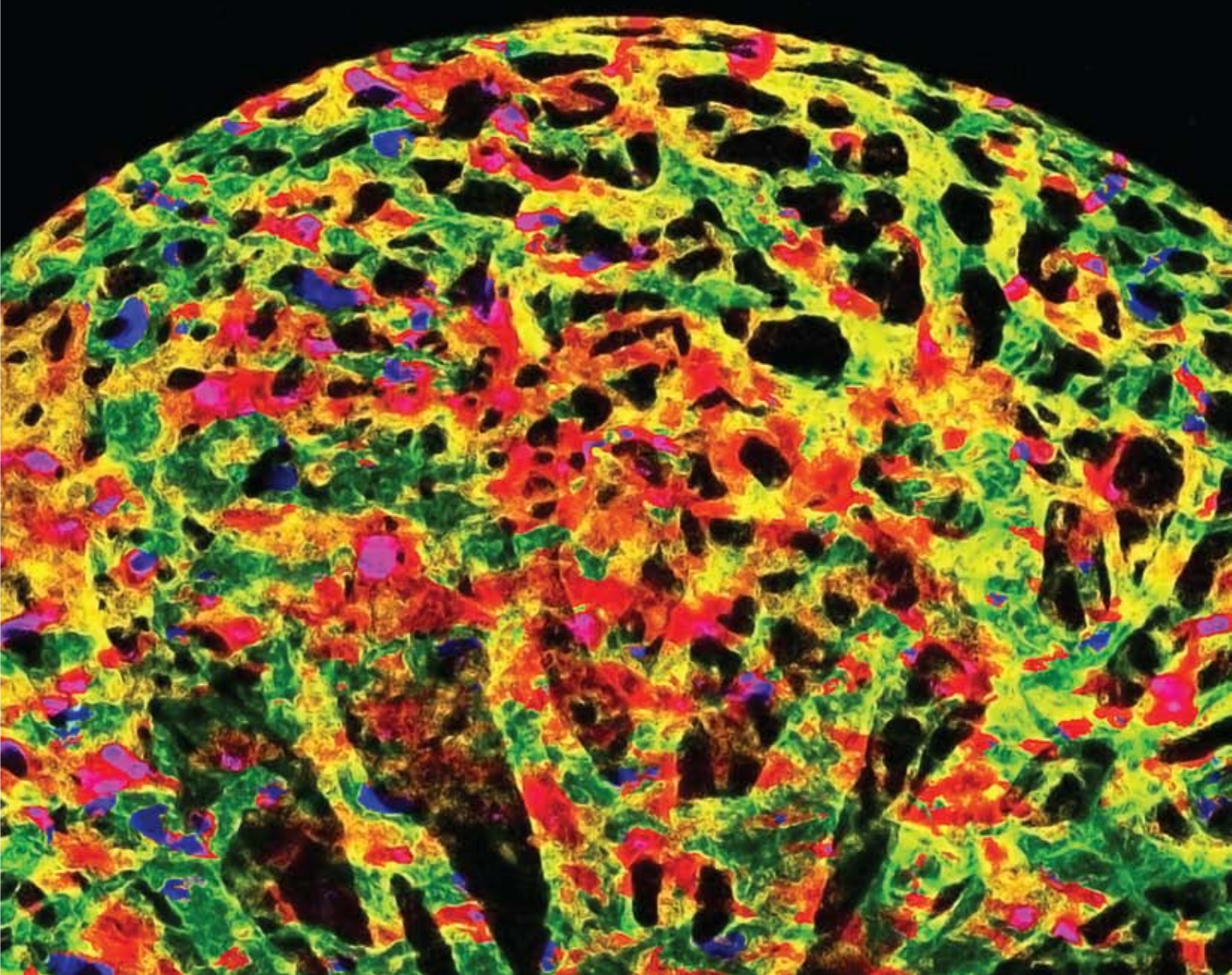
MODEL ANIMAL RESEARCH CENTER OF NANJING UNIVERSITY
MOE KEY LABORATORY OF MODEL ANIMAL FOR DISEASE STUDY
NANJING BIOMEDICAL RESEARCH INSTITUTE OF NANJING UNIVERSITY
NATIONAL RESOURCE CENTER FOR MUTANT MICE

ANNUAL REPORT

2014



Director's Words

As scientists, we are constantly pre-occupied with research and teaching obligations, such as writing grants and manuscripts, teaching classes, staying current with the latest publications, mentoring students on their research progress, and more. Often, we unconsciously ignore the beauty of our surroundings. One early morning as I walked across campus toward our research building, I suddenly realized that spring had arrived and the campus was beautiful! Blooming roses were colored in bright red; soft green grass carpeted the ground; pretty willow leaves were reflected in the lake; and verdant trees overlooked the new research building. Even the birds were singing in the woods. What a charming place!

In September, we moved into a brand new, 13 floor research building with an affiliated state-of-the-art mouse facility. It was only ten short years ago that the founder of MARC, Dr. Xiang Gao, worried about how he would recruit enough scientists to fill a smaller 2-floor research building. It was beyond his imagination to think that within a few short years, the 2-floor building would be filled with 22 research laboratories! The newly recruited principal investigators (PIs) had to share the limited bench space in the laboratories of the older PIs. Happily, there is now ample space for everyone to perform experiments on their own benches.

Science at MARC, like spring, has also been blooming. Intriguing discoveries with high impact in biomedical research have been made in the past year. Some of the paradigms are highlighted in this report. We are delighted that these achievements were published or accepted in many prestigious journals, including *Cell, Nature Methods, Nature Communications, Cell Reports, PLoS Genetics* and *Development.*

Recently, we have grouped the 22 laboratories into 4 research fields: 1.Developmental Biology; 2.Metabolic Homeostasis; 3.Cancer and Stem Cell Biology; and 4.Neurobiology. Our vision is to promote inter-laboratory collaborations in each of the 4 fields for better and stronger science, a strategy that was developed at the institutional brainstorm meeting this year. We look forward to in-depth collaborations among MARC groups in the coming year, which will lead to groundbreaking findings with translational applications. We are fully confident that MARC is poised to become an ideal place to bridge fundamental studies of gene function and regulatory mechanisms with clinical applications, leading to novel therapeutic approaches and improved patient care.

Zhongzhou Yang

Director

Cover photo by Qi XIAO

CONTENTS

Research Highlight in 2014

Group Xiang GAO

Stem Cell Factor as a Potential Anti-Obesity Agent

ARTICLE
Received 13 Nov 2013 | Accepted 3 Jun 2014 | Published 7 Jul 2014

The stem cell factor/Kit signalling pathway regulates mitochondrial function and energy expenditure

Huang Z, Ruan H-B, Xian L, Chen W, Jiang S, Song A, Wang Q, Shi P, Gu X, Gao X

nature COMMUNICATIONS

Background:

Obesity is a major risk factor for many diseases, including type 2 diabetes, cardiovascular disease, neurodegenerative disease, and some types of cancers, making it one of the leading preventable causes of death in the world. Obesity develops when people are in positive energy balance, i.e. calorie intake is greater than energy expenditure. Prevention and treatment of obesity are difficult, largely because changes in food intake and body weight are associated with compensatory changes in energy expenditure, which keep body weight at the original "set point". In other words, we need to increase (or at least maintain) energy expenditure, in combination with lifestyle interventions and medications that prevent energy over-intake to effectively treat obesity.

