2. He WQ, Qiao YN, Peng YJ, Zha JM, Zhang CH, Chen C, Chen CP, Wang P, Yang X, Li CJ,, Kamm KE, Stull JT, Zhu MS* Alteration of contractile phenotypes of intestinal smooth muscle from mice deficient in myosin phosphatase target subunit 1. Gastroenterology 2013; 144(7):1456-65.

3. Zhang CH, Chen C, Zhao Wei, Zhu MS*, ZhuGe R*. The TMEM16A Ca2+-activated Cl- channel in airway smooth muscle contributes to airway hyperresponsiveness. American Journal of Respiratory and Critical Care Medicine 2013;187(4):374-813

4. Qiao YN, He WQ, Chen CP, Zhang CH, Zhao W, Wang P, Zhang L, Yang X. Peng YJ, Gao JM, Kamm KE, Stull JT, Zhu MS*. MYPT1 regulates vascular smooth muscle contractility and blood pressure maintenance. J Biol. Chem. 2013 (revised)

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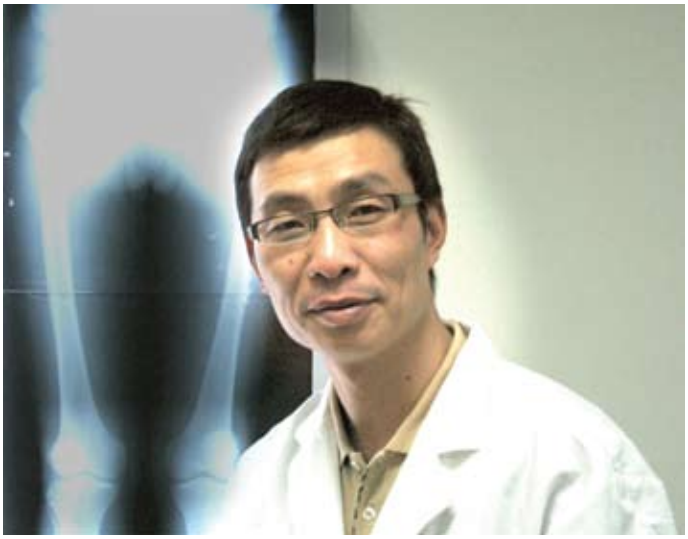

Qing Jiang, MD, Ph.D.

Qing Jiang received his MD degree in Nanjing Medical University in 1989 and PhD degree in Beijing Medical University in 1999. In 2008, he was appointed professor in Nanjing University and moved to Model Animal Research Center (MARC) as Adjunct Professor for research on bone and joint disease. Qing Jiang's group has established human gene bank of bone and joint disease including osteoarthritis (OA), developmental dysplasia of the hip (DDH), deep venous thrombosis (DVT), ankylosing spondylitis (AS) and osteoporosis (OP).

Jiang's research interests mainly focus on bone and joint disease. Besides the previous projects involved in GWAS study of developmental dysplasia of the hip (DDH) and deep vein thrombosis (DVT), a couple of new research projects concerned with translational medicine in osteoarthritis were launched at our lab in 2013.

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Research on bone and joint disease

1.GWAS study of DDH

The incidence of DDH in China was estimated about 0.1% to 0.5%. Persistent DDH may result in chronic pain, gait abnormalities and degenerative arthritis. Hereditary factors had been paid more attention to the development of DDH. Our lab has established gene bank of DDH in recent years. Different from previous SNP studies, in 2013, we have performed GWAS study of DDH. Subsequent replication studies showed that UQCC gene was a candidate gene associated with DDH occurrence. Replication studies also demonstrated a missense mutation in TXNDC3 gene as a risk factor for DDH.

2.DVT related studies

DVT is a severe complication after total hip arthroplasty (THA) or total knee arthroplasty (TKA). It leads to acute pulmonary embolism which can threaten patients' life. The prevalence of DVT after THA or TKA ranged from 15% to 80%. It has been suggested that methods to prevent the occurrence or to early discover of DVT are absolutely meaningful. In 2012, we have investigated if microRNA-186 and microRNA-19b could be available molecular biomarkers of DVT. This year, we paid more attention to DVT's prevention and early diagnosis using duplex ultrasonography.

2.1.Researches about DVT prevention

The usually used prophylactic anticoagulants after arthroplasty were low molecular weight heparin or direct inhibitor of factor Xa. However, these kinds of drugs could increase the risk of major bleeding. Now our group conducted a rat model to test the effect of ischemic preconditioning (IPC) on preventing deep vein thrombosis. After laprotomy, four circles of respectively 5 minutes of Clamp/Unclamp of the vena cava were performed before ligation of the iliac vein to generate local thrombi. In the control group, a 40-minute blank treatment was performed after laparotomy before iliacligatio. Significant difference in thrombi weight, length and density was observed in the IPC group and control group,(Fig.1)indicating IPC can reduce venous thrombi formation in mice, justifying further experiment to assess effects of IPC or hypoxic pretreatment on venous endothelial cells.

2.2.Researches about early diagnosis and dissolution of DVT

Recently, venography was considered as the gold standard for confirmation of DVT. But, it is an invasive procedure. Duplex ultrasonography which was used as a non-invasive method to diagnose DVT has low sensitivity. Now, our group established a kind of lipidicmicrobubbles wrapped with L-arginine (L-Arg) targeting to P-selectin to enhance the diagnostic sensitivity of ultrasound and accelerate DVT dissolution simultaneously without complications of bleeding risk. At current stage, our results demonstrate that both thrombus weight and length significantly decreased in L-Argmicrobubbles group. (Fig.2) In the future study, we will evaluate the impact on coagulation function as well as the difference of diagnostic sensitivity between targeted L-Argmicrobubbles and conventional microbubbles.

3.Cartilage defects repair

Osteoarthritis (OA) is a common skeletal disease characterized by the progressive loss of articular cartilage in synovial joints. The prevalence of OA ranged from 50% to 65% in people in their 60s. But due to avascular nature, articular hyaline cartilage has a limited intrinsic capacity for self-repair. Several traditional techniques like osteochondral allograft transplantation and microfrcture have been clinically used to repair damaged cartilage. However, the quality of repaired tissue from these procedures is still poor.

3.1.Drugs about preventing OA or cartilage defects repair

Clinically safe and effective drugs to treat OA or prevent the occurrence of OA are lacking. Now, our lab studied several effective drugs that are potential to be clinically used.(1)As is known, Chromatin higher order structure is affected by the recruitment of several post-transcriptionally modifying enzymes and plays an essential role in determining gene expression in eukaryotic cell. Two opposing enzyme activities, histone acetyl transferases and histonedeacetylases (HDACs), determine the acetylation status of the ε-amino groups of the lysine residues at the N-terminalhistone tails extending out of the nucleosome particle. Inhibition of HDAC leading to histone hyperacetylation is therefore associated with transcriptional activation. Actually, trichostatin A (TSA) is a kind of HDAC inhibitors. Nowadays, large amounts of studies about TSA and cartilage have been reported.TSA could block MMP and ADAMTS expression and inhibit cartilage resorption induced by IL-1a. At the moment, we suspect that whether TSA is able to prevent the degeneration of autologous osteochondral transplantation grafts. So we design experiments involving animal research and cell test. Upon animal research, we inject TSA into both knees to prevent osteochondral grafts degeneration in autologous osteochondral transplantation rabbit model. Finally, we make a conclusion that TSA can suppress synthesis of cartilage extracellular matrix in the fresh osteochondral graft. However, it can meet the demand that it play a role in maintaining articular cartilage phenotype, suppressing degeneration of the old osteochondral graft.

(2)Several studies demonstrated that hypoxic preconditioning could enhance chondrocytes differentiation of mesenchymal stem cells. Also, hypoxia-inducible factors (HIFs) including HIF-1α and HIF-2α appear to be critical in chondrocytes development. Dimethyloxaloylglycine (DMOG), a non-specific hydroxylase inhibitor upregulates hypoxia-inducible factor (HIF)-1α, HIF-2α, and SOX9 transcription factors in chondrocytes. We made a presumption that whether intra-articular administration of DMOG could improve articular cartilage repair in cooperation with microfracture. We intra-articular injected of DMOG and its solvent, PBS once a week in rabbit microfracture model. After three months' injection, we found that macroscopic appearance of control group is inferior to that of the DMOG group. (Fig.3)

(3)AS SCIENCE reported a small molecule compound kartogenin (KGN) has the ability to induce mesenchymal stem cells from bone marrow into chondrocytes in 2012. We cooperated with Professor Yixiang Cheng from the institute of chemical industry, Nanjing University, to synthesis this compound. Human SMSCs were pellets cultured and induced with or without addition of KGN. Pellets size, HE staining, immunoflurescence staining, cartilage related gene expression were used to measure the chondrocytes differentiation ability. Our results showed that KGN could significantly enhance the chondrocytes differentiation and proliferation in a cartilage microenvironment. (Fig. 4)We also established an animal model of cartilage defect, and intra-articular injection of KGN to investigate if it can direct SMSCs from the synovial fluid into chondrocytes and repair the defect. We found that after three months' repeated injection, cartilage defects were properly restored. (Fig.5)

3.2. Cartilage tissue engineering

It have been suggested that stem cells based tissue engineering is a promising strategy for restoring cartilage defects. However, recent cartilage tissue engineering needs an open operation to transplant the tissue engineered cartilage into cartilage defects. Our group focuses on non-invasive methods about using cartilage tissue engineering for cartilage repair. Right now, we use injectable self-assembling peptide nanofibers to deliver KGN and SMSCs for cartilage defects repair. The self-assembling peptides we used are short peptides, typically 8 to 16amino acids long. They are insolution at low pH and osmolarity but rapidly form fibers on the order of 5 to 10 nm and assemble into a 3-dimensional scaffold at physiological pH and osmolarity. In this way, they can be injected into cartilage defects and self-assemble into tissue engineered cartilage. In the future, we will use a photoreactivehydrogel-SMSCs composite for cartilage defects repair. We hope that these kinds of methods could be used clinically.

4.International collaboration

In 2013, we have cooperated with the University of Western Australia to conduct studies about bone metabolism related disease. Right now, we mainly focuses on osteoporosis related researches including treatment measures and relevant molecular mechanisms.

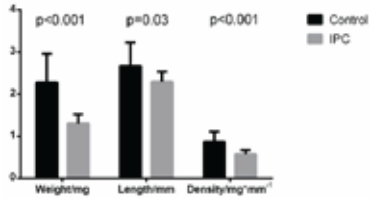


Fig1. Thrombus formation in control and IPC

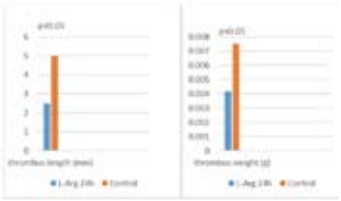
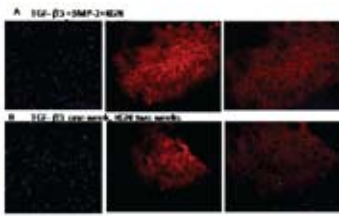


Fig2. thrombus weight and length in L-Arg group and control group

Fig3. Pellets size of human SMSCs cultured in different medium



Immunofluorescence staining for collagen type **II** after pellets was frozen sectioned

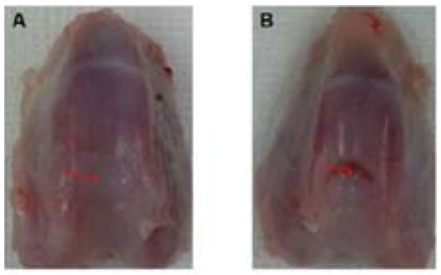


Fig.4: A:Three months after intro-articular injection of KGN
B: Three months after intro-articular injection of DMSO

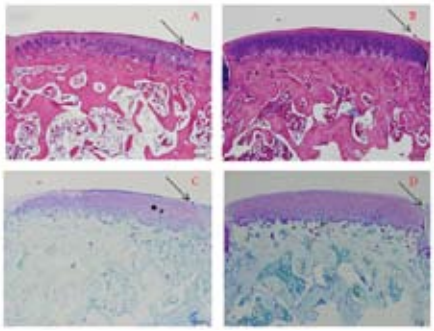
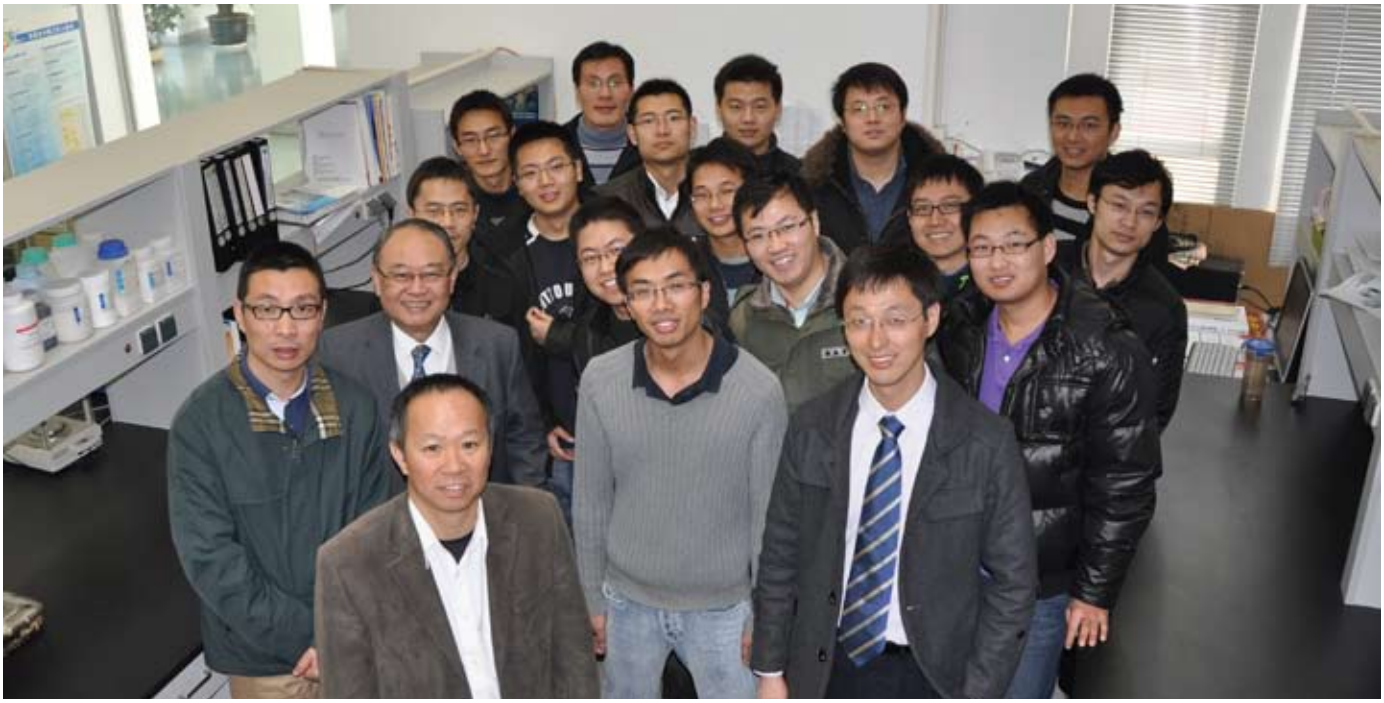


Fig.5: Histologic appearance of a graft in trochlear groove in TSA(A,C) and control group (B,D). (HE:A,Bx4; toluidine blue:C,Dx4)

Selected publications

1. Sun Y, Chen D, Xu Z, et al. The incidence of deep venous thrombosis after arthroscopically assisted anterior cruciate ligament reconstruction. Arthroscopy. 2013, 29(4):742-7.
2. Qin J, Xu Z, Shi D, et al. Deep Vein Thrombosis After Total Hip Arthroplasty and Total Knee Arthroplasty in Patients With Previous Ischemic Stroke. Int J Low Extrem Wounds. 2013 Sep 16. [Epub ahead of print]
3. Xu Z, Shi D, Zhang C, et al. Postoperative plasma D-dimer value for predicting deep venous thrombosis following hip arthroplasty with nadroparin prophylaxis. Hip Int. 2013, 23(4):411-6.
4. Qin J, Dai J, Xu Z, et al. Genetic polymorphism of NOS3 with susceptibility to deep vein thrombosis after orthopedic surgery: a case-control study in Chinese Han population. PLoS One. 2013, 8(7):e70033.
5. Wang C, Yang F, Xu Z, et al. Intravenous release of NO fromlipidicmicrobubbles accelerates deep vein thrombosis resolution in a rat model. Thromb Res. 2013, 131(1): e31-8.



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  Qiting Ge

Geng Liu, Ph.D.

Geng Liu received his B.S. degree in Biochemistry from Wuhan University, China and his Ph.D. degree in Gene & Development from University of Texas Graduate School of Biomedical Sciences at Houston in 1999. He continued his postdoctoral training at University of Texas M.D. Anderson Cancer Center under the guidance of Dr. Gigi Lozano where he studied the tumor suppression mechanism of p53 in vivo using genetically engineered mouse models. Dr. Geng Liu joined the Model Animal Research Center of Nanjing University as principal investigator and professor of Genetics in 2006.