Significance:

The aim of this study was to identify novel hormones that regulate energy homeostasis. We found that levels of stem cell factor (SCF), a hormone critical for the growth and survival of multiple cell lineages, are elevated in conditions where energy expenditure is increased in a compensatory manner, such as feeding, cold exposure, and obesity, indicating that SCF is a potential regulator of weight control. We further demonstrated that SCF and its receptor Kit promote transcription of the Ppargc1a gene (encoding the PGC-1α protein) and mitochondrial biogenesis in skeletal muscle and brown adipose tissue (BAT). Genetic disruption or chemical inhibition of SCF/Kit signaling reduced PGC-1α expression, mitochondrial function, and energy expenditure, leading to obesity in mice. On the other hand, overexpressing SCF systemically or specifically in BAT increased thermogenesis and thus reduced weight gain. Based on these findings, we propose that SCF is a "satiety hormone" that acts peripherally to counteract obesity. With this knowledge, the SCF/Kit pathway can be considered as a potential therapeutic target for treating obesity and related metabolic diseases.

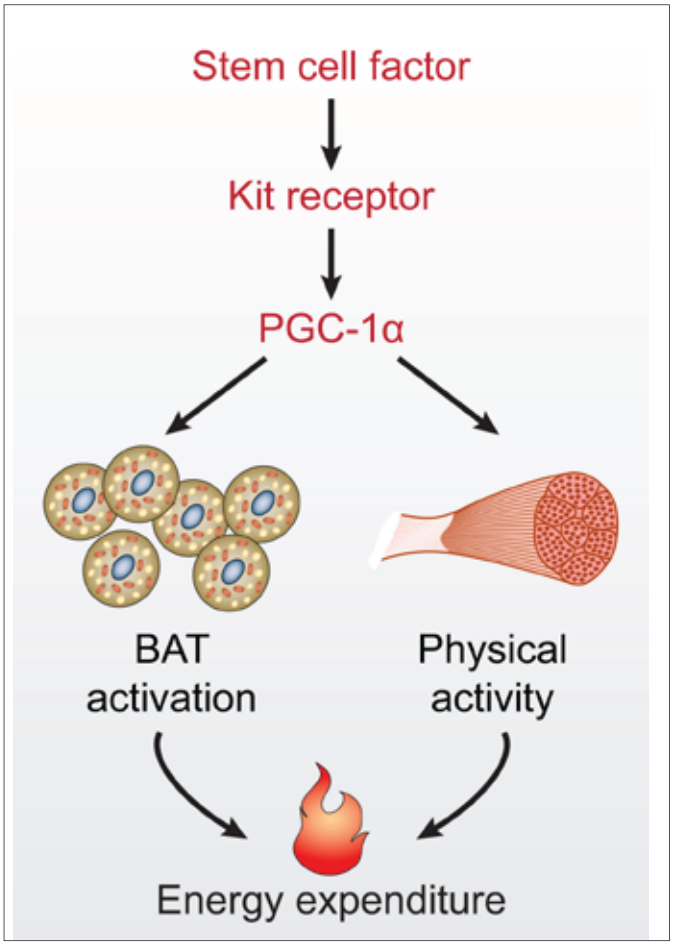


Figure. Regulation of energy expenditure by the SCF/Kit pathway.

Group Ying XU

PER1 Phosphorylation Specifies Feeding Rhythm in Mice

Zhiwei Liu, Moli Huang, Xi Wu, Guangsen Shi, Lijuan Xing, Zhen Dong, Zhipeng Qu, Jie Yan, Ling Yang, Satchidananda Panda, Ying Xu

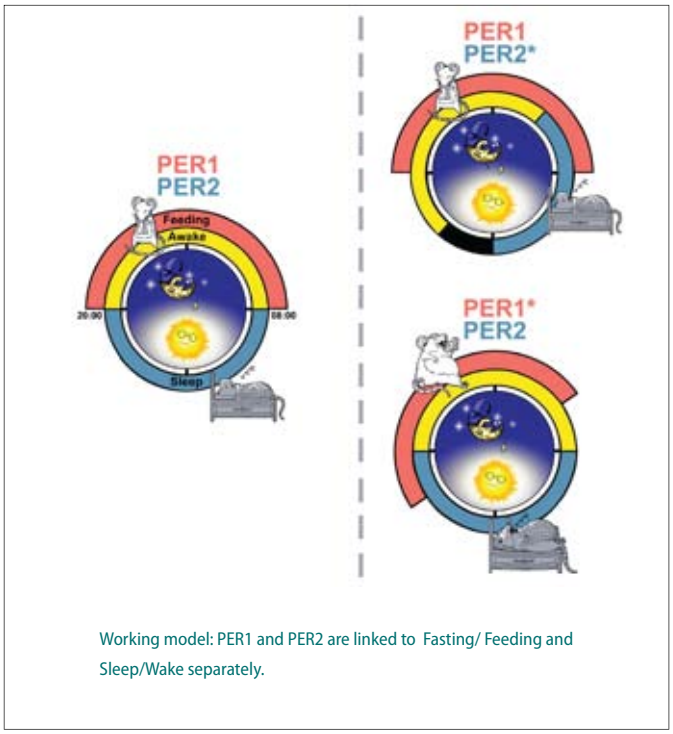
Cell Reports

Highlights

- S714G mutation in PER1 causes internal clock misalignment
- S714G mutation in PER1 is associated with feeding rhythms
- PER1S714G mice rapidly develop obesity
- PER1 is an important regulator of interactions between clock and energy metabolism

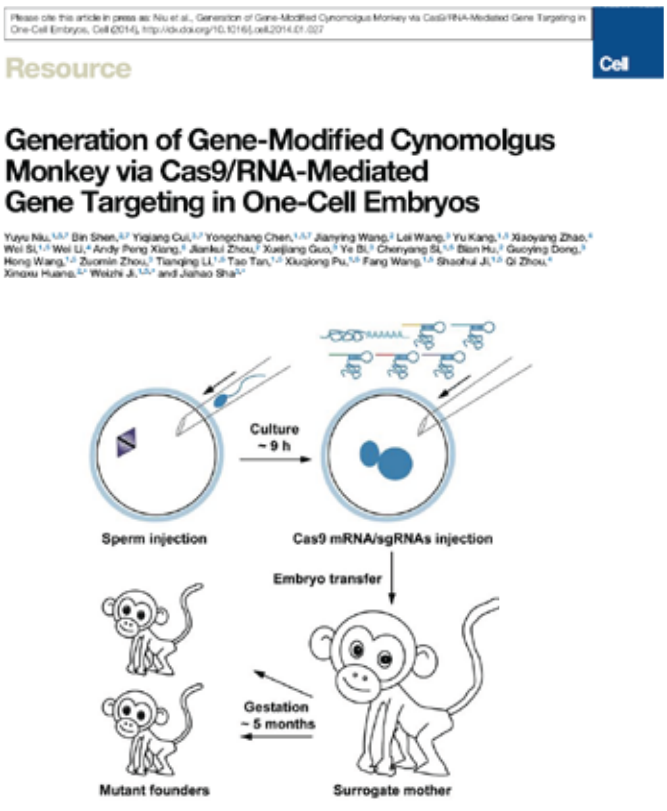
Summary

Organization of circadian behavior, physiology, and metabolism is important for human health. An S662G mutation in hPER2 has been linked to familial advanced sleep-phase syndrome (FASPS). Although the paralogous phosphorylation site S714 in PER1 is conserved in mice, its specific function in circadian organization remains unknown. Here, we find that the PER1S714G mutation accelerates the molecular feedback loop. Furthermore, hPER1S714G mice, but not hPER2S662G mice, exhibit peak time of food intake that is several hours before daily energy expenditure peaks. Both the advanced feeding behavior and the accelerated clock disrupt the phase of expression of several key metabolic regulators in the liver and adipose tissue. Consequently, hPER1S714G mice rapidly develop obesity on a high-fat diet. Our studies demonstrate that PER1 and PER2 are linked to different downstream pathways and that PER1 maintains coherence between the circadian clock and energy metabolism.



Working model: PER1 and PER2 are linked to Fasting/ Feeding and Sleep/Wake separately.

Group Xingxu Huang


Please cite this article in press as: Niu et al., Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos, Cell (2014), http://dx.doi.org/10.1016/j.cell.2014.01.027

Resource

Cell

Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos

Yuyu Niu,[1,6,7] Bin Shen,[2,7] Yiqiang Cui,[2,7] Yongchang Chen,[1,6,7] Jianying Wang,[3] Lei Wang,[3] Yu Kang,[1,6] Xiaoyang Zhao,[4] Wei Si,[1,6] Wei Li,[4] Andy Peng Xiang,[5] Jiankui Zhou,[4] Xuejiang Guo,[5] Ye Bi,[1] Chenyang Si,[1,6] Bian Hu,[2] Guoying Dong,[3] Hong Wang,[1,6] Zuomin Zhou,[1] Tianqing Li,[1,6] Tao Tan,[1,6] Xiuqiong Pu,[1,6] Fang Wang,[1,6] Shaohui Ji,[1,6] Qi Zhou,[4] Xingxu Huang,[2,*] Weizhi Ji,[1,6,*] and Jiahao Sha[2,*]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NATURE | NEWS

First monkeys with customized mutations born

Milestone for targeted gene-editing technology promises better models for human diseases.

Helen Shen

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

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First CRISPR-Tinkered Primates Born

Twin macaques are the first primates born whose genomes were edited using CRISPR technology.

By Kerry Grens | February 3, 2014

Researchers achieved precise gene modification in monkeys.

Student of the Year 2014

Zan Huang

Zan Huang was awarded the 2014 Student of MARC for his excellent research on the stem cell factor/Kit signalling pathway that regulates mitochondrial function and energy expenditure.

Zan Huang received his Bachelor's degree of Biological Science and Technology in 2007 from School of Life Sciences, Nanjing Agriculture University. He joined Dr. Xiang Gao's Lab at the year of 2007 to investigate the mechanism of energy homeostasis.

For the past few years, his study focused on the relationship between SCF/Kit signaling pathway and mitochondria functions. Using an unbiased genetic approach, he and his colleagues found expression of SCF and Kit in adipose tissues is responsive to food availability and environmental temperature, and is altered in obese mice and human patients. Mice carrying a loss-of-function mutation in Kit develop obesity as a result of decreased energy expenditure. Collectively, these data provide mechanistic insight into the regulation of mitochondrial function by SCF/Kit signaling and lay a foundation for exploring SCF/Kit signalling as a therapeutic target for metabolic diseases.

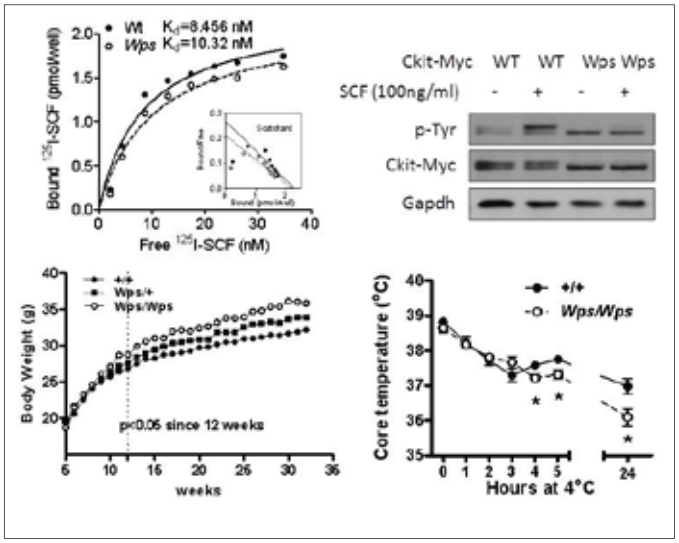


Fig1.Mice carrying a loss-of-function mutation in Kit develop obesity as a result of decreased energy expenditure.