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Tumor suppression and mouse tumor models

Improvements on both early detection and therapeutic strategies of cancer remain as a huge challenge and require a thorough understanding of the complex tumorigenic processes involving both the tumor and host tissues, and the intricate interplays of oncogenic and tumor suppression signaling pathways. Better animal models should help to elucidate the key steps and dynamic changes during tumorigenesis, pinpoint the underlying mechanistic bases and find important therapeutic clues and opportunities that can be applied to human. We aim to understand the functional mode and regulatory mechanisms in tumor development, emphasizing on the dynamic and multifaceted aspects of tumorigenesis using genetic modified mouse models.

Tumor microenvironment has been increasingly recognized to play critical roles in tumor progression, maintenance and metastasis. Chronic inflammation is one of the major mechanisms mediating the tumor promoting effects of the microenvironment. We are interested in probing the role of tumor suppressor in counteracting inflammation and suppressing tumor formation in spontaneous tumor models. p53 tumor suppressor is a central player in the defense against aberrant proliferation and malignant transformation. We found that activation of p53 in myeloid lineage significantly down-regulated inflammatory cytokine productions from macrophages upon stimulation. In addition, we also observed dampened inflammatory response and resistance to tissue damage during colitis induction in these mice (Figure 1). We further demonstrated that p53 activation in specific compartment of tumor microenvironment attenuated colitis-associated tumorigenesis (Figure 1) as well as intestinal adenoma formation in the *APCmin* mice. In contrast, we found that p53 deficiency accelerated adenoma formation in *APCmin* mice. These and other evidences support a significant role of tumor suppressor in modulating tumor microenvironment, which in turn have impacts on tumorigenesis.

Genetic labeling represents a powerful strategy to study cell lineages, cell fate and hierarchy during tissue homeostasis, regeneration and wound repair. Importantly, this strategy can also be used in tracking the evolvement of tumor cells during tumor initiation and maintenance. In combination with advanced reporting systems, distinct and dynamic cell populations could be identified in vivo and characterized in more details. We are poised to generate novel genetic labeling systems to facilitate clonal analysis of cells of various origins, including those of tumors (Figure 2).

In addition, we recently developed an interest in generating novel mouse models to study cancer metabolism. Being a core hallmark of cancer, cancer metabolic reprogramming is crucial for the growth, survival and drug resistance of tumor cells. A deeper understanding of the plasticity and interplay of cancer metabolic pathways may help to unveil the "Achilles' Heel" of cancer for successful therapies. These studies are currently in progress.

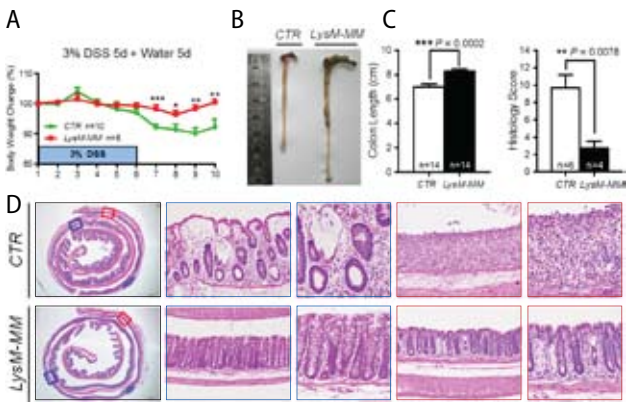
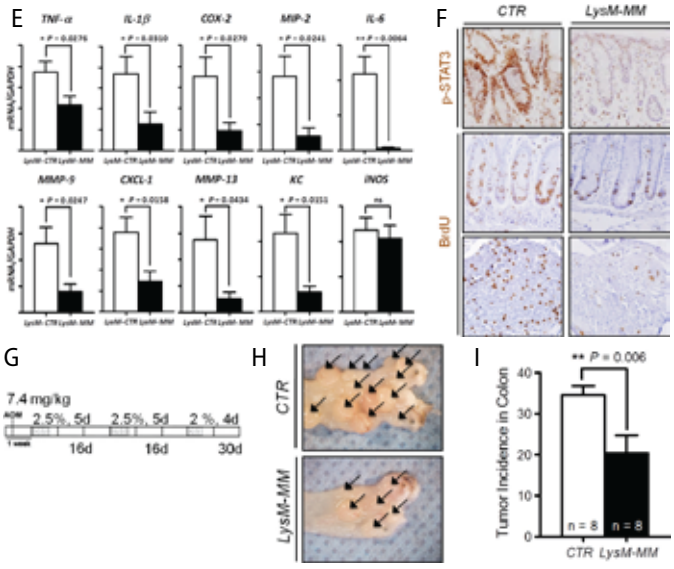


Fig 1. Mice with Myeloid-Specific Activation of p53 (LysM-MM) Were More Resistant to DSS-Induced Colitis and Colitis Associated Tumorigenesis.

(A) Mice were fed with 3% DSS for 5 days, followed by regular water for 4 days. Body weight was monitored daily.
(B) Mice were sacrificed on day 9 to measure colon length as an indicator of severity of inflammation.
(C, D) Histology score and representative colon section at day 9 after DSS administration. Note the loss of crypt structure, infiltration of inflammatory cells and ulceration in control (LysM-CTR) mice.
(E) Relative mRNA expressions of inflammatory cytokines in colons of DSS treated mice (n ≥ 4).
(F) P-STAT3 immunostaining and BrdU labeling of colon on day 15 after AOM and DSS administration from LysM-CTR and LysM-MM mice.
(G, H, I) Tumor incidences in colons of LysM-CTR and LysM-MM mice under an AOM+DSS treatment scheme.

Figure 1. continue



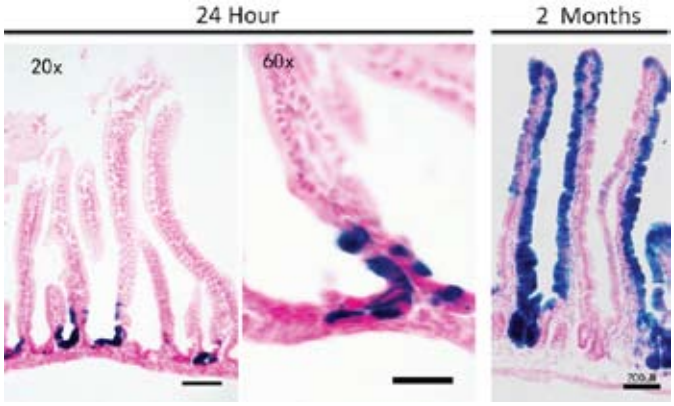


Fig 2. Development of a novel inducible genetic labeling system coupled with Rosa 26 reporter.

X-gal staining for Rosa-LacZ on the small intestine of mice through a pulse-chase experiment at the indicated time length after Tamoxifen induction. Note the limited labeling of the cells at the crypt area of the small intestine at 24 hours (pulse) and the contribution of the initially labeled cells to tissue homeostasis after a long period (chase).

Selected publications

1. Zhang Q, He X, Chen L, Zhang C, Gao X, Yang Z, Liu G. (2012) Synergistic regulation of p53 by Mdm2 and Mdm4 is critical in cardiac endocardial cushion morphogenesis during heart development. J Pathol 228(3):416-28.
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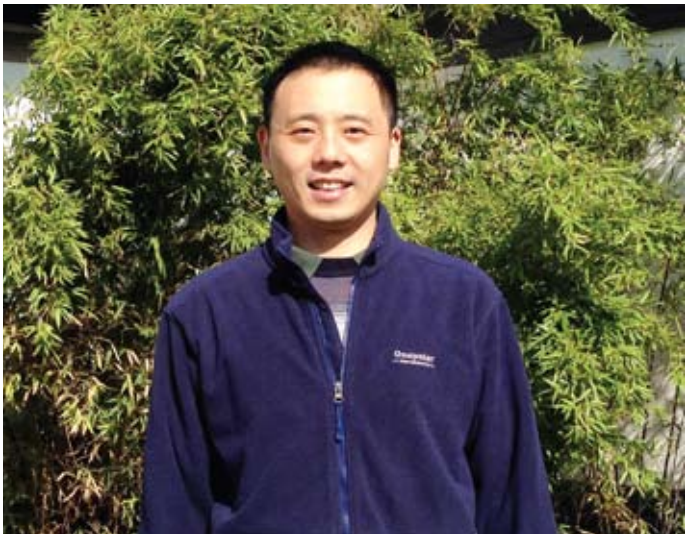

Jianghuai Liu, Ph.D.

Jianghuai Liu received his Ph.D. degree in Biochemistry from Boston University School of Medicine in 2005. Upon completion of his postdoctoral fellowship at University of Pennsylvania in 2009, he joined MARC as a principle investigator and professor of genetics.

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Regulation of immunity and reproduction

Most cellular processes, on a holistic scale, are driven by coordinated actions by multiple regulatory networks. Our understandings to life and disease can be improved by examining how these regulatory networks communicate with each other to shape the eventual biological outcomes. The general research goal of our lab is to unveil novel regulatory circuits within two biological processes, i.e. innate immune activation and female germline cell development. Using cell and animal models, we are currently analyzing the functional outputs by several fundamental regulatory pathways under these specialized biological contexts.

The ongoing projects of the lab are outlined in the following section:

1.Metabolic licensing of innate immune activation: Innate immune recognition of pathogens can be mediated by the membrane-bound pattern recognition receptors (e.g. TLRs) and their cytosol-localized counterparts (e.g. RLRs), which would in general inform the cells to engage defensive programs against bacterial and viral infections, respectively. Despite a conceivably critical role of energy metabolism in regulating the innate immune programs and pathogen replication, the metabolic aspects of innate immune activation are relatively understudied. We hypothesize that due to the different localization of infecting bacterium and viruses in relation to the protective plasma/phagocytic membrane shield, differential modes of metabolic regulation may be engaged following TLR or RLR activation.

Confirming some recent studies, we observed that TLR activation triggers increased glucose flux via glycolysis (Fig 1A). Moreover, our data suggest that allosteric activation of pyruvate kinase, presumably conferred by a PFKFB3-mediated positive feed-forward regulation, may contribute to regulation of some cytokine genes (Fig 1B, C).

Distinct from the latter scenario of a 'Warburg'-like effect, viral infection of the cells instead leads to down-regulation of PFKFB3, which may limit the carbon flux through lower glycolytic steps (Fig 1D, E, F). Further experiments are underway to clarify whether such distinct modes of metabolic regulation contribute to bacteria- or virus-specific innate immune programs.

2.Mechanisms controlling selective ovarian follicle growth: In mammals, resting female oocytes reside in primordial ovarian follicles. An individual primordial follicle may stay quiescent for a protracted period of time before initiating follicular growth, also termed 'activation'. Female reproductive capacity is sustained by the gradual, streamlined activation of the entire population of primordial follicles. How only a portion of primordial follicles are selected to grow within a given time frame is incompletely understood. Recently, we examined the necessity of mTORC1 signaling in mediating physiological primordial follicle activation in mice, based on previous reports showing the sufficiency of oocyte mTORC1 activity in causing pre-mature primordial follicle growth. We found that induction of oocyte mTORC1 activity is associated with early follicular growth in neonatal mouse ovaries (Fig 2A). Pharmacological inhibition of mTORC1 activity *in vivo* by rapamycin treatment leads to a marked, but partial, suppression of primordial follicle activation (Fig 2B). Mechanistically, our study implies that mTORC1 signaling in oocytes may engage a Cyclin A/CDK regulatory network that promotes primordial follicle activation (Fig 2C). Our study establishes a critical, early signaling node that fuels the initial oocyte growth, which may help the future research to unravel the detailed mechanisms underlying selective primordial follicle activation.

As selective growth also applies to late-stage ovarian follicles, another ongoing project in the lab is to understand the mechanisms that cause growth advantages of some pre-antral ovaraian follicles.

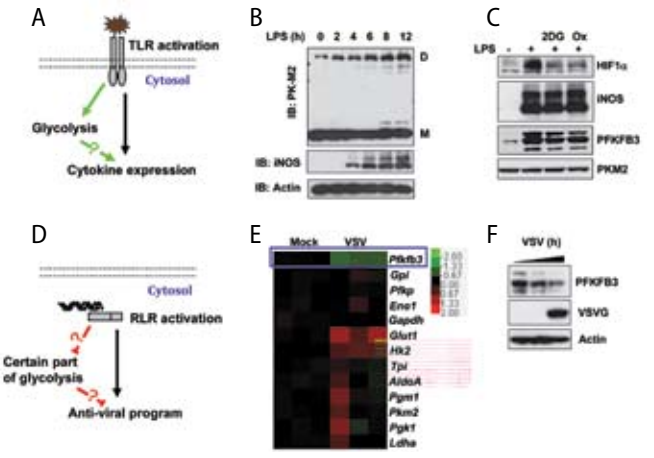

Fig.1 Metabolic changes associated with TLR or RLR activation

(A) A model for TLR-mediated induction of glycolysis.

(B) LPS treatment of Raw264.7 cells leads to an apparent increase in oligomerized PK-M2

(C) LPS treatment up-regulates PFKFB3,which may trigger an increase of glycolytic flux that sustains the induction of HIF1α.

(D) A model for RLR-mediated regulation of glycolysis that is different from the above TLR-related scenario.

(E) Among a panel of mRNAs encoding regulators/enzymes of early glucose breakdown,the mRNA of *pfkfb3* was most reliably down-regulated by viral infection.

(F) Down-regulation of PFKFB3 protein by viral infection.

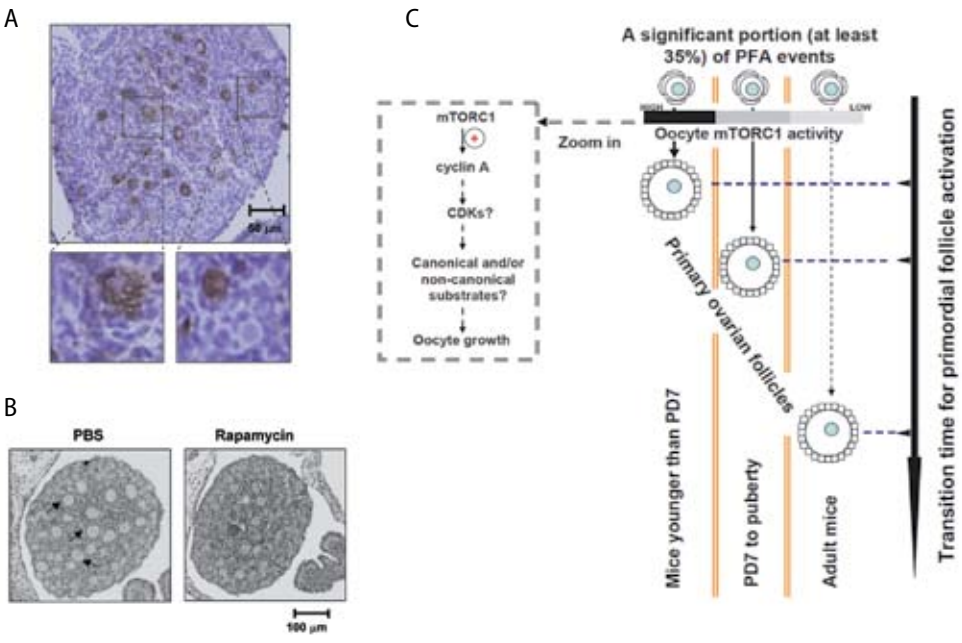

Fig.2 mTORC1 signaling is involved in physiological activation of primordial ovarian follicles in mice

(A) Oocyte mTORC1 activity is associated with primordial follicle activiation in neonatal mice (PD4)

(B) Rapamycin treatment significantly suppressed primordial follicle activation.

(C) A model for mTORC1-mediated regulation of Cyclin A in determining the rate of primordial follicle activation.

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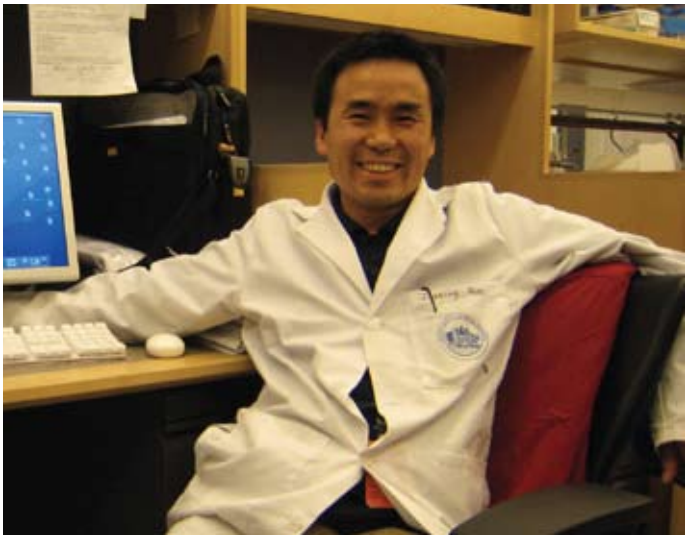

Jinzhong Qin, Ph.D.