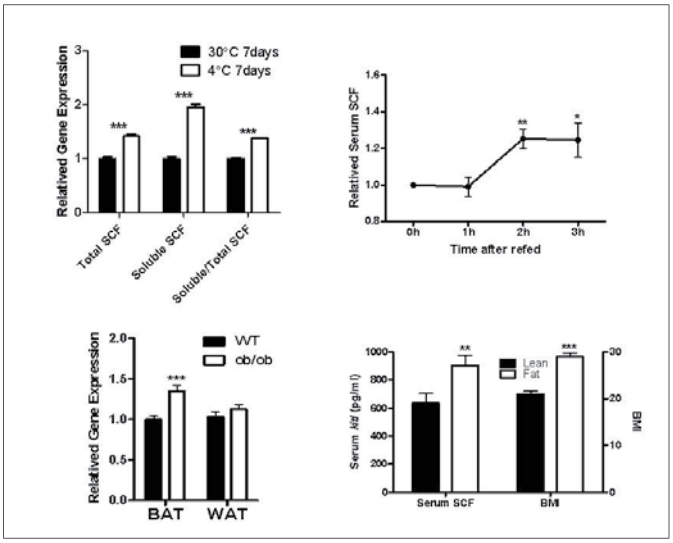
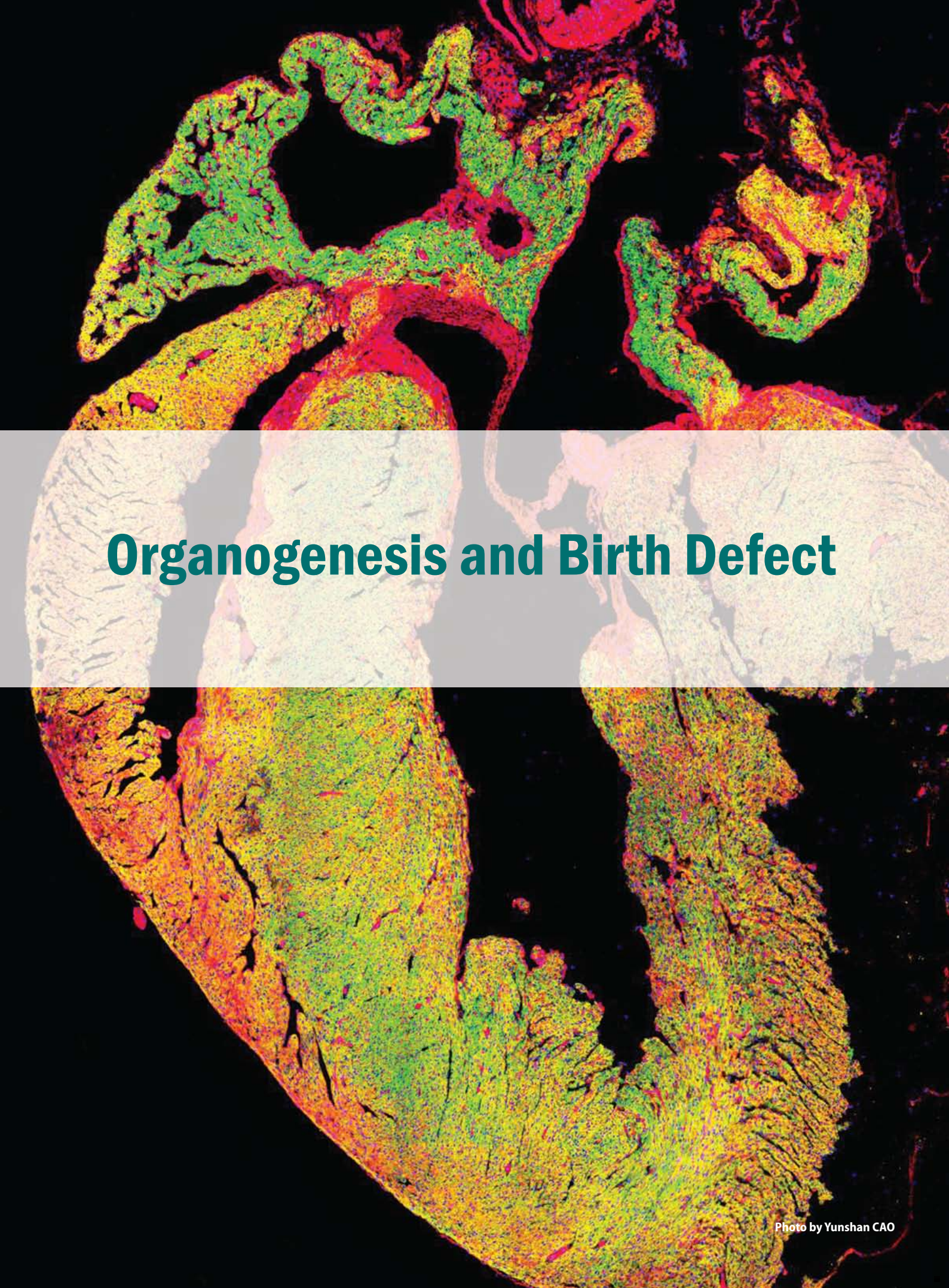


Fig2.The expression of SCF and Kit in adipose tissues is responsive to food availability and environmental temperature, and is altered in obese mice and human patients.

Selected publications

1. Zan Huang, Hai-Bin Ruan, Zengdi Zhang, Weiqian Chen, Zhaoyu Lin, Hu Zeng and Xiang Gao. Mutation in the first immunoglobulin-like domain of c-Kit leads to constitutive JAK2 activation and myeloproliferation in mice.
   The American Journal of Pathology, Vol. 184, No. 1, January 2014.
2. Zan Huang, Hai-Bin Ruan, Li Xian, Weiqian Chen, Shujun Jiang, Anying Song, Qinghua Wang, XingxingGu, Xiang Gao. Stem cell factor/Kit signaling pathway regulates mitochondrial function and energy expenditure.
   Nature Communications, 2014 Jul 7; 5:4282.



Organogenesis and Birth Defect

Photo by Yunshan CAO

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 Embrynoic Development

a) Kdm2a/b regulate canonical Wnt signaling via mediating the stability of nuclear eta-Catenin

In this year, one major thing is that we have continued our experiments on the role of the protein lysine demethylaes Jhdm1a/b (Kdm2a/b) in the regulation of Wnt/eta-catenin signaling. With these results, we have proposed a model for the regulation of -catenin. Without Wnt activation, eta-Catenin is phosphorylated and targeted for proteasomal degradation in cytoplasm. Consequently, Wnt target genes are not transcribed (Figure 1, left panel). Otherwise, Wnt activation leads to stabilization and translocation of -Catenin into nucleus, where it is methylated at the lysine residues within the fourth and fifth armadillo repeats by unknown factor(s). eta-Catenin forms a complex with Tcf/Lef transcription factors to activate transcription (Figure 1, middle panel). To limit accumulation of eta-Catenin in nucleus and hence target gene activation or to remove the protein at the end of signaling, Jhdm1a/b competes with Tcf/Lef for -Catenin binding and removes the methyl marks from -Catenin, which is subsequently degraded via ubiquitylation. As a result, Wnt target gene transcription is attenuated or turned off (Figure 1, right panel).

Since the Wnt/beta-catenin signaling is an important pathway in regulating dorso-ventral axis patterning, we therefore have tested the effect of jhdm1a/b on the secondary axis formation induced by ectopic expression of beta-catenin. In Xenopus embryos, ventral injection of beta-catenin caused secondary axis formation (Figure 2B). When either Jhdm1a (Figure 2C) or Jhmd1b (Figure 2D) mRNA was coinjected, the secondary axis was not formed anymore (Figure 2C and D). Therefore, Jhdm1a/b regulates body axis formation via regulating the activity of beta-catenin. These results have been submitted to Developmental Cell and the manuscript is now under revision.

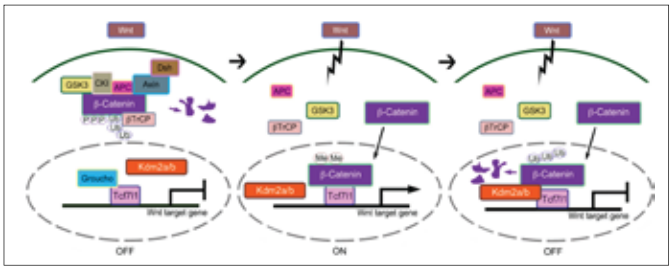

Figure 1. A proposed model for the Jhdm1a/b regulated stability of nuclear -Catenin

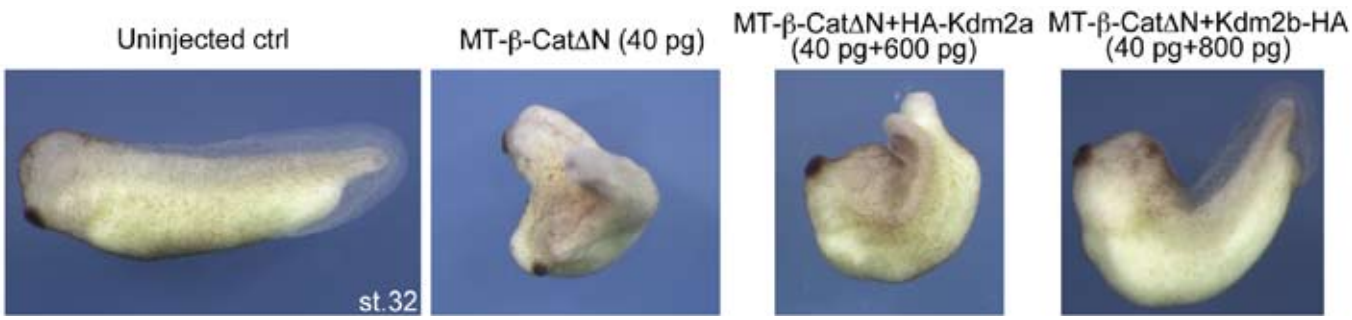

Figure 2. Jhdm1a/b (Kdm2a/b) inhibits beta-catenin induced secondary axis formation during Xenopus embryogenesis.

b) Investigation of Klf family factors during Xenopus germ layer formation and body axis patterning

Klf family proteins play crucial roles in the regulation of cell proliferation, apoptosis, differentiation, and are correlated to the development of many diseases. There are 17 members of Klf family in mammals, Klf1-klf17. Their roles in embryonic development are largely unknown. Previously our lab investigated the function and mechanism of the puripotency factor Klf4 during Xenopus germ layer and body axis formation, we further analyzed the expression and functions of other members of Klf members, Klf2, Klf5, Klf6, Klf7, Klf8, Klf11, Klf15 and Klf17, in Xenopus embryonic development. Using morpholino oligo knockdown, we found that inhibition of each of these Klf factors led to different developmental defects. Gene expression analyses demonstrated that these factors generated different effects on germ layer formation (Figure 3). More experiments have demonstrated that Klf factors regulate the transcription of genes that control germ layer induction and body axis patterning, including dkk1, cer1, chrd, gsc, wnt8, nodal5. These data imply that Klf factors are required for the fine-tuning of key genes during early embryogenesis, so as to ensure that correct development of early embryos. The data are submitted to Developmental Biology.

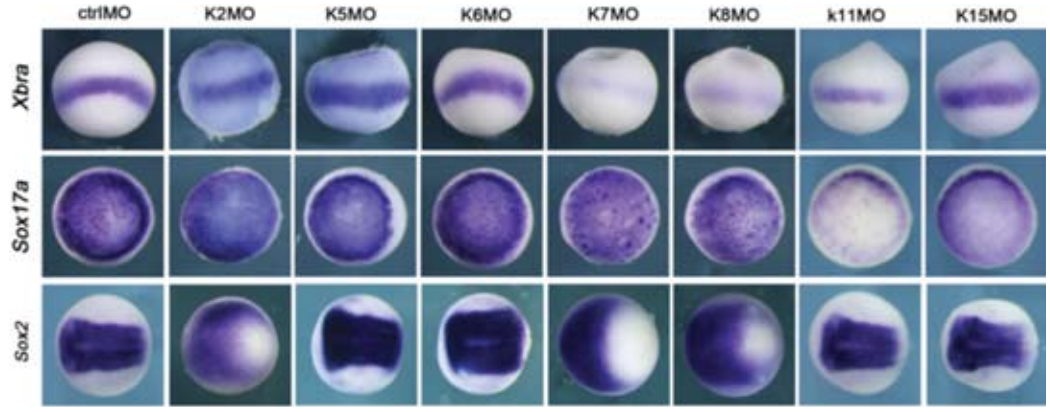

Figure 3. Knockdown of Klf2 (K2MO), Klf5 (K5MO), Klf6 (K6MO), Klf7 (K7MO), Klf8 (K8MO), Klf11 (K1MO) and Klf15 (K15MO) leads to malformation of the three germ layers, as indicated by the expression of Xbra (mesoderm), Sox17a (endoderm) and Sox2 (neuroectoderm).

Selected publications (*Correspondence author)

Yan Gao, Qing Cao, Lei Lu, Xuena Zhang, Xiaohua Dong, Wenshuang Jia, Zan Zhang, Ying Cao*. 2014. The Kruppel-like factor (Klf) family is involved in Xenopus germ layer formation and body axis patterning. Dev. Biol. (Under review).

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 -catenin. Dev. Cell (Under revision).

Cao Y*. (2013). Regulation of germ layer formation by pluripotency factors during embryogenesis (Review). Cell Biosci. 3(1):15.

Cao Q, Zhang X, Lu L, Yang L, Gao J, Gao Y, Ma H, Cao Y*. (2012) Klf4 is required for germ-layer differentiation and body axis patterning during Xenopus embryogenesis. Development 139:3950-3961.

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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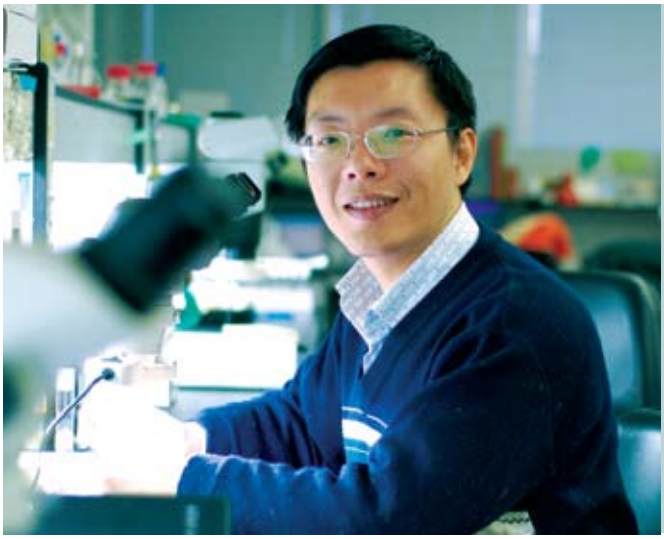

Jiong Chen Ph.D.

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 Principal 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 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 4 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 (Project 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 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.Generation of distinct cell polarities during collective migration of border cells.

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

5.Establish various mouse models with AIP1 conditional knockout.