Jinzhong Qin received his Ph.D. from Cleveland State University (Ohio, USA) in 2004 after completing a research project at Department of Immunology, Cleveland Clinic Foundation. His research at Cleveland Clinic was focused on the regulation of innate immune signaling pathways. From 2005 to 2008, Jinzhong did his postdoctoral fellowship at the Massachusetts General Hospital Cancer Center, Harvard Medical School in Boston, USA, and he was promoted to Assistant in Genetics within the same institution in 2008. Using murine genetics, he described an essential role of L3mbtl2-containing atypical Polycomb Repressive Complex 1 (PRC1) in embryonic stem cells (ESCs) proliferation and early embryonic development. He joined the Faculty of Model Animal Research Center (MARC), Nanjing University in 2013. He is now a professor of genetics and developmental biology and a Principal Investigator in MARC.

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Roles of the polycomb group proteins in stem cells & early development

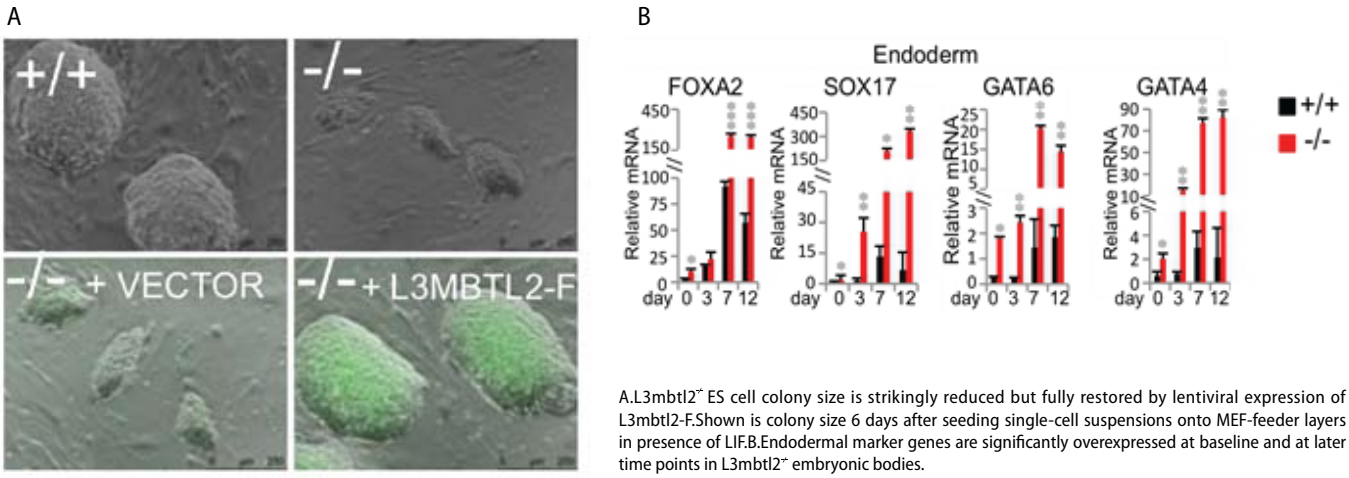
Pluripotent stem cells are capable of differentiating into any cell type in the body and therefore hold tremendous promise for the future of regenerative medicine. However, a detailed understanding of the molecular mechanisms that regulate the pluripotent state is currently lacking. Recently, we show that L3mbtl2, a mbt family member without previously known biological functions, is critical for early embryonic development as well as ES cell proliferation and differentiation and provide first insights into its unique molecular role (see figure below). In ESCs, L3mbtl2 establishes an atypical PRC1 complex that includes Oct4, G9A and several components of the E2F6 and NuRD repressor complexes. Intriguingly, the majority of genes bound and repressed by L3mbtl2 in ESCs are not occupied by canonical PRC1 and PRC2, although a small set of lineage commitment genes are co-occupied by all three complexes.

The main objective of our future study is to comprehensively establish the role of L3mbtl2-containing atypical PRC1 in stem cells, embryonic development, and cancer and to define its function at a molecular, mechanistic level. The success of our study will not only contribute to uncovering novel and essential molecular mechanism for governing stem cell pluripotency but also provide basic knowledge that in the long term is required for realizing the therapeutic potential of stem cells. Our ongoing studies address the following specific aims:

1. Elucidate the precise molecular mechanisms of L3mbtl2-mediated transcriptional repressive complex.

2. Defines the roles of other components of L3mbtl2-containing repressive complex in ESC self-renewal by genetic approaches.

3. Identify functions of L3mbtl2-mediated complex in cancer and other diseases.



A.L3mbtl2$^{-/-}$ ES cell colony size is strikingly reduced but fully restored by lentiviral expression of L3mbtl2-F.Shown is colony size 6 days after seeding single-cell suspensions onto MEF-feeder layers in presence of LIF.B.Endodermal marker genes are significantly overexpressed at baseline and at later time points in L3mbtl2$^{-/-}$ embryonic bodies.

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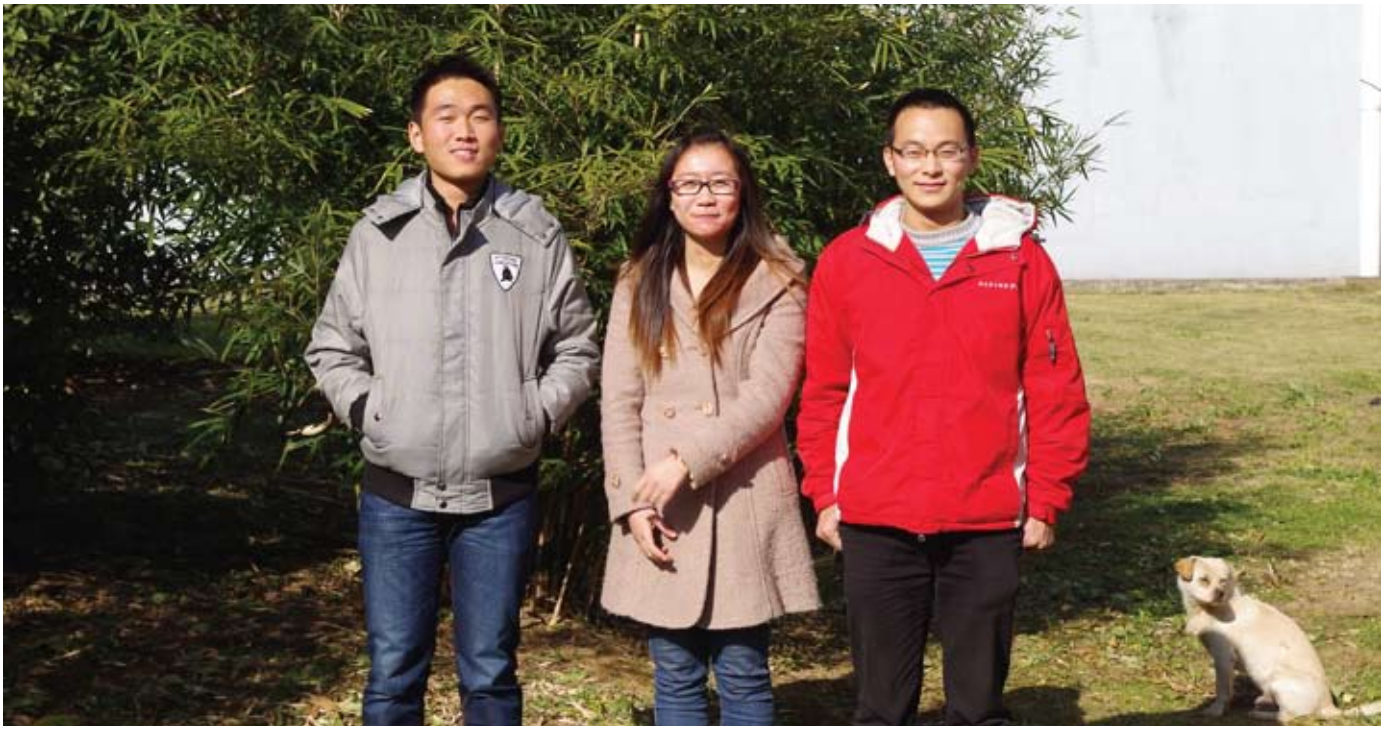

Lab members

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  Huan Tong
- **Graduate Students**
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Pingping Shen, Ph.D.

Pingping Shen received her PhD degree at Nanjing University in 2000. From 2002 to 2003, she studied at University of California at San Diego as a visiting scholar. In 2004, she was appointed as a professor in Nanjing University and moved to Model Animal Research Center (MARC) as Adjunct Professor for research on Inflammation and related diseases. Research in Pingping Shen's Lab is mainly focused on two fields: regulation of macrophage functions in tumor progression and development of new clinical immunoassay techniques.

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Macrophage functions in tumor associated inflammation

PPAR-γ Phosphorylation in Functional Regulations of Macrophage and Tumor Cell

Post-translational regulation plays a critical role in the control of the phenotype, function and fate of cells. The phosphorylation is one of the most important modifications for peroxisome proliferator-activated receptor-γ (PPAR-γ). Phosphorylated PPAR-γ exerts new biological functions comparing with PPAR-γ. We are focusing on studying the PPAR-γ phosphorylation model and the role of Phosphorylated PPAR-γ in the regulation of macrophage function and tumor progression.

Phosphorylated PPAR-γ switches phenotype of macrophage

PPAR-γ can be phosphorylated at several sites by different up-stream kinases, resulting diverse biological effects. Such as some sites phosphorylating may further decrease ligand-binding affinity, reduce the transcriptional activity, and eventually impair adipogenesis; the other sites may be involved in the pathogenesis of insulin-resistance, and present an opportunity for development of an improved generation of anti-diabetic drugs through PPAR-γ activity. Therefore, discovering new phosphorylation sites of PPAR-γ and related functions is meaningful.

We have predicted three new phosphorylation sites (NS1, NS2 and NS3) of PPAR-γ by using combinational strategy and found that phosphorylation of NS1 could inhibit PPAR-γ activity. We have illustrated the correlation between PPAR-γ's phosphorylation and macrophage function. Phosphorylation of NS1 can change M1/M2 macrophage status and propelled the M2 polarization, which has being been an important regulator of TAM(tumor associated macrophage) differentiation. We demonstrated that an upstream kinase (PK) which could interact with AF1 domain of PPAR-γ and initiated phosphorylation of NS1, further inhibit the M2 phenotype of macrophage. Furthermore, we have screened serious inhibitors which could be the potential drug by using the PK/NS1 model.

PPAR-γ phosphorylation promotes hepatocarcinogenesis

We have investigated the pivotal role of PPAR-γ phosphorylation in hepatocellular carcinomas genesis. By using the nonphosphorylation (Ser84 to alanine, S84A) and phosphorylation (Ser84 to aspartic acid, S84D) mutant of PPAR-γ, spontaneous hepatocellular carcinoma model and nude mouse model, we found that phosphorylation attenuated PPAR-γ transcriptional activity and changed the transcriptional profile, resulting in an increased tumor cell proliferation. Moreover, phosphorylated PPAR-γ promoted antiapoptotic capacity of hepatoma carcinoma cells.

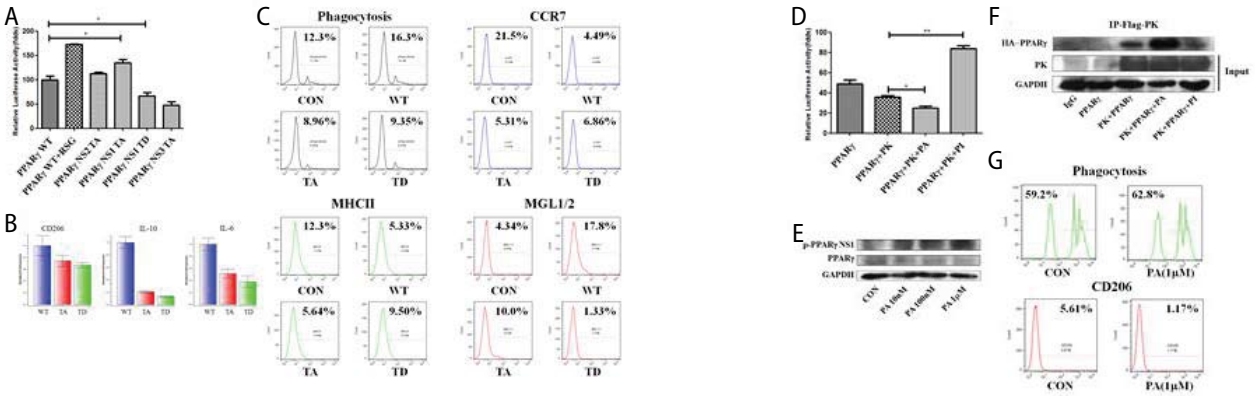

Fig.1 Phosphorylation of PPAR-γ new site 1 switches phenotype of macrophage

(A) Screen of PPAR-γ possible phosphorylation new sites

(B-C) Phosphorylation of PPAR-γ NS1 might switch the phenotype of macrophage from M2 to M1.

(D-G) Possible kinase interact with PPAR-γ and regulate the phenotype of macrophage

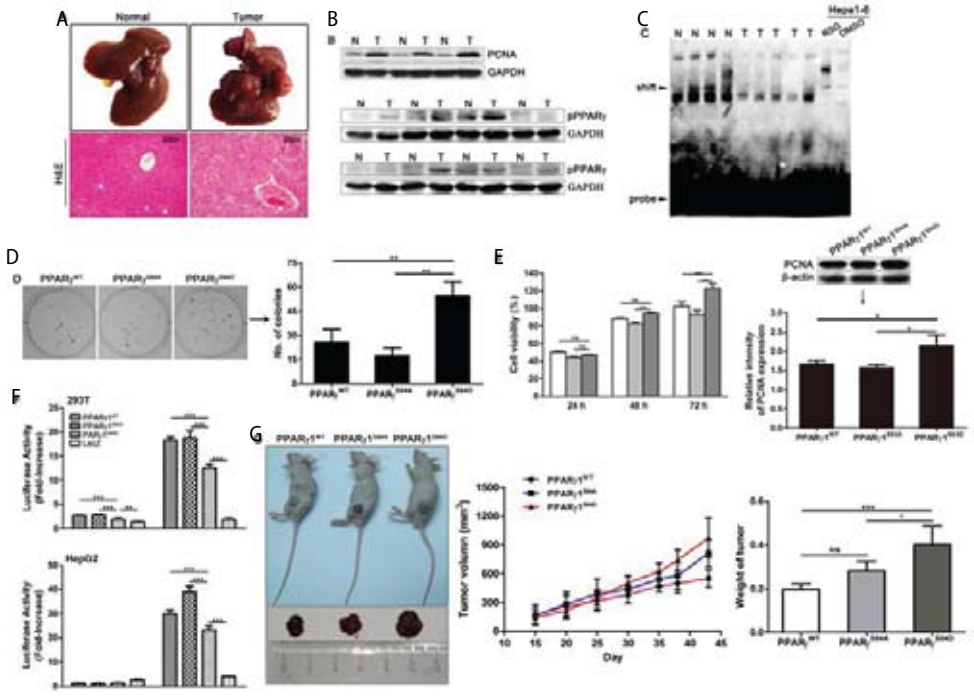

Fig.2 PPAR-γ phosphorylation promotes hepatocarcinogenesis in vivo and vitro.

A: DEN-induced hepatocellular carcinomas (HCCs) in C57BL/6 mice.

B: Increased expression of PPAR-γ phosphorylation in mouse HCCs.

C: Transcriptional activity of PPAR-γ in mouse HCCs and normal livers.

D and E: Ectopic expression of PPAR-γ phosphorylation promoted colony formation and cell proliferation in HepG2 cells.

F: Phosphorylation attenuated PPAR-γ transcriptional activity.

G: PPAR-γ phosphorylation promoted tumorigenesis in vivo.

Selected publications

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  Yinna Wei
  Chun Chen
  Hanwen Xu
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  Xiaojuan Pang
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  Xiujing Feng
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  Sen Ding
- **Technician**
  Wei Zheng

Jun Yan, Ph.D.

Jun received his Bachelor in Genetics at Fudan University in Shanghai in 1997 and his Ph.D. degree in Cell Biology at Institute of Biochemistry and Cell Biology, Shanghai Institutes for Life Sciences, Chinese Academy of Sciences in 2003. Afterwards, he pursued his postdoctoral training at Baylor College of Medicine in Houston and moved to Columbia University in New York as Associate Research Scientist. Recently, Jun joined the Model Animal Research Center of Nanjing University as Associate Professor and principal investigator.