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

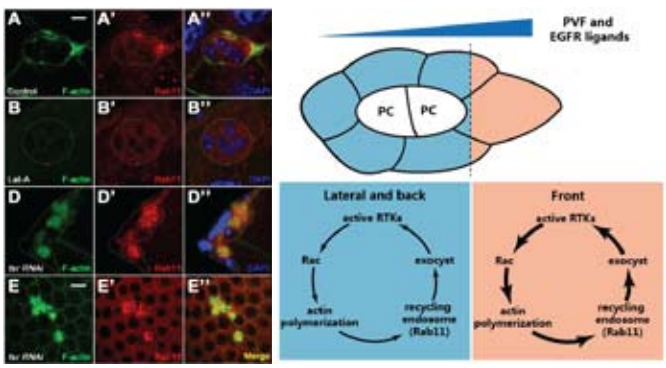

Figure 1. A model showing a positive-feedback loop promoting guided collective migration of border cells. (Taken from Wan et al, 2013)

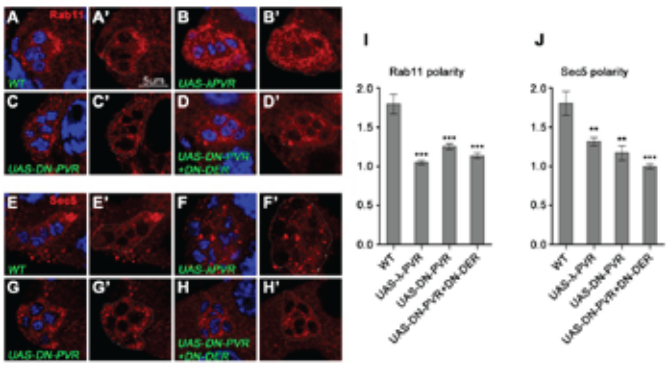

Figure 2. The guidance receptors PVR and EGFR mediate front-back asymmetry of Rab 11 (recycling endosome) and Sec 5 (exocyst) within a border cell cluster. (Taken from Wan et al, 2013)

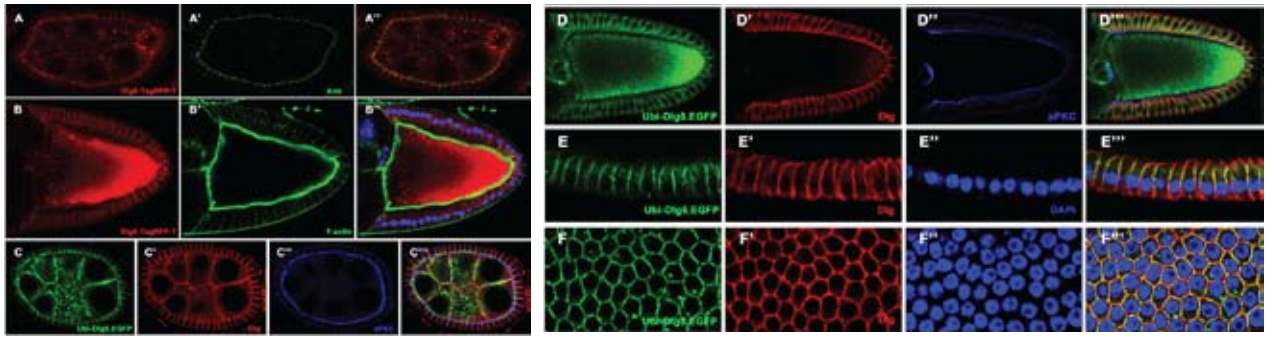

Figure 3. Dlg5 is involved in the formation and maintenance of apical polarity of the follicle epithelium in Drosophila ovary

Selected Publications (*corresponding author)

1. Yuan, B., Wan, P., Chu, D., Nie, J., Cao, Y., Luo, W., Lu, S., Chen, J.*, Yang, Z*. A Cardiomyocyte-Specific Wdr1 Knockout Demonstrates Essential Functional Roles for Actin Disassembly during Myocardial Growth and Maintenance in Mice. American Journal of Pathology (2014)
2. Luo, J., Zuo, J., Wu, J., Wan, P., Kang, D., Xiang C., Zhu H., Chen J.* in vivo RNAi screen identifies candidate signaling genes required for collective cell migration in Drosophila ovary. Science China Life Sciences (2014 accepted)
3. Wan, P., Wang, D., Luo, J., Chu, D., Wang, H., Zhang, L., and Chen, J.* Guidance receptor promotes asymmetric distribution of Exocyst and recycling endosome during collective cell migration Development (2013).
4. Zeng, L., Wan, Y., Li, D., Wu, J., Shao, M., Chen, J., Hui, L., Ji, H., and Zhu, X*. The m-subunit of murine translation initiation factor eIF3 maintains the integrity of the eIF3 complex and is required for embryonic development, homeostasis, and organ size control Journal of Biological Chemistry (2013)
5. Chu, D., Pan, H., Wan, P., Wu, J., Luo, J., Zhu, H. and Chen, J.* AIP1 Acts with Cofilin to Control Actin Dynamics during Epithelial Morphogenesis Development 139:3561-3571. (2012)
6. Zhang, Lijun, Luo, J., Wan, P., Wu, J., Laski, F., Chen, J*. Regulation of cofilin phosphorylation and asymmetry in collective cell migration during morphogenesis Development 138:455-464. (2011)
7. Dan Wang, Zhang, L., Zhao, G., Heino, H., Chen, J.* and Zhang, Y.* Drosophila twinfilin is required for cell migration and synaptic endocytosis Journal of Cell Sciences (2010)
8. Chen, Jiong, Call, G., Undergraduate Research Consortium in Functional Genomics (URCFC), and Banerjee, U. Discovery-based science education: functional genomic dissection in Drosophila by undergraduates. PLoS Biology. 3(2): e59 (2005).
9. 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).

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Wang Dou
Wu Jing
Wu Mengqi
Xu Zehao

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Chu Dandan (Ph.D)
Wan Ping (Ph.D)
Zuo Juntao (MS)

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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Understanding and manipulating the transcriptional and epigenetic regulation of germ cell development

The germ cell ensures the perpetuating and diversifying the genetic and epigenetic information across the generations. The germ cell development is accompanied by several fundamental biological processes such as reprogramming and lineage commitment which are regulated by transcriptional network and epigenetic modifications. After profiling the global transcriptome and epigenome, one of the next frontiers in biology is to site-specifically define and manipulate the transcriptional and epigenetic regulation of different biological processes. To this end, we have been developing genetic and epigenetic manipulation approaches, and applying such approaches combined with genome-wide analysis to understand the site-specific transcriptional and epigenetic regulation of germ cell development, including the elucidation and manipulation of the epigenetic and transcriptional network for reprogramming and lineage commitment.

1.Establishing an efficient programmable nuclease system

The strategies for precise genome modifications were limited in certain organism using ES cell-mediated gene targeting by homologous recombination, which hampers their use for research and application. Recently, the engineered nucleases such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) have provided a much simpler and economic way for targeted modification. These engineered nucleases generate DNA double strand break (DSB) at the targeted genome locus. These nuclease-induced DSBs can be repaired by two different ways including nonhomologous end-joining (NHEJ) and homology-directed repair (HDR). Small deletion or insertion (indels) could be generated at the cleavage sites through error-prone NHEJ repair. Such indels may cause frame shift mutation in the coding region to disrupt the target gene, while HDR-mediated modification is a precise editing in the target site including site-specific mutation, replacement or insertion (Figure 1).

Now, we focus most of our efforts on Cas9. We have successfully developed our own Cas9 system, and improved our system by minimizing the off-target mutagenesis through dual Cas9 nickase/dual sgRNAs strategy (Figure 2). Applying our system, we were the first to accomplish single and multiple gene knockout, conditional knockout and knock-in in mouse and rat (Shen et al. Cell Research 2013, 23(5): 720-723; Ma et al. Cell Research 2014, 24(1): 122-125.). We were also the first to accomplish genetic modification in monkey (Niu et al. Cell 2014, 156 (4): 836-843.) (Figure 3).

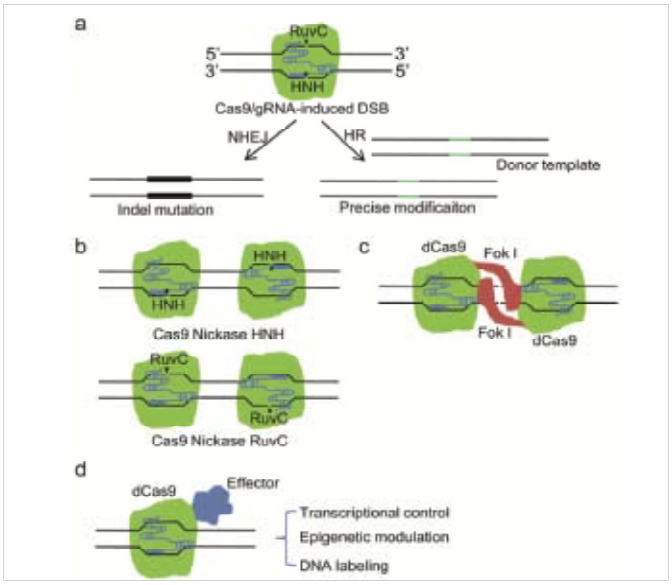

Figure 1 CRISPR/Cas9-mediated genome editing

a. Cas9/gRNA induced double strand breaks (DSBs) can be repaired by either non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways. The Cas9 contains RuvC and HNH nuclease domains, each of which is responsible for one strand DNA cleavage. b. Paired nickases were used to improve the specificity in the genome editing. Cas9 nickase (HNH) cleaves only the strand DNA (complementary strands of the target DNA) recognized by the gRNA. Cas9 nickase (RuvC) cleaves the strand DNA (non-complementary strands of the target DNA) not interacting with the gRNA. c. dCas9 ('dead' Cas9, both HNH and RuvC nuclease domain are inactivated by mutation) is fused with Fok I nuclease to improve the specificity of genome editing. d. dCas9 fused with an effector domain, such as DNA methylases, demethylases, histone acetylases, deaceylases, and kinases to modify the specific chromatin modification for desired effects.

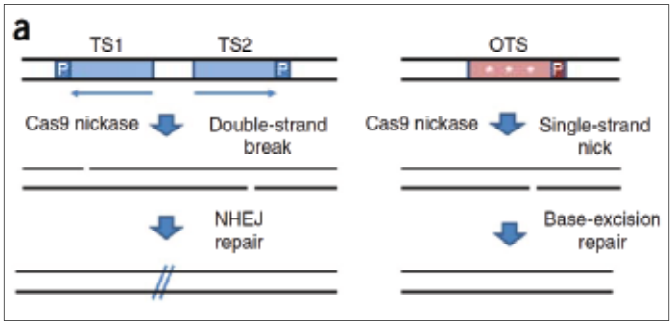

Figure 2 Strategy of Cas9 nickase/Dual sgRNAs

Strategy for introducing a double-strand break with Cas9 nickase and paired sgRNAs to adjacent target sites (TS1 and TS2) on opposite DNA strands. Single-strand nicks on opposing strands create a double-strand break and result in mutation of the target locus by NHEJ. Nicks generated at off-target sites (OTS) for each sgRNA will be corrected by the base-excision repair pathway. P, PAM site.

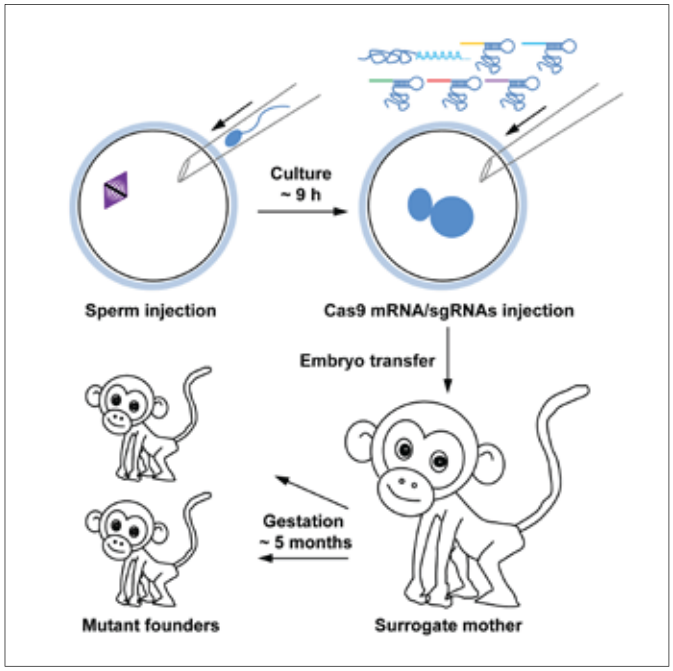

Figure 3 CRISPR/Cas9-mediated simultaneous targeting of multiple genes in monkeys

2. Defining the precise DNA methylome of gametes and early embryos

In addition to genetic targeting, we are also interested in site-specific epigenetic engineering. We started our epigenetic targeting effort by defining the single site 5-methylcytosines (5mCs) over the whole genome. In mammals, paternal 5-methylcytosines (5mCs) have been proposed to be actively converted to oxidized bases. These paternal oxidized bases and maternal 5mCs are believed to be passively diluted by cell divisions. By generating single-base resolution, allele-specific DNA methylomes from mouse gametes, early embryos and primordial germ cell (PGC), as well as single-base resolution maps of oxidized cytosine bases for early embryos, we demonstrated the existence of 5hmC and 5fC in both maternal and paternal genomes and find that 5mC or its oxidized derivatives, at the majority of demethylated CpGs, are converted to unmodified cytosines independent of passive dilution from gametes to 4-cell embryos. The paternal methylome and at least a significant proportion of maternal methylome go through active de-methylation during embryonic development (Wang et al. Cell 2014, 157(4): 979-991.) (Figure 4).