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Nuclear receptor cofactors in disease models

Cancer heterogeneity refers to the existence of subpopulations of cells with distinct genotypes and phenotypes, which is a widely accepted phenomenon in solid tumors. Clonal evolutionary model of carcinogenesis, which was first put forward by Nowell in 1976 and elaborated by Darwinian models of natural selection, explains cancer heterogeneity which permitting the tumor as a whole to adapt to a fluctuating microenvironment. This also can explain for drug resistance and metastasis, which are the reasons for the increase of the mortality rate.

At molecular level, carcinogenesis is a multiple-step process intertwined with genetic and epigenetic alterations. Recent novel techniques, such as whole-genome sequencing and RNA-seq, have provided new perspective for cancer biologists to fully understand the process of carcinogenesis, especially to identify the promising driver genes, which are essential for cancer development. Notably, besides frequent altered genes, such as tp53 and ras, there exists a group of high frequent deregulated genes, involved in epigenetic modifications. They include histone modifiers and noncoding RNAs. To understand their cellular functions and the networks regulated by them, will provide us a real picture of cancer development. Of particular interest, identification of these molecular alterations may give us novel diagnostic biomarkers and potential therapeutic targets in near future.

Our lab is interested in the elucidation of the molecular mechanisms underlying cancer recurrence and metastasis, especially the epigenetic alterations involved in these processes. Active projects include: (1) As a small population in solid tumor, cancer stem cells are resistant to conventional chemotherapeutic agents. Hypoxia is one of the major factors for the maintenance of cancer stem cells. We are now characterizing the epigenetic alterations involved in cancer stem cell maintenance under hypoxic microenvironment. (2) Histone H3K9 and H3K27 are two important sites for post-translational modifications. EHMT2 and EZH2, two histone methyl transferases, have been implicated as driver genes in cancer development, especially in metastasis. Therefore, we strive to determine the regulatory networks formed by these two histone modifiers to keep metastatic capacity of cancer cells. In addition, we found out a chemical derived from herbal extract can suppress tumorigenicity through targeting EZH2. (3) To establish novel mouse cancer models recapitulating human cancer development. These mouse models will provide excellent platforms for pre-clinical study sometime in the future.

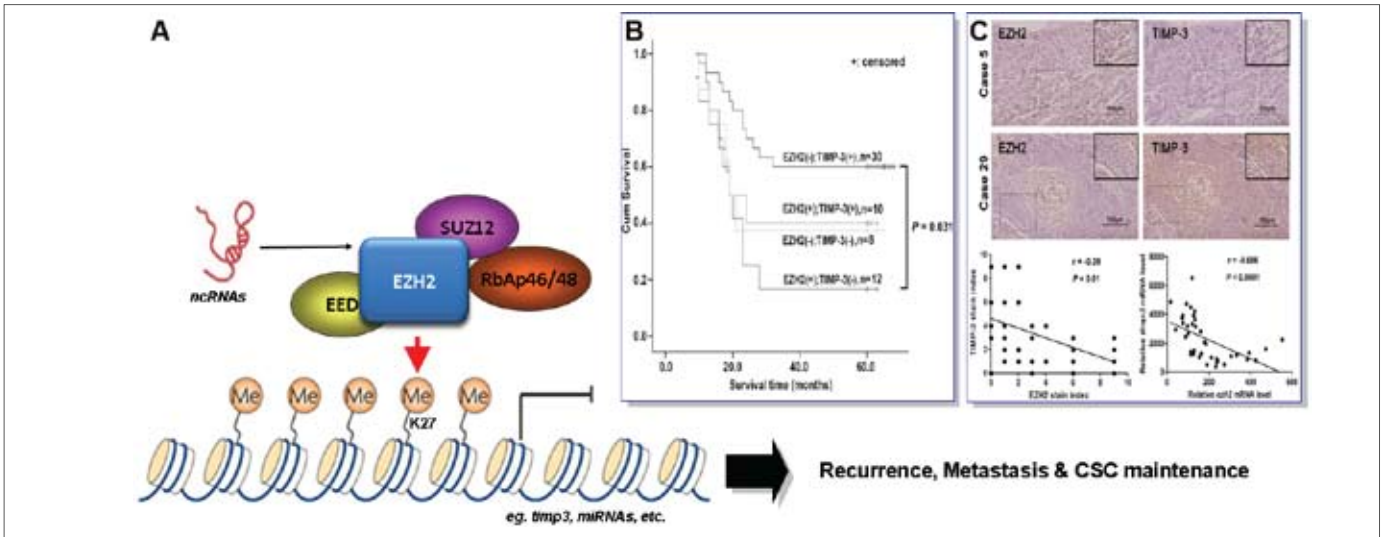

Fig 1. EZH2 represses TIMP-3 to promote cancer malignance.

A) cartoon of EZH2 network.

B) EZH2/TIMP3 ratio predicts cancer patients outcome.

C) Inverse relationship between EZH2 and TIMP3 in clinical samples at protein and mRNA levels.

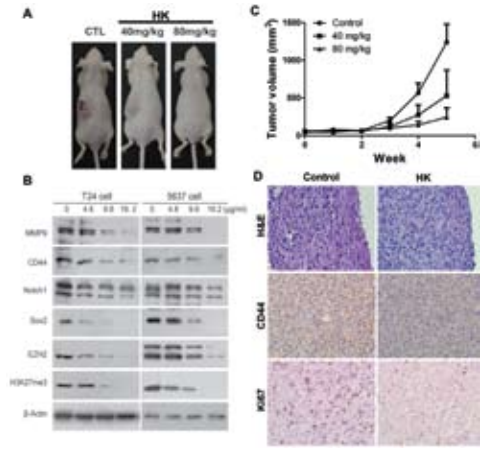

Fig 2. HK, derived from herb extract, inhibits tumor formation through suppressing EZH2 expression.

A)HK inhibits tumorigenecity.

B) HK suppresses the expressions of EZH2 and related CSC markers.

C) Tumor growth curve.

D) H&E and IHC analyses on control and HK treated xenografts.

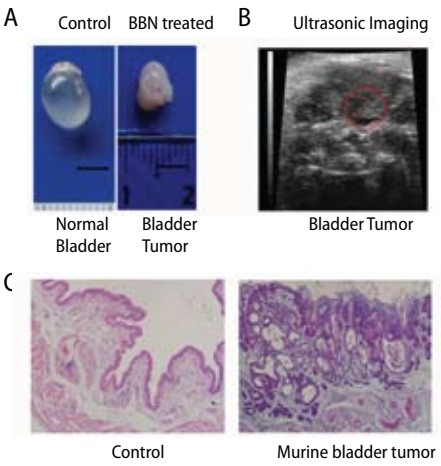

Fig.3 Bladder cancer mouse model.

A) photograph of mouse bladder.

B) in vivo US imaging.

C) histologic analysis of normal bladder and bladder tumor.

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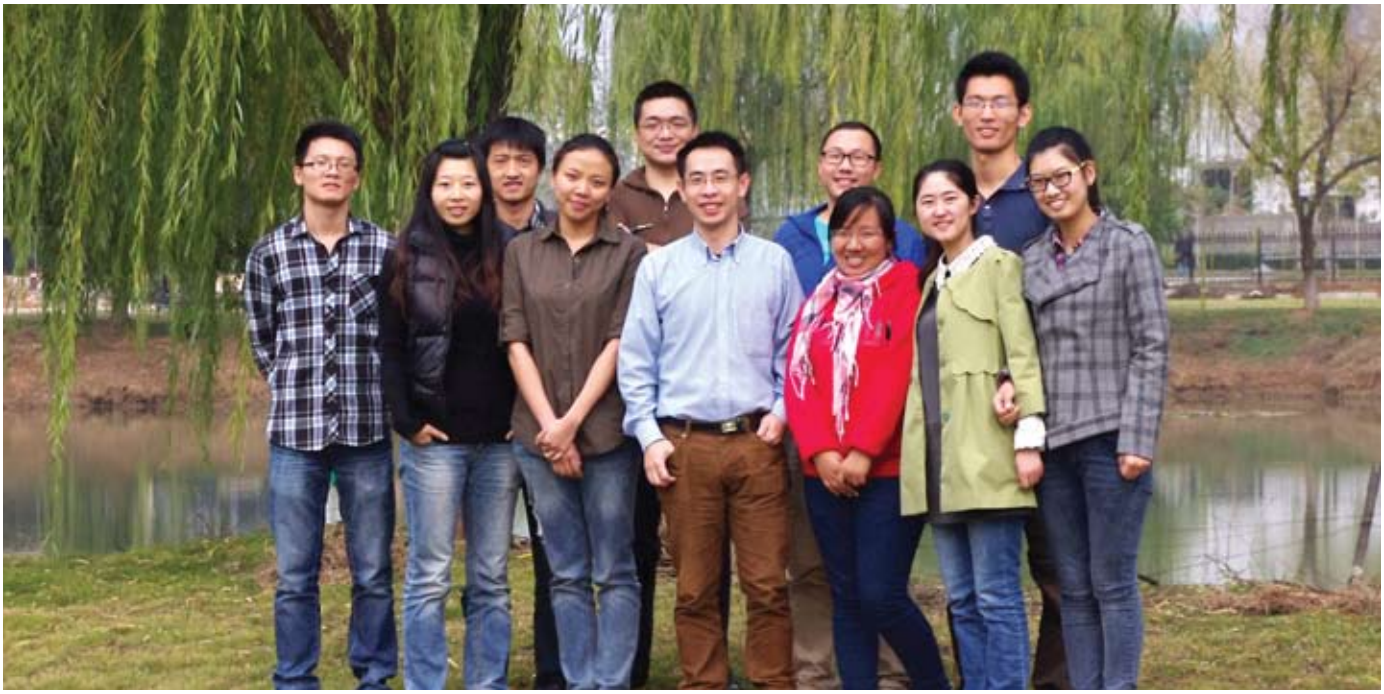

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Guiquan Chen, Ph.D.

Guiquan obtained his PhD in Neuroscience at Edinburgh University in Scotland and then did postdoctoral research for several years at Harvard University and Edinburgh University. He joined the MARC of Nanjing University as a principle investigator in December 2011. His research interest is molecular mechanisms underlying γ–secretase-dependent neurodegeneration. His lab uses a combination of mouse genetics, molecular biology, cellular and behavioral neuroscience techniques. Elucidation of molecular mechanisms by which γ–secretase promotes neuronal survival in the adult brain may help identify novel therapeutic targets for the prevention and the treatment of Alzheimer's disease, a major form of neurodegenerative diseases.

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Neuroinflammation and tau hyperphosphorylation in neurodegenerative diseases

Alzheimer's disease (AD) is the most common form of dementia, characterized by progressive memory loss, neurodegeneration and the presence of amyloid plaques and neurofibrillary tangles. Presenilin and nicastrin are two essential components of the γ-secretase complex, a key enzyme that cleaves amyloid precursor protein (APP) to produce β-amyloid. γ–Secretase is believed to be involved in the pathogenesis of neurodegeneration, as numerous genetic mutations on various subunits of this protease complex have been reported in familial cases of AD.

Our recent data have indicated that nicastrin plays a critical role in neuronal survival in adult brain. To generate a loss-of-γ–secretase-function animal model, we crossed floxed nicastrin to a CaMKIIa-Cre transgenic mouse and obtained viable neuron-specific nicastrin conditional knockout (cKO) mice. First, significantly reduced protein levels for nicastrin were found in the cortex. Second, in situ hybridization revealed decreased nicastrin mRNAs levels in the cortex but not in the cerebellum of nicastrin cKO mice. Third, levels of APP C-terminal fragments (CTFs), a direct substrate of γ-secretase, were dramatically increased in the cortex of nicastrin cKO mice.

To determine whether nicastrin is required for the maintenance of neuronal survival in adult brain, we performed Nissl staining using brain sections of nicastrin cKO mice at 2 (young group) and 6-9 (old group) months of age, and we observed significant brain atrophy in old but not in young cKO mice. We performed biochemical analyses on a neuronal marker, NeuN and found that protein levels of NeuN in old cKO cortical samples were about 30% less than those in age-matched control samples (Fig. 1A), suggesting age-dependent neuron loss. As abnormal tau phosphorylation is believed to have a critical role on neurodegeneration, we then conducted biochemical analyses on protein levels of tau phosphorylated at different epitopes (Fig. 1B). Interestingly, although young cKO mice did not show changes, old cKO mice displayed massive increases on p-tau levels, suggesting age-dependent tau hyperphosphorylation.

Neuroinflammation has long been believed to be a driving force for neurodegeneration. We further examined inflammatory responses in cKO mice. We first conducted immunohistochemical analysis on GFAP, a marker for astrocytes. At young age, there was significant increase in GFAP immuno-reactivity in the cortex and the hippocampus of cKO mice (Fig. 2A). Our Western analysis confirmed about 40% increase in cKO mice at this age. At old age, there was massively enhanced immuno-reactivity on GFAP in cKO mice. Western data were consistent (~4 folds of increase). Next, we examined immuno-reactivity on Iba1, a marker for microglia, in cKO mice. Interestingly, there were small increases on immuno-reactivity and protein levels of Iba1 (Fig. 2B) in cKO mice at young age. Protein levels of Iba1 in old cKO mice showed a significant increase (~6 folds) relative to those in controls. Therefore, loss of nicastrin causes progressive neuroinflammation. The occurrence of neuroinflammation precedes that of neurodegeneration in nicastrin cKO mice.

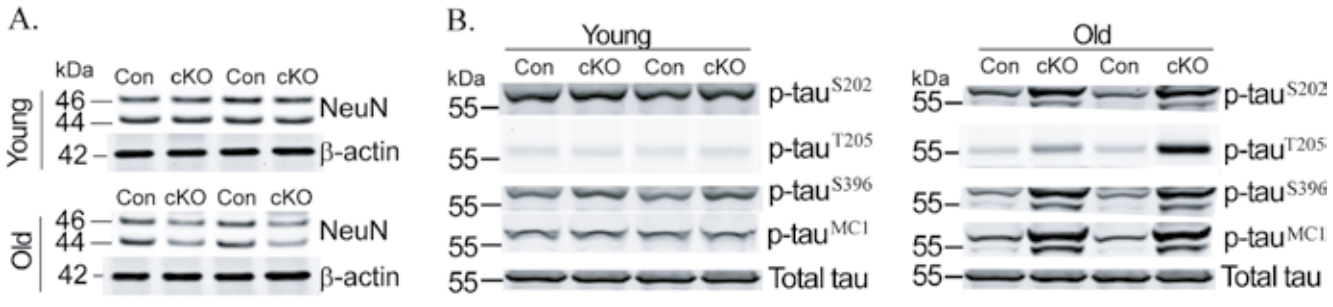

Figure 1. Age-dependent neuron loss and tau hyperphosphorylation in neuron-specific nicastrin conditional knockout (cKO) mice.

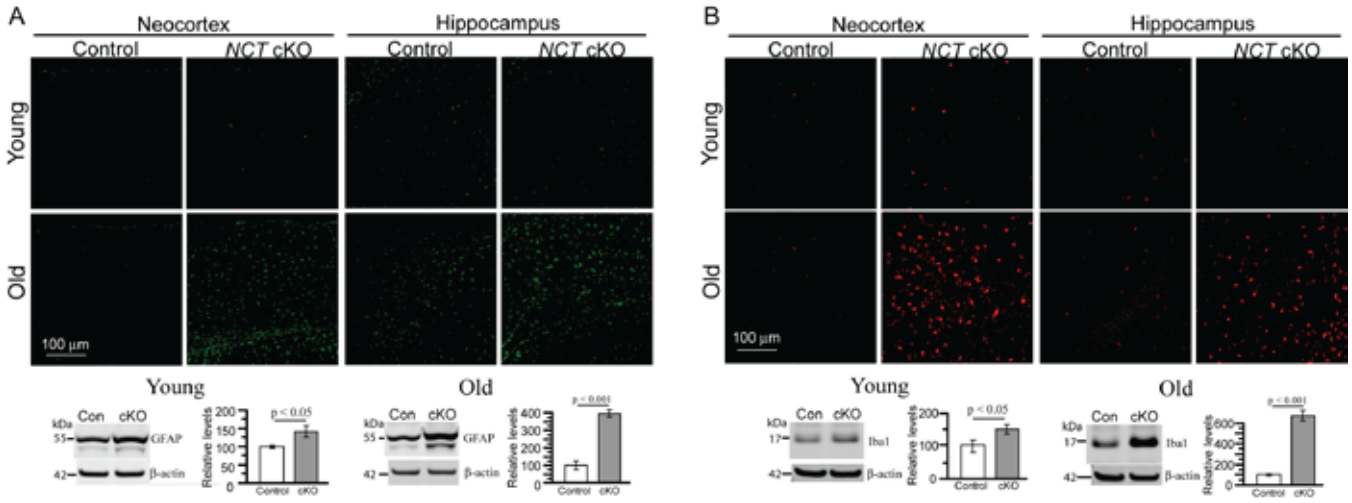

Figure 2. Progressive neuroinflammation in neuron-specific *nicastrin* cKO mice

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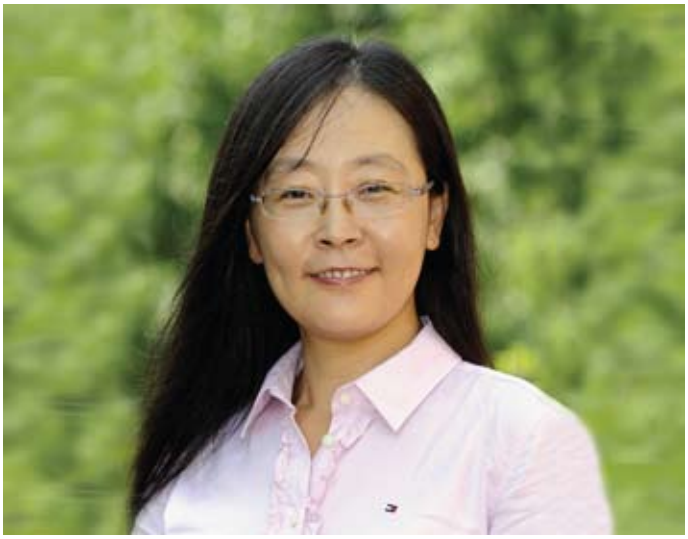

Huiming Gao, M.D., Ph.D.

Huiming Gao received her M.D. in 1993 and Ph.D. in 2003 from Dalian Medial University. Her Ph.D. thesis was carried out at the National Institute of Environmental Health Sciences (NIEHS)/ National Institutes of Health (NIH) in the USA. After her postdoctoral training at University of Pennsylvania School of Medicine and NIEHS/NIH, Dr. Gao joined the Faculty of Model Animal Research Center (MARC), Nanjing University in 2013. She is now a professor and a principle investigator in MARC.

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Neuroinflammation and neurodegeneration

More than 36 million people worldwide suffer from Alzheimer's disease (AD) and Parkinson's disease (PD), the two most common neurodegenerative diseases. Chronic, irreversible degeneration of brain neurons causes progressive memory loss in AD and movement impairment (e.g. tremor and rigidity) in PD. Importantly, what drives the decades-long progression of neurodegeneration in neurodegenerative diseases remains unknown. At present, no treatment can slow down or retard such neurodegeneration. Many previous studies including ours have elucidated that neuroinflammation (inflammation in the brain) is an important contributor to chronic neurodegeneration. The goal of our research is to investigate a potential driving role for chronic neuroinflammation in progressive neurodegeneration, to identify new therapeutic targets, and to develop novel anti-inflammatory and neuroprotective therapeutics for neurodegenerative diseases.

During viral infection, extracellular dsRNA is a potent signaling molecule that activates many innate immune cells, including macrophages. TLR3 is a well-known receptor for extracellular dsRNA. Preserved inflammatory responses of TLR3-deficient macrophages to extracellular dsRNA support a TLR3-independent mechanism. We recently identified Mac-1 (macrophage antigen complex 1) as a novel surface pattern recognition receptor (PRR) for extracellular dsRNA. dsRNA-binding assay and confocal immunofluorescence showed interaction and colocalization of the CD11b subunit of Mac-1 with poly I:C (polyinosinic:polycytidylic acid; a synthetic dsRNA) on the surface of macrophages (Figure 1). CD11b deficiency reduced poly I:C-elicited cytokine production in mouse sera and cultured peritoneal macrophages. Further mechanistic studies revealed that Mac-1 facilitated poly I:C internalization through activation of PI3K signaling and enhanced TLR3-dependent IRF3 activation. Poly I:C stimulates phagocyte NADPH oxidase (NOX2) and triggers inflammatory oxidative signaling in a TLR3-independent, but Mac-1–dependent, manner (Figure 2). This study identifies a novel mechanistic basis for macrophages to recognize extracellular dsRNA and implicates Mac-1 as a potential therapeutic target for virus-related inflammatory diseases. In order to determine the role of virus-related inflammation in the development of PD, we will further investigate roles of dsRNA-Mac1 interaction on activation of brain immune cells microglia and neuronal survival. We will also develop and screen anti-inflammatory neuroprotective drugs (e.g. Mac-1 or NOX2 inhibitors). Since neuroinflammation is the most common feature shared by all neurodegenerative diseases, our research will present hope for therapeutic advances that ameliorate many neurodegenerative disorders simultaneously.

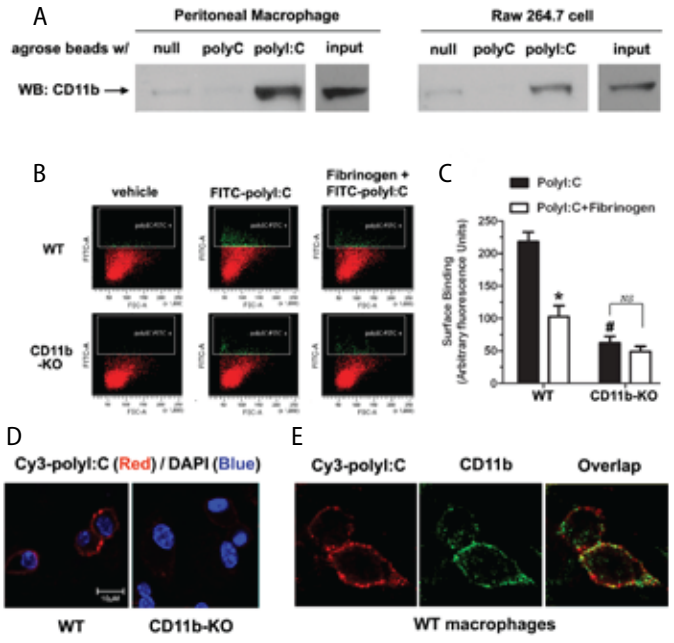

Figure 1. poly I:C (a synthetic dsRNA) bound to CD11b subunit of Mac-1 on the cell surface.

(A) dsRNA-binding assay showed only poly I:C–coated beads can pull down the CD11b subunit of Mac-1.

(B-D) Flow cytometry and confocal images revealed higher surface binding of fluorescence-labeled poly I:C in wildtype (WT) macrophages than CD11b-/- macrophages; the poly I:C binding was inhibited by fibrinogen (an endogenous ligand of Mac-1) only in WT macrophages.

(E) Mac-1 colocalized with surface-bound Cy3–poly I:C.

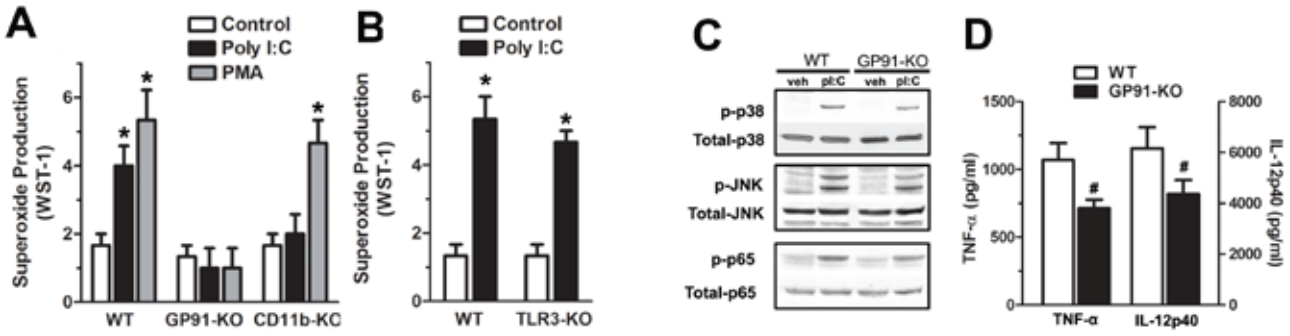

Figure 2. Mac-1–dependent activation of NOX2 enhanced poly I:C–elicited immune response.

(A, B) Deficiency in CD11b and gp91, but not TLR3, abolished poly I:C-elicited extracellular superoxide release.

(C, D) gp91 knockout attenuated poly I:C-induced activation of MAPK and NK-kB and secretion of TNF-α and IL-12p40 in macrophages.

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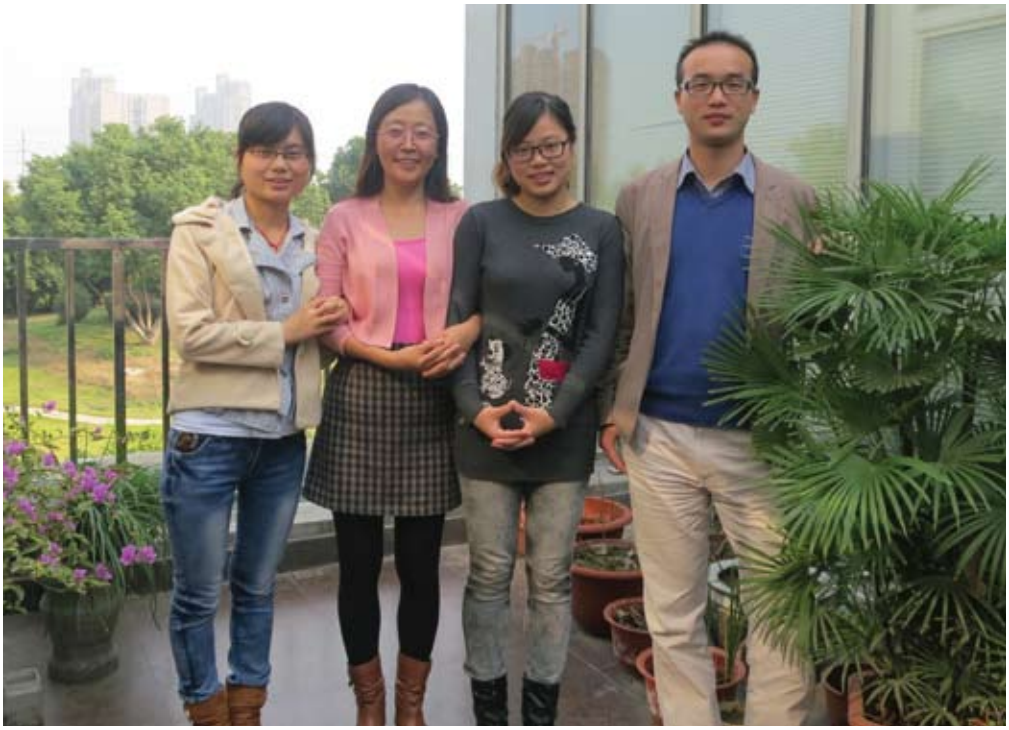

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Yun Shi, Ph.D

Yun Shi received his Ph.D degree in Georgia State University under the advisor of Dr. Chun Jiang at Atlanta, USA in 2007. He then had postdoctoral training with Dr. Roger Nicoll in UCSF. In 2013, he joined the Model Animal Research Center, Nanjing University as a professor and principle investigator.

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The fundamental mechanisms of neural plasticity

The central neural system (CNS) is a complex network, in which the appropriate functions rely on the information exchange among neurons through a specified structure named synapses. The neurotransmitters released from pre-synaptic neurons bind to post-synaptic receptors, enhancing or inhibiting postsynaptic activity. Meanwhile, postsynaptic neurons also release certain regulatory information and modulate presynaptic activity, allowing the feedback occurs. Many of high neuronal functions result from the changes of neurotransmission, or so called plasticity. Dysfunction of synaptic activity is the major causes of neuro-degeneration diseases. Therefore, studying the synaptic transmission not only help unreal human high neural functions but also improve our understanding on the mechanisms of neural diseases and provide cues for cure.

Glutamate is the major excitatory neurotransmitter in CNS. Two types of glutamate receptors are located on the post-synaptic membrane, i.e., ionotropic and metabotropic glutamate receptors. Ionotropic receptors include AMPA, NMDA and Kainate receptors, each are composed of different subunits. The normal function of excitatory synapses and the generation of neural plasticity are dependent of the composition and expression level of postsynaptic glutamate receptors. However, how the receptors correctly traffic to post-synaptic membrane and how the expression level is appropriately regulated remain unclear.

Hippocampus is a relative simple structure in brain, which is believed to play an essential role in learning and memory. The CA3-CA1 synapses are arguably the best studied synapses in brain. The plasticity of those synapses, including long-term potentiation (LTP) and long-term depression (LTD), are believed to be the fundament of learning and memory mechanisms. During my postdoctoral training in Dr. Roger Nicoll's laboratory, I had studied two family of AMPA receptors auxiliary subunits, the TARPs and the Cornichons, in regulation of AMPA receptor trafficking using the hippocampus model. In the study of LTP, we also found Neto proteins enhances the trafficking of kainate receptors (Nature 2013). I will continue to study those topics.

The projects in our lab are: 1. The fundament of long-term potentiation. 2. Synaptic trafficking of NMDA-type glutamate receptors. 3. Kainate receptor trafficking, synaptic targeting and function regulation.

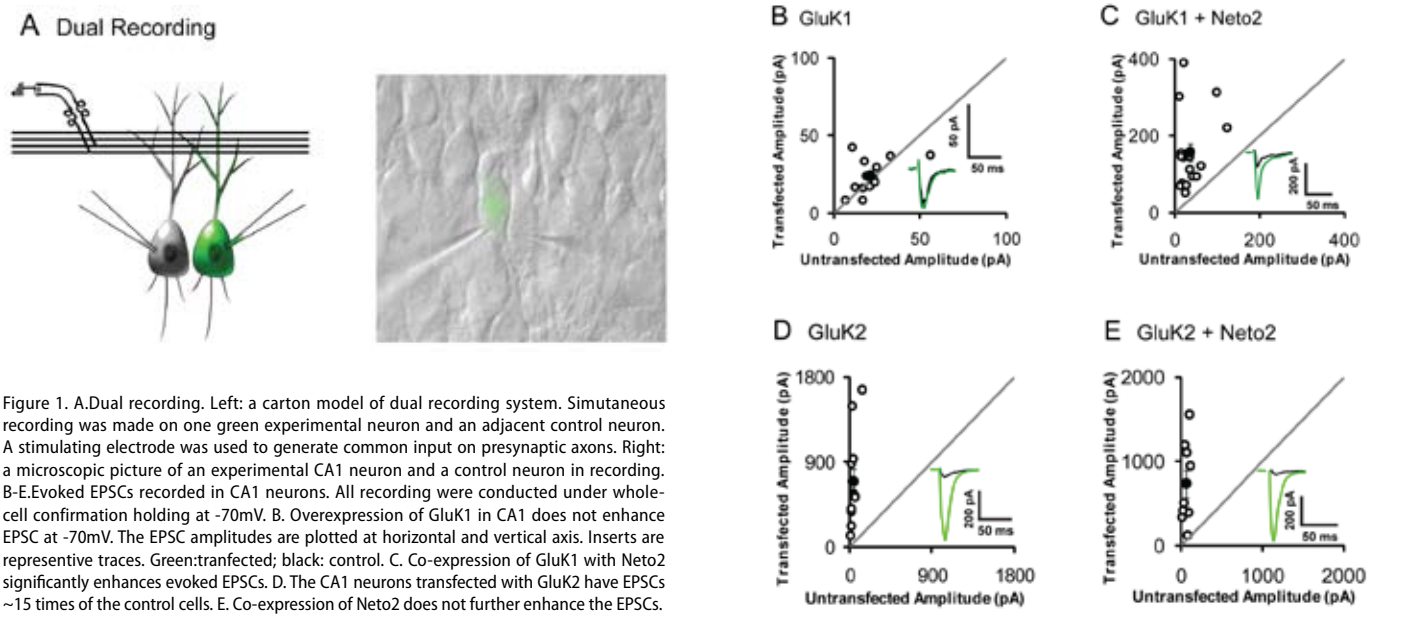

Figure 1. A.Dual recording. Left: a carton model of dual recording system. Simutaneous recording was made on one green experimental neuron and an adjacent control neuron. A stimulating electrode was used to generate common input on presynaptic axons. Right: a microscopic picture of an experimental CA1 neuron and a control neuron in recording. B-E.Evoked EPSCs recorded in CA1 neurons. All recording were conducted under whole-cell confirmation holding at -70mV. B. Overexpression of GluK1 in CA1 does not enhance EPSC at -70mV. The EPSC amplitudes are plotted at horizontal and vertical axis. Inserts are representive traces. Green:tranfected; black: control. C. Co-expression of GluK1 with Neto2 significantly enhances evoked EPSCs. D. The CA1 neurons transfected with GluK2 have EPSCs ~15 times of the control cells. E. Co-expression of Neto2 does not further enhance the EPSCs.

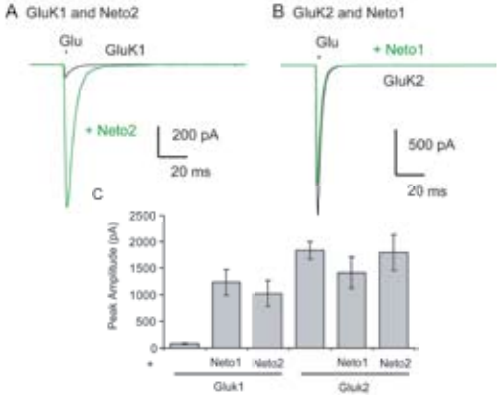

Figure 2. Extrasynaptic currents. Outside-out patches were pulled from transfected CA1 neurons. Glutamate (10mM) was applied to patches for 1 ms using a piezor switcher in the presence of gyki53655. A. CA1 cells transfected with GluK1 has small currents compared to GluK1+Neto2. Shown are representive traces. Green: GluK1 + Neto2; Black: GluK1. It should be noted that patches from wt CA1 neurons generally produces no currents. B. Neto1 does not enhanced GluK2 extrasynaptic currents. C. Summary of peak current amplitudes induced by 1 ms glutamate.

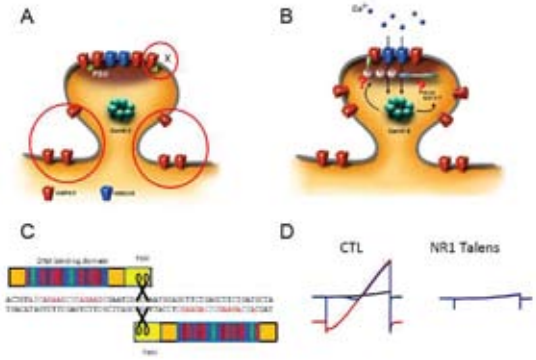

Figure 3. The mechanisms of LTP. A. Our previous study demonstrates that the phosphorylation of AMPA receptors is not required for LTP induction (Nature 2013). Instead, the extrasynaptic pool of AMPARs is essential for LTP. B. Our new hypothesis about LTP induction. The question marks indicate some of our hypothetic molecules. C. The schematic demonstration about Talens. D. Our preliminary data about Talens efficiency in cultured Neurons. Left, the NMDA receptors mediated currents recorded in a control cerebellar granule neuron (CGN). Black: baseline; Red: Glutamate induced NMDAR currents in the absence of Mg2+; Blue: NMD currents in the presence of 1mM Mg2+. Right, the same recordings in a CGN with Talens targeting NMDAR subunit NR1. Note that NMDAR currents are all gone.

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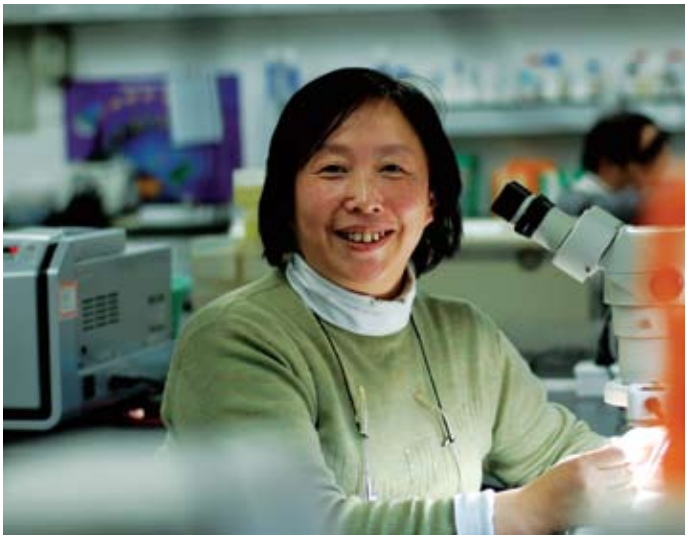
- **Visiting students**
  Lili Qiu
- **Techanicians**
  Yanyu Zang
  Huanhuan Hou (intern)



Ying Xu, Ph.D.

She received a bachelor's degree in Pharmacology from Shanghai Medical University in 1985. Then she earned her first Ph.D. in Dept. of Pathology, Saitama Medical School in 1996, and second Ph.D. under Dr. Nobutaka Hirokawa, in Dept of Cell Biology and Anatomy, University of Tokyo in 2001. After she worked in The YS Institute as Chief Scientist for two years, she moved to Dept of Neuroscience, University of California, San Francisco in Fu and Ptacek lab as visiting postdoctoral from 2003-2006. In 2006, she was recruited to the Model Animal Research Center, Nanjing University.

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Circadian rhythm and health research

Circadian behavior is endogenous rhythms in physiology or behavior with a period length near 24 hours in all genetically studied organisms. In most cases, these rhythms are generated by endogenous processes referred to as circadian oscillators. These oscillators provide temporal structure to an organism's physiological processes. Nearly all functions of the body show significant daily variations including arousal, cognition, learning, memory, motor performance and perception. This temporal variation obviously plays an important role in the body' homeostatic mechanisms and has a major impact on the physiological processes.

Our laboratory is using cellular, molecular, genetic, evolutional and behavioural approaches to more fully understand the circadian system, with a focus on identifying linkers between circadian system and peripheral tissues such as ovary. Another major project is on understanding why and how extant traits in species may have evolved to elucidate broad principles of how adaptive evolution occurs in response to some selection pressures.

Active projects include: (1) Mammalian circadian clock is composed of interlocking feedback loops. How these two loops are coupled together to generate robust circadian rhythms is unclear. We are carrying out phenotype-driven genetic screens and genetic interaction screens to the basic mechanism of oscillator function. (2) In the past decades, it has become clear that signalling cascades contributing to various physiological regulations respond to both central and cellular timing signals. Disruption in the normal circadian rhythms of an animal result in changes in sleep, activity, metabolism, cell cycle etc., and may ultimately lead to a number of diseases. We are trying to elucidate the integral role of the circadian clock in normal physiology as well as disease. Our studies demonstrated that the clock affects heart performance, and cell cycle progression. (3) Some new clock models were been generating including Drosophila, Zebrafish and mice to understand the multiple oscillators and construct PER family function network and their evolution.

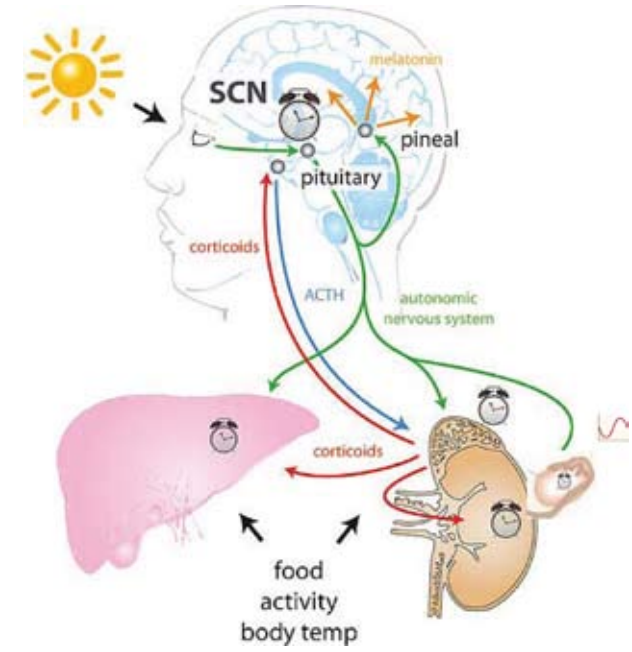

Fig.1 Overview of Circadian Clock:

It has been known that the principle of mammalian circadian clock is located in the hypothalamus called suprachiasmatic nucleus (SCN). The SCN receives input from external cues and conveys information via neural and chemical messages to the circadian clock of peripheral tissues. Every day the cues from the environment such as light/dark cycles and food entrain the body to external time.

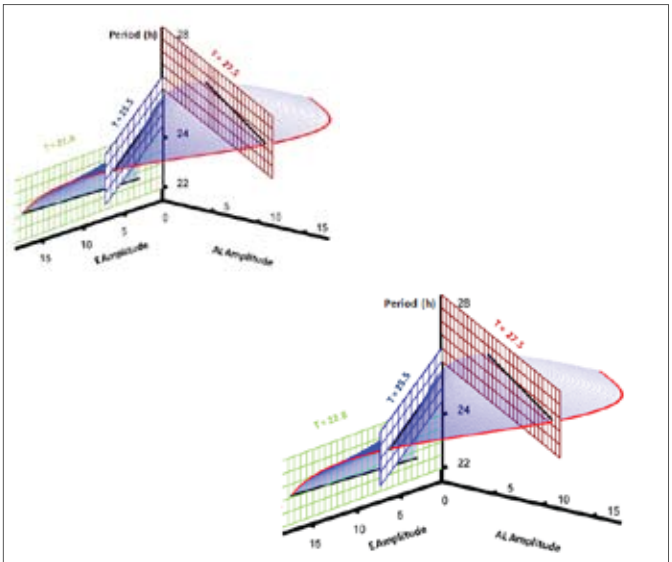

Fig.2 Model illustrating E-box and RRE codependency for effective circadian period determination

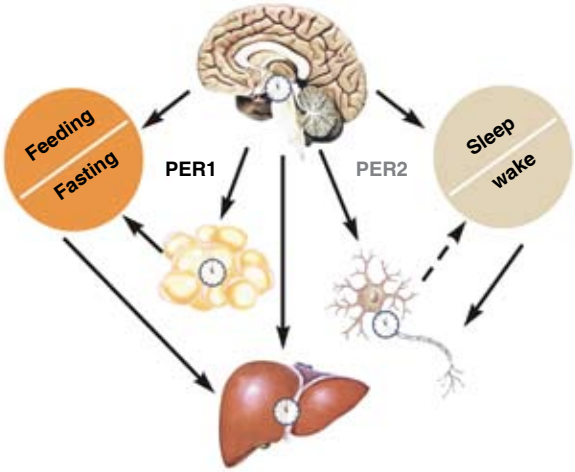

Fig. 3 PER1 and PER2 are linked to different physiological pathways

Selected publications

1. Wu X, Wang B, Dong Z, Zhou S, Liu Z, Shi G, Cao Y, Xu Y. A nanos3 mutation linked to protein degradation causes premature ovarian insufficiency. Cell Death Dis. 2013;4:e825
2. Shi G, Xing L, Liu Z, Qu Z, Wu X, Dong Z, Wang X, Gao X, Huang M, Yan J, Yang L, Liu Y, Ptacek LJ, Xu Y. Dual roles of fbxl3 in the mammalian circadian feedback loops are important for period determination and robustness of the clock. Proceedings of the National Academy of Sciences of the United States of America. 2013;110:4750-4755
3. Lee HY, Nakayama J, Xu Y, Fan X, Karouani M, Shen Y, Pothos EN, Hess EJ, Fu YH, Edwards RH, Ptacek LJ. Dopamine dysregulation in a mouse model of paroxysmal nonkinesigenic dyskinesia. The Journal of clinical investigation. 2012;122:507-518
4. Gu X, Xing L, Shi G, Liu Z, Wang X, Qu Z, Wu X, Dong Z, Gao X, Liu G, Yang L, Xu Y. The circadian mutation per2(s662g) is linked to cell cycle progression and tumorigenesis. Cell death and differentiation. 2012;19:397-405
5. Wu X, Liu Z, Shi G, Xing L, Wang X, Gu X, Qu Z, Dong Z, Xiong J, Gao X, Zhang C, Xu Y. The circadian clock influences heart performance. Journal of biological rhythms. 2011;26:402-411
6. Wang X, Gao X, Xu Y. Maged1: Molecular insights and clinical implications. Ann Med. 2011;43:347-355
7. Tao W, Chen S, Shi G, Guo J, Xu Y, Liu C. Switch/sucrose nonfermentable (swi/snf) complex subunit baf60a integrates hepatic circadian clock and energy metabolism. Hepatology. 2011;54:1410-1420
8. Wang X, Tang J, Xing L, Shi G, Ruan H, Gu X, Liu Z, Wu X, Gao X, Xu Y. Interaction of maged1 with nuclear receptors affects circadian clock function. The EMBO journal. 2010;29:1389-1400
9. He Y, Jones CR, Fujiki N, Xu Y, Guo B, Holder JL, Jr., Rossner MJ, Nishino S, Fu YH. The transcriptional repressor dec2 regulates sleep length in mammals. Science. 2009;325:866-870
10. Xu Y, Toh KL, Jones CR, Shin JY, Fu YH, Ptacek LJ. Modeling of a human circadian mutation yields insights into clock regulation by per2. Cell. 2007;128:59-70
11. Xu Y, Padiath QS, Shapiro RE, Jones CR, Wu SC, Saigoh N, Saigoh K, Ptacek LJ, Fu YH. Functional consequences of a ckidelta mutation causing familial advanced sleep phase syndrome. Nature. 2005;434:640-644

Lab members

- **Group leader**
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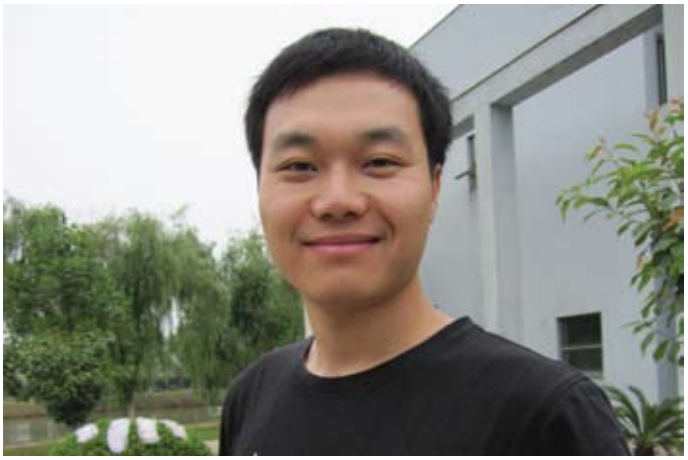
- **Technical assistants**
  Lingming Zhang
  Yumei Wang
- **Graduate students**
  Guangsen Shi
  Xi Wu
  Zhen Dong
  Zhipeng Qu
  Pancheng Xie
  Zhihui Zhang
  Yang An
  Dongchuan Liu
- **Research Associates**
  Ping Wang
  Lijun Xing
  Zhiwei Liu
- **Former graduate students**
  Xiwen Gu
  Xiaohan Wang

Student of the Year 2013



Guangsen Shi

Guangsen Shi received his Bachelor's degree of Biochemistry in 2008 from School of Life Science, Nanjing University. He joined Dr. Ying Xu's Lab at the year of 2007 to investigate the mechanism of mammalian circadian clock.

For the past few years, his study focused on the regulation of mammalian circadian clock. Using an unbiased genetic approach, he and his colleagues revealed an unexpected genetic interaction between the clock genes Fbxl3 (F-box and leucine rich repeat protein 3) and Rev-erba (orphan nuclear receptor, Nr1d1). This work extended our knowledge about how the network of the molecular circadian clock works within our bodies and provided new insight into manipulating the clock pharmaceutically.

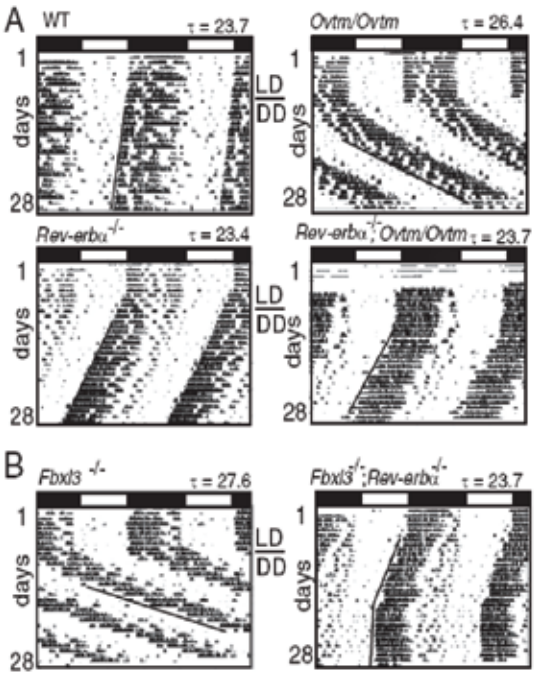


Fig1.Representative actograms of locomotor activty in established mice across the circadian day.Figures illustrate that Fbxl3 genetically interacts with Rev-erbα.

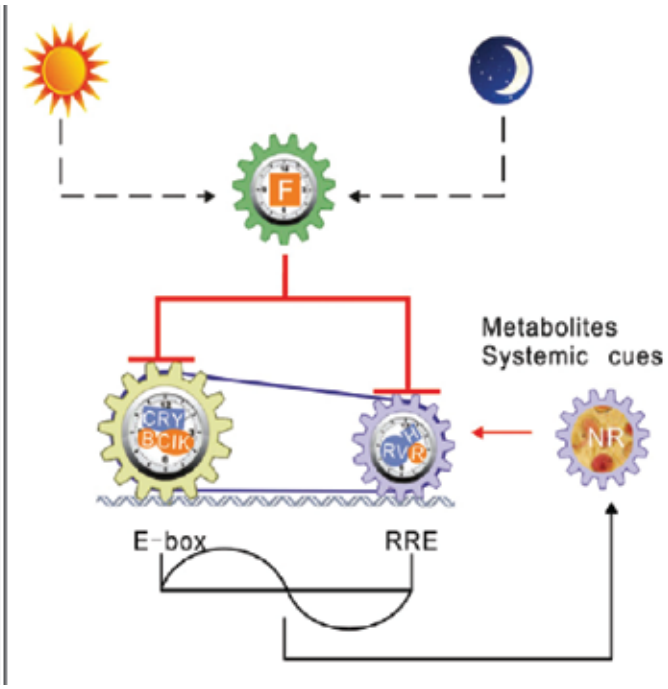


Fig2.Model illustrating FBXL3 on E-box and RRE codepedence for effective circadian period determination.

Selected publications

1. Jie Yan†, Guangsen Shi†, Zhihui Zhang†, Xi Wu, Zhiwei Liu, Lijuan Xing, Zhipeng Qu, Zhen Dong, Ling Yang and Ying Xu. Robust Period of Mammalian Circadian Oscillator from Amplitude Balance between Feedback Loops (to be submitted)
2. Shi G, Xing L, Liu Z, Qu Z, Wu X, Dong Z, Wang X, Gao X, Huang M, Yan J, Yang L, Liu Y, Ptacek LJ, Xu Y. Dual roles of fbxl3 in the mammalian circadian feedback loops are important for period determination and robustness of the clock. PNAS 2013;110:4750-4755
3. Wu X, Wang B, Dong Z, Zhou S, Liu Z, Shi G, Cao Y, Xu Y. A nanos3 mutation linked to protein degradation causes premature ovarian insufficiency. Cell Death Dis. 2013;4:e825
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5. Wu X, Liu Z, Shi G, Xing L, Wang X, Gu X, Qu Z, Dong Z, Xiong J, Gao X, Zhang C, Xu Y. The circadian clock influences heart performance. Journal of biological rhythms. 2011;26:402-411
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National Resource Center For Mutant Mice (NRCMM)

NRCMM is recognized as a leading mammalian genetics research center focusing on mouse genetics research and a unique resource center supporting the science community. Through 12 years' development, NRCMM has developed advanced and reliable platforms of mouse genome modifications such as transgene, gene knock-out and knock-in, ENU chemical mutagenesis, and etc.

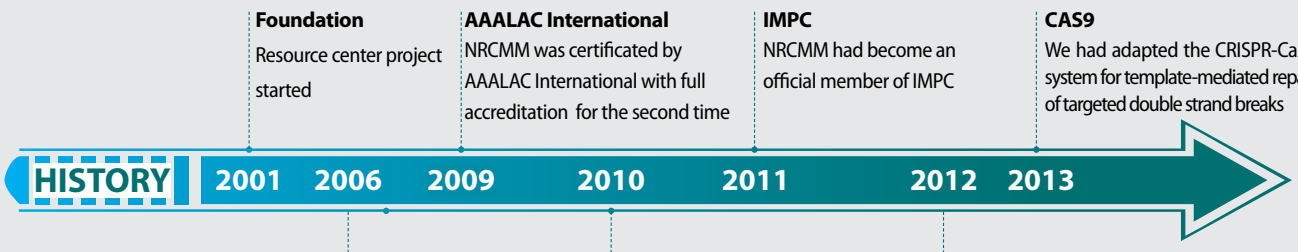
NRCMM is now harboring 1500 mouse strains, at the same time distributing ~150,000 genetically engineered mice including cardiovascular diseases, tumor, metabolic diseases, immunodeficiency and neurodegenerating disease models to research institutes and laboratories all over the world. We also help import and export mouse strains for customers worldwide.

Through years of dedicated work, NRCMM has established good relationships and long-term cooperations with world's leading pharmaceutical and biomedical companies and research institutes like Eli Lilly, GSK, Pfizer, UC Davis and the Beatson Institute for Cancer Research, the University of Western Australia, MRC, Schepens Eye Research Institute, and Harvard Medical School.

We are proud to be:

- The **only** national resource center for mutant mice authorized by the Ministry of Science and Technology of China
- The **first** research center which certificated by AAALAC International Institution and keep the **longest** certification until now
- As the **largest** mouse preservation center and the **largest** gene knockout service organization in Asia,it has 90000 IVC cages in SPF level facilities
- The **only** formal member from China joined in the International Mouse Phenotyping Consortium(IMPC)
- The **first** strategic cooperative research institution with Medical Research Council(MRC) and National Laboratory Animal Center(NLAC) in China
- Knockout service guaranted by germline transmission(GLT);**100%** success rate for TG service
- **High** quality animals(free of over 43 pathogens including all types required by the GB standard).
- **Integration** service(from IDEA to DATA).

HISTORY OF NRCMM



Foundation
Resource center project started

AAALAC International
NRCMM was certificated by AAALAC International with full accreditation for the second time

IMPC
NRCMM had become an official member of IMPC

CAS9
We had adapted the CRISPR-Cas9 system for template-mediated repair of targeted double strand breaks

2001 Foundation
2006 AAALAC International
2009 AAALAC International
2010 National resource center for mutant mice
2011 IMPC
2012 AAALAC International
2013 CAS9

AAALAC International
NRCMM was certificated by AAALAC International with full accreditation

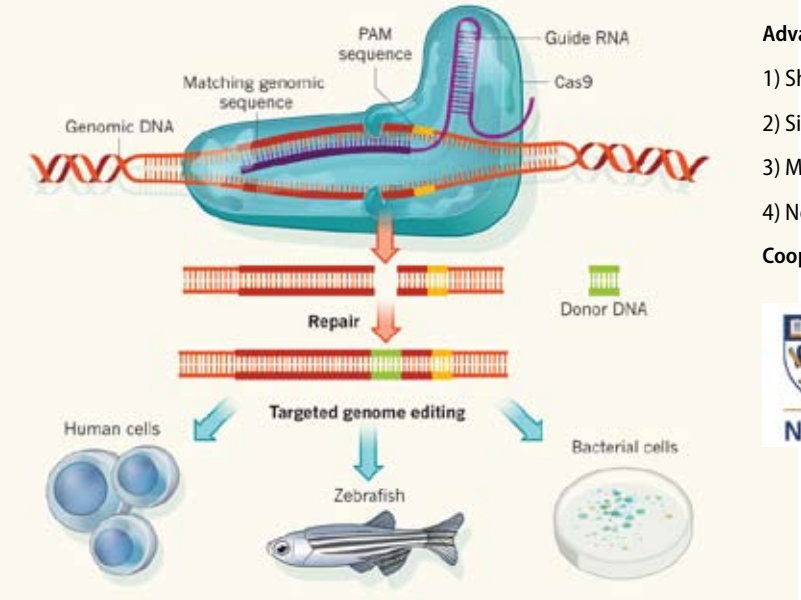
National resource center for mutant mice
NRCMM was authorized national resource center of mutant mice by the Ministry of Science and Technoloogy of the People's Republic of China

AAALAC International
NRCMM was certificated by AAALAC International with full accreditation for the third time

1.2013 Highlights

The CRISPR-Cas9 method provides an efficient and facile strategy to create loss-of-function gene mutations which are also known as conventional knock-out; we also adapted the CRISPR-Cas9 system for template-mediated repair of targeted double strand breaks via homologous recombination in mice, enabling customized and efficient genome editing such as conventional knock-out, conditional knock-out, knock-in and point mutation.

Technology roadmap



Advantages compared with traditional method:

1) Short cycle: skip ES cell targeted step, 4-6 months;

2) Simpler: one step pronuclear micro-injection;

3) More efficient: 50-80% efficiency rate;

4) No species limited;

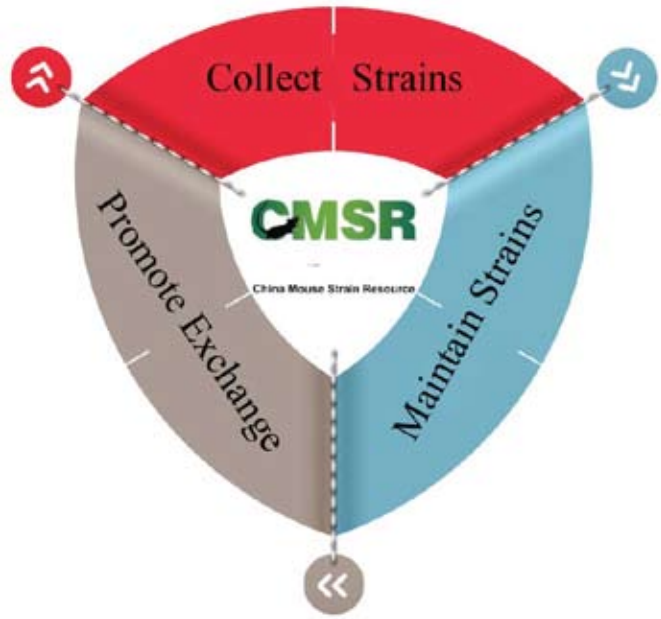
Cooperation organization:

NUS National University of Singapore
NUS Enterprise

FUDAN UNIVERSITY 1905

IBCB

2.Resource Sharing Alliance



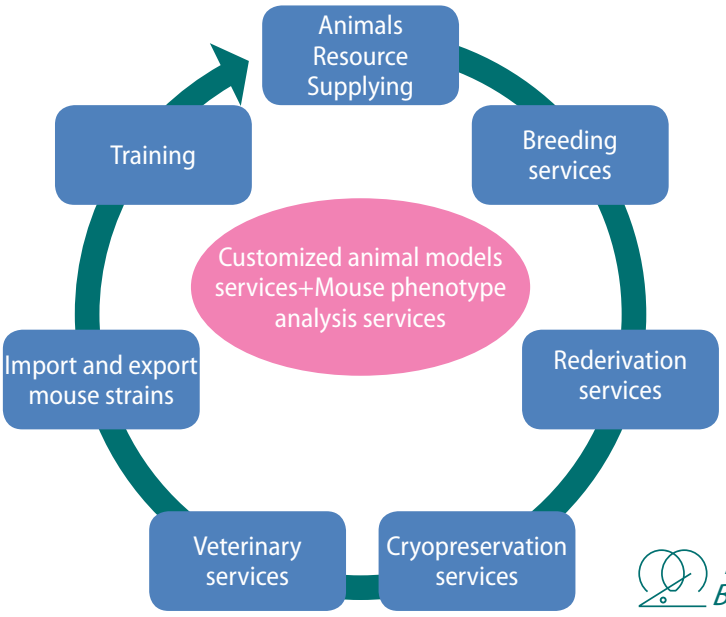
Http://CMSR.NRCMM.CN

Sharing and collaborative utilization of model mouse resource have become a global trend. At present, developed countries like UK, USA and Japan have already established complete repository and resource systems. Within this global atmosphere, Chinese Mouse Strain Resource (CMSR) was established.

- Ensure equal access to valuable research resources in China
- Promote the sharing and collaborations of model mouse resources
- Dedicate to avoiding reimporting and regenerating mouse models based on the assertions of proper intellectual property rights
- Collect and organize available mouse resources through a complete and centralized network system
- Ensure that every alliance member is capable of finding desired mice strains by searching CMSR's online database at any time any place

THEME: Targeting resource integration and research serving, CMSR is devoted to establishing an active platform which would provide all alliance members with services including one-step inquiries and resource sharing of available model mice strains, and thus further promote the development of biomedical researches in China

3.One-Stop Service Provided By Us



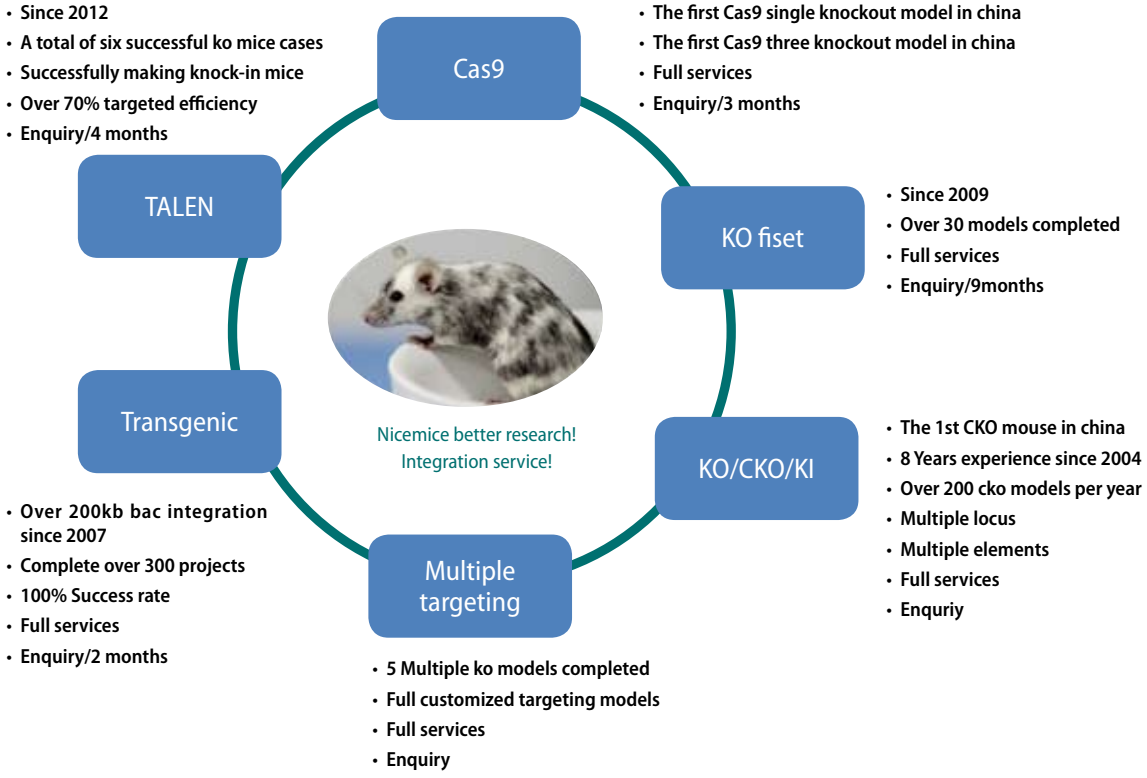
4.Transgenic/Knockout Mice Services

NRCMM provides services for generating transgenic mice (including large fragment transgenic mice, such as BAC transgenic mice) , knockout mice (including conventional KO, conditional KO, Double KO and KO First, knock in mice and more complicated targeting mutated mice). Nowadays,

CRISPR/Cas9 is an important new tool for genome engineering by using RNA-guided DNA endonuclease Cas9, the type II (clustered regularly interspaced short palindromic repeat) .CRISPR–Cas system uses CRISPR RNA (crRNA) as a guide to locate the DNA target and the Cas9 protein to cut DNA then generates DSBs in the target DNA. Easy programmability of the Cas9 endonuclease using customizable RNAs brings unprecedented flexibility and versatility for targeted genome modification.

Moreover, our services are flexible to offer partial services of whole package and/or adapt to customer requirements.

Tel: 86-025-58641559 Contact: Lu Ying Email: yinglu@nicemice.cn



5.International Standardized Phenotyping Platform

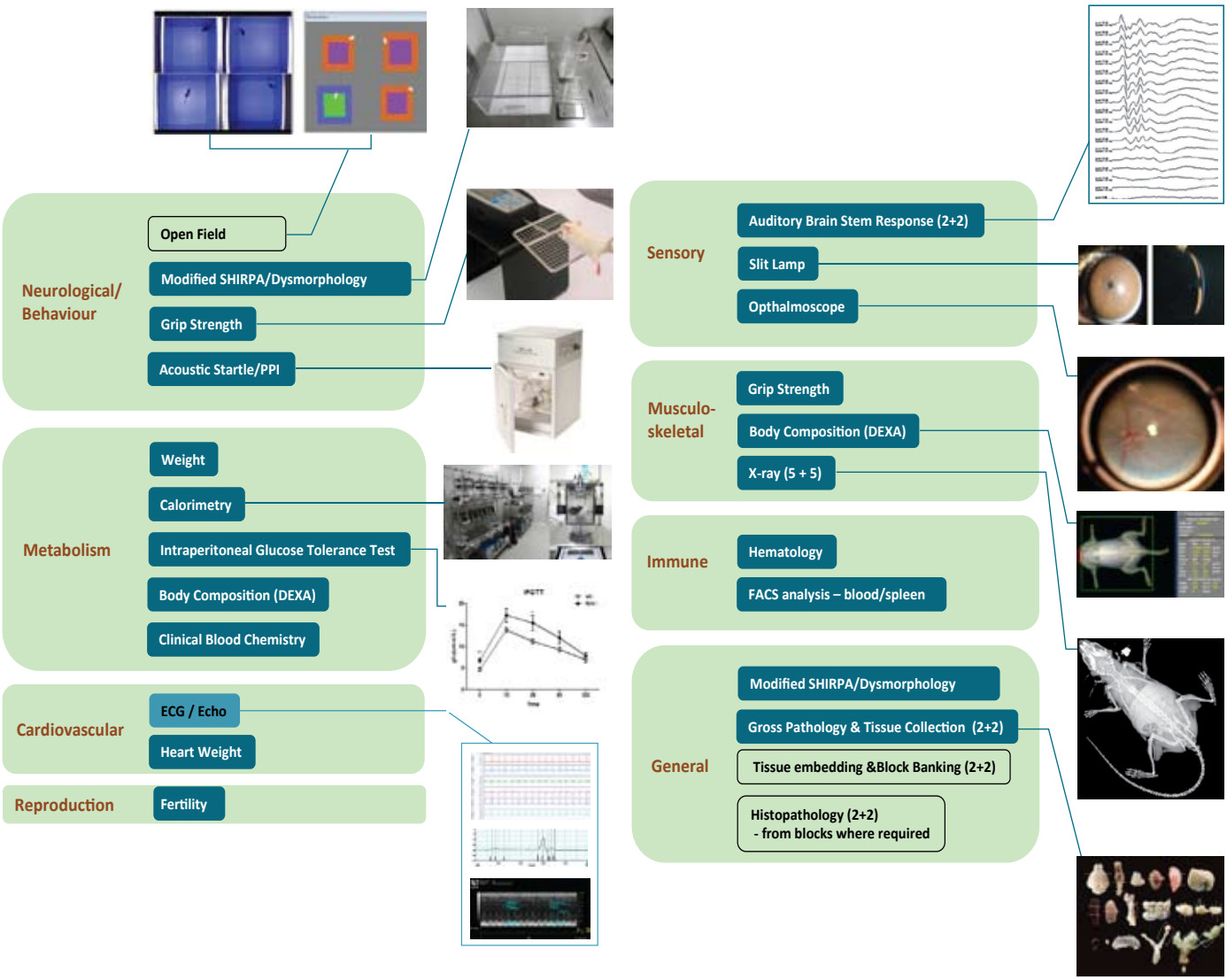
NRCMM is the only qualified research institute of IMPC in China.

This year we have imported more than 100 gene targeting ES cells for IMPC program from KOMP and Eucomm. After strict QC procedure, the QC passed clones were miroinjected. >80% GLT ratio was achieved for our chimera. Crossing with ACTB-Cre strain converted the tm1a hetrozygous mouse into tm1b convetional knockout mouse. Up to now, our IMPC phenotyping platform has completed baseline data of B6/N background line, whole set data of three well-established mutant lines and 15 mutant lines of IMPC project.

Our services include:

- Systemic basic screening of potential phenotypes of mutant mice, covering behavior, metabolism, cardiovascular, sensory, skeletal and immune system.
- Professional molecular and histological analysis of individual mouse/tissue, including QPCR, western blot, HE staining and immunohistochemistry.
- Secondary screen platform of behavior and metabolism under construction and coming soon.

Tel:86-025-58641511 Contact: Xin Qi Email: qixin@nicemice.cn



6.Supplying SPF Laboratory Mouse Strains

NRCMM holds hundreds of strains of inbred strains, mutant strains, disease models (including cardiovascular disease, adiposity, diabetes mellitus, immunodeficiency, alzheimer disease, tumor, etc.). We also provide many "tool mice" such as tissue-specific Cre transgenic mice and fluorescent protein report mice.

Throughout the year of 2013, MARC has accomplished an output of around 50,000 model mice including commercial purchases and exporting service. At the same time, we have also imported 72 new mice strains and gratefully received 35 strains from donations to add in our resource center.

Tel: 86-025-58641520 Contact: XinLiu Email: liuxin@nicemice.cn nicemice@163.com

Star mice

B6.Cg-Lep^{ob}/JNju

Type II Diabetic mouse model

NOD.Cg-Prkdc^{scid}B2m^{tmiUnc}/JNju

Combined immunodeficiency, B,T cell deficiency

BKS.Cg-Dock7^{m}+/+Lepr^{db}/JNju

Type II diabetic mice model

B6.129P2-Apoe^{tm1Unc}/JNju

Atherosclerosis

7.Breeding Services and Cryopreservation

Cryopreservation saves the expense and space associated with maintaining live breeding colonies, and provides a backup against the loss of the mouse colonies due to equipment failure, genetic contamination or diseases, or natural disasters such as earthquakes and fire.

In 2013, NRCMM has finished approximately 400 strains for cryopreservation. We can supply the cryopreservation services. We can also help customers to breed, house, genotype and maintain mouse colonies. Furthermore, we also provide custom breeding services to meet your specific requirements. Below are some routine services provided by our animal facilities: Ideal for strains whose males have short lifespan or breeding windows.

1) Rederivation to improve the health status of your mice.

2) Embryo or sperm cryopreservation and recovery to ensure the safety of your valuable research models.

3) Mice health test: A platform to test the health conditions of the animals systematically.

4) Speed Expansion Service: We can quickly produce hundreds of SPF, same-age mice. Requires only two males, 2-3 months.

5) Strain Rescue Service: Through IVF or ovary transfer, we can further check the state of the sperm. Our success rate is over 80%.

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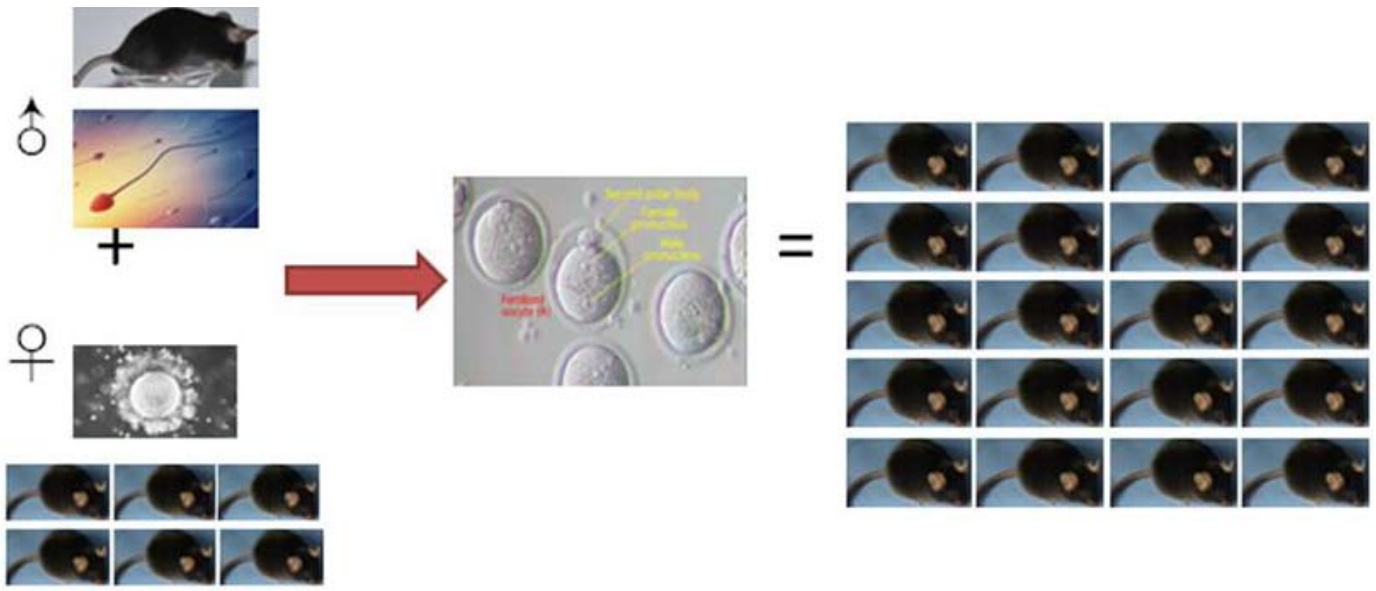
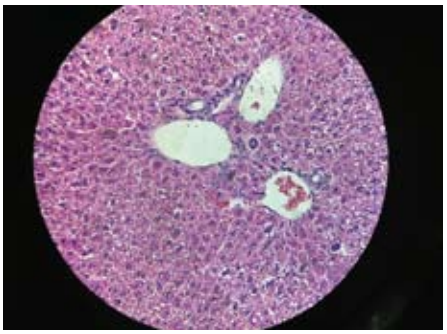
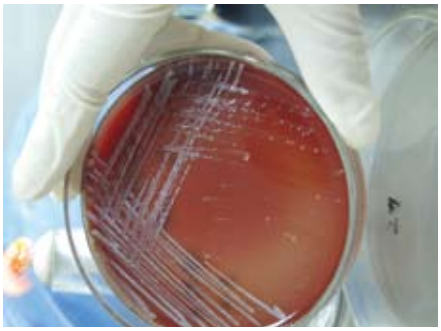


8.Laboratory Animal Pathogen Detection and Veterinary Services

The veterinaries ensure the MARC's animal welfare and health by supervising all animal health report review and approval program, by assisting in establishment and/or monitoring animal quarantine procedures, by taking charge of Health Monitoring Program which include disease detection and surveillance, prevention, diagnosis, treatment,and resolution. Veterinary services include serological, microbiological, parasitological testing services, as well as facility inspection program.

Tel: 86-025-58641506-801 Contact: Huixin Yang Email: yanghx@nicemice.cn


9.Agency Services

We have a high-quality service team, with expertise and technical capability, which has successfully imported mice more than 100 times from USA, Britain, Germany, France, Japan, Netherlands, Austria, Australia, Hongkong and other countries, at the same time, exported mice nearly 50 times to USA, Britain, South Korea, Japan, Sweden, Switzerland, Singapore, Hongkong and other countries and regions. We work as an import and export service agent of live mice approved by overseas and domestic research institutions and mouse resource center. Since 2010, we have progressively improved our exporting and importing services of nonliving materials, such as mice cells, embryos, sperm, etc. We are always committed to providing perfect and professional services to both domestic and foreign customers.

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10.Training Services

The institute will provide training services about molecular biology technology, generating transgenic/knockout mice, maintaining barrier system, breeding mice. The trainers will learn the principle of the experimental and the specific experimental techniques through observing and practicing the experiment.

The training programs

- BAC-retrieve-based gene knockout vector construction
- The knowledge of ES cell culture
- ES cell blastocyst injection
- Transgenic prokaryotic injection
- The knowledge of breeding, managing of SPF mice, gene identification
- Embryo cryopreservation, recovery and biological purification
- Environmental monitoring and microbiological testing of barrier system

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IACUC

Expert technological Consultation

Administrative Office

Jing Zhao Ph.D.
Chief of Animal Facility
IACUC Chairman

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RIKEN BRC/Nanjing University MARC 2nd International Short Summer Course of the Mouse Research.

RIKEN BioResource Center (BRC), Japan (Director: Dr. Yuichi Obata) and Model Animal Research Center (MARC), Nanjing University, China (Founder Director: Dr. Xiang Gao) co-organized a short educational program focusing on mouse genetics and related experimental technologies for young scientists. RIKEN BRC hosted the 1st International Short Summer Course of the Mouse from Aug 27 to Aug 29 in Tsukuba, Japan. This summer, Nanjing University MARC hosted the 2nd program from July 29 to July 31 in Nanjing, China.

Place: Model Animal Research Center, Nanjing University. **Periods:** From July 29 to 31, 2013. **Students:** 64 students from China, Korea, France, USA and Canada.

Speakers: Prof.David Wasserman from the USA(Vanderbilt University), Dr.Tom Weaver from the UK(MRC), Prof. Je Kyung Seong from Korea (Soul National University), 10 from China (Nanjing University MARC), and 7 from Japan (RIKEN BRC) including: Dr. Yuichi Obata (Director), Dr. Kuniya Abe, Dr. Atsushi Yoshiki, Dr. Atsuo Ogura, Dr. Shigeharu Wakana, Dr. Yoichi Gondo, and Dr. Hiroshi Masuya.

Themes of lectures:

With the following 4 themes, 21 lectures were held in total.

1) Genome manipulation and mouse models.

2) Mouse Resource and international consortium.

3) Disease Model and application I.

4) Disease Model and application II.

During sessions for open discussion when students were allowed to ask questions to the lectures, many of them actively joined in the discussion.

Three experimental courses on July 31.

1) SHIRPA Operator Training.

2) Microinjection.

3) Visiting the Phenotypic analysis room and echocardiography.

The 3rd International Short Summer Course was scheduled to take place from July 28 (Mon) to 30 (Wed), 2014 at RIKEN BRC in Tsukuba.

Dr. Gao, Founder Director of Nanjing University MARC and Dr. Obata, Director of RIKEN BRC awarding certificate to a participant.

2013 China Conference of Developmental Biology

To promote basic research on developmental biology in China, MARC and Chinese Society for Cell Biology (CSCB) organized the 2013 China Conference of Developmental Biology which was held in Nanjing from September 26th-29th. There are around 70 participants who are professors/PIs from Nanjing University , Tsinghua University, Tongji University, Zhejiang University, Suzhou(Soochow) University, Xiamen University, Beijing Normal University, Sichuan University, and Chinese Academy of Science, as well as the administrative staffs of National Natural Science Foundation of China.

At the opening ceremony, Dr. Xiang Gao, Chairman of the conference and founder director of MARC, extended a welcome and summary talk about the status of developmental research in China. Dr. Bin Zhou , professor and director of The Institute for Nutritional Sciences, Chinese Academy of Sciences(CAS), gave the first talk titled Coronary arteries bring blood flow to the heart muscle.

39 talks from domestic scientists, were divided into six session: Organogenesis, Stem Cells and Regenerative Medicine, Neuron development, Developing epigenetic regulation, Molecular Cell Biology, The signal-regulated during development. These sessions were chaired by Dr. Ying Jin(investigator at SIBS, CAS), Dr. Naihe Jing(investigator at SIBS, CAS), Dr.Steven Y. Cheng(Nanjing Medical University) Dr. Lingfei Luo(Southwest University), Dr. Anming Meng(Institute of Zoology, CAS). The speaker presented latest research progress and scientific ideas from their laboratories, which were followed by heated discussion between the speaker and the audience.

2013 Annual Conference of MARC

The 2013 MARC Annual Conference was held in Yangzhou Shita Hotel (Yangzhou, Jiangsu province) from October 26th to 28th. This meeting was organized by Drs. Ying Cao and Xingxu Huang's laboratories. More than 190 scientists and students from MARC attended the conference, which hold in the Hall of Mulanyuan in the hotel. Dr. Zhongzhou Yang, director of MARC and chairman of the conference, summarized the research and education advances of MARC in the past year at the opening ceremony. In the following sessions, PIs and student representatives presented latest research progresses and scientific ideas from their laboratories, followed by lively discussions between the speakers and the audience. Dr. Jing Zhao, director of NRCMM also presented the 2013 annual report of NRCMM. About 83 posters were presented by senior students to exhibit their research results. In the Teacher-Student Interaction session, interested issues and topics were discussed.

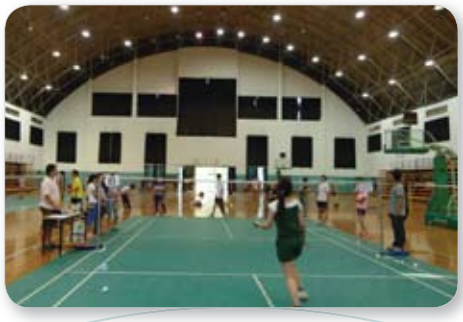
Guangsen Shi from Dr. Ying Xu's laboratory, was awarded the 2013 Student of MARC for his excellent research on circadian rhythm and health research. Bin Shen from Xingxu Huang's laboratory and Xueyan He from Geng Liu's laboratory were nominated.

Ten students received 2013 Outstanding Poster Prize.

2013 Summer School

Graduate students are always our treasure at MARC; therefore we treat the recruitment and education of students as a top priority. This year, we held the 4th Summer Camp from July 8–12. 38 excellent undergraduates were selected from a pool of 109 applicants from 40 universities nationwide. In order to increase the interaction between undergraduate students and our faculty members/graduate students, we have re-organized the programs. 9 faculty members gave lectures on the current progress in biomedical researches, ranging from circadian rhythms, cell migration to heart regeneration and neurodegeneration. At nights, special academic salons on three topics covering metabolism, neuron biology, and developmental biology were held. Moreover, 3 of our outstanding graduate students, Ning Shen, Zhaoyu Lin and Bing Shen, communicated with the Summer Camp students on their own research lives at MARC. The purpose of the Summer Camp is to train and attract students for future biomedical researches involving model animals both at MARC and at other institutes in China.

2013 Students Union


In MARC, annual badminton game and table tennis game is held in May and December, respectively. The champion for these two games is animal facility and Qingshun Zhao lab, respectively. The two games are so attractive that most of us are involved in them with full enthusiasm. In addition, we also have our own basketball and soccer team with weak activities. It is firmly believed that we would make better science research with a more healthy body.


In July, we held a 2013-2012 new to old communication meeting to welcome our new students. More importantly, we could help them answer some associated doubts and offer several ways to solve difficulties which they are facing while coming to a new environment.

On Oct. 27th, "MARC' Night" game party was held in the 2013 annual retreat. At that evening, students had fun with all PIs, and everyone enjoyed a wonderful time. The program "caiqiqiu" attracted much laughter and impressed us profoundly.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