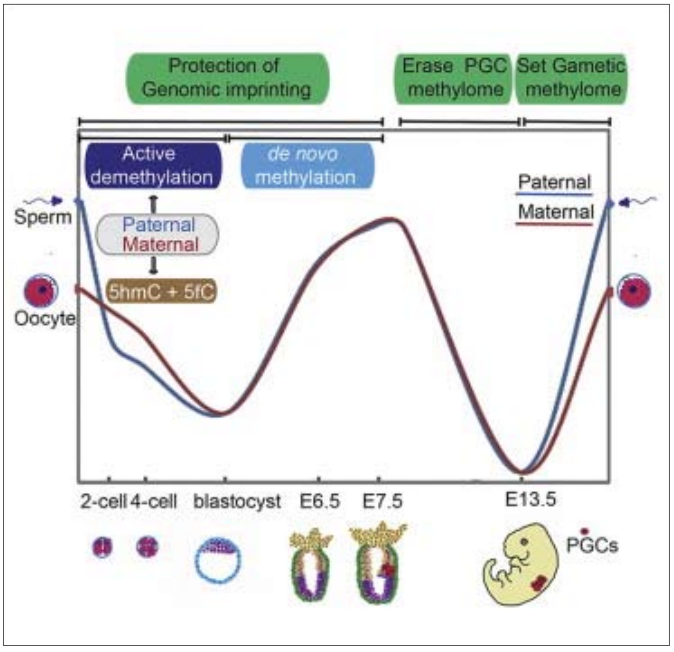

Figure 4 Programming and inheritance of parental DNA methylomes in mammals

Selected Publications (#co-first author; *co-corresponding author)

1.Han J#, Zhang J#, Chen L, Shen B, Zhou J, Hu B, Du Y, Tate PH, Huang X*, Zhang W*. Efficient in vivo deletion of a large imprinted lncRNA by CRISPR/Cas9. RNA Biology 2014, 11(7): 829-835.

2.Ma Y, Ma J, Zhang X, Chen W, Yu L, Lu Y, Bai L, Shen B, Huang X*, Zhang L*. Generation of eGFP and Cre knockin rats by CRISPR/Cas9. FEBS Journal 2014, 281(17): 3779-3790.

3.Xie S, Shen B, Zhang C, Huang X*, Zhang Y*. sgRNAcas9: a software package for designing CRISPR sgRNA and evaluating potential off-target cleavage sites. PLoS One 2014, 9(6): e100448.

4.Wang L#, Zhang J#, Duan J#, Gao X#, Zhu W, Song C, Yang L, Zhang J, Li G, Ci W, Li W, Zhou Q, Tang F, He C, Huang X*, Liu J*. Programming and inheritance of parental DNA methylome in mammal. Cell 2014, 157(4): 979-991.

5.Zhou J#, Wang J#, Shen B, Chen L, Yang J, Zhang W, Tian X*, Huang X*. Dual sgRNAs facilitate CRISPR/Cas9 mediated mouse genome targeting. FEBS Journal 2014, 281(7):1717-1725.

6.Ma Y, Shen B, Zhang X, Lu Y, Chen W, Ma J, Huang X*, Zhang L*. Heritable multiplex genetic engineering in rats using CRISPR/Cas9. PLoS One 2014, 9(3): e89413.

7.Shen B#, Zhang J#, Zhang W#, Zhou J, Wang J, Chen L, Wang L, Hogkins A, Iyer V, Huang X*, Skarnes W*. Efficient genome modification in mice by CRISPR/Cas9 nickase without off-target effects. Nature Methods 2014, 11(4): 399-402.

8.Niu Y#, Shen B#, Cui Y#, Chen Y#, Wang J, Wang L, Kang Y, Zhao X, Si W, Li W, Xiang AP, Zhou J, Guo X, Si C, Hu B, Dong G, Wang H, Zhou Z, Li T, Tan T, Pu X, Wang F, Ji S, Zhou Q, Huang X*, Ji W*, Sha J*. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell 2014, 156(4): 836-843.

9.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 2014, 24(1): 122-125.

10.Zhou J#, Shen B#, Zhang W#, Wang J, Yang J, Chen L, Zhang N, Zhu K, Xu J, Hu B, Leng Q*, Huang X*. One-step generation of different immunodeficient mice with multiple gene modifications by CRISPR/Cas9 mediated genome engineering. International Journal of Biochemistry & Cell Biology 2014, 46: 49-55.

11.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. Journal of Hepatology 2014, 60(2): 306-312.

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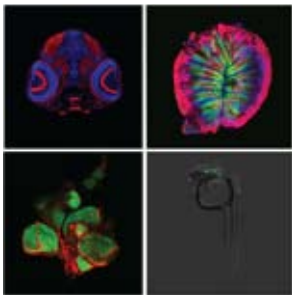
Vertebrate Organogenesis and regeneration



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

1)REVEAL THE DYNAMIC CHANGE AND ROLE OF EPIGENETIC REGULATION IN HEART DEVELOPMENT AND 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.

2)IDENTIFICATION OF NOVEL REGULATORS OF ORGANOGENESIS.

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.

Selected Publications

1. Lou, X., Deshwar, A. R., Crump, J. G. and Scott, I. C. (2011). Smarcd3b and Gata5 promote a cardiac progenitor fate in the zebrafish embryo. Development 138, 3113-23.
2. Takeuchi, J. K.*, Lou, X.*, Alexander, J. M., Sugizaki, H., Delgado-Olguin, P., Holloway, A. K., Mori, A. D., Wylie, J. N., Munson, C., Zhu, Y. et al. (2011). Chromatin remodelling complex dosage modulates transcription factor function in heart development. Nat Commun 2, 187. (* Co-first author)

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	Lingling Zhang	Qianyue Chen

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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Cardiovascular Development

The cardiovascular system is the first to develop and to function in mammals, and its development involves cell fate specification, cell proliferation and differentiation, and migration. We are interested in the developmental processes of the cardiovascular system and the underlying regulatory mechanisms. A variety of mouse models are uterlized to address these questions.

Regulation of the second heart field development

Lineage tracing and retrospective clonal analysis have identified two populations of cardiac progenitors during early mouse heart development. These two pools of cardiac progenitors are localized in the first heart field (FHF or primary heart field) and the second heart field. While the FHF contributes mainly to the left ventricle, the SHF develops into the right ventricle, inflow tract and outflow tract (OFT).

Starting at embryonic day 8.5 (E8.5), the migration of SHF progenitors from the pharyngeal mesoderm (PM) and splanchnic mesoderm (SM) into the linear heart tube is essential for heart development in mice. Genetic studies in mice have revealed that disruption of SHF formation and migration severely impairs heart development. For instance, deletion of Isl1, Tbx5, Mef2c and Nkx2.5 affects SHF development, resulting in developmental heart defects, with a single ventricle (the left ventricle) and an absence of SHF derivatives, the right ventricle and the OFT being observed.

SHF progenitors exhibit continued proliferation and a delay in differentiation. Fgf10, the first molecular marker of the murine SHF, and Fgf8 are the two important regulators that promote SHF proliferation. Canonical Wnt/β-catenin signaling also drives SHF progenitor cell proliferation. Bone morphogenetic protein (BMP) signaling is required to induce SHF formation and to subsequently inhibit cardiac cell proliferation.

PTEN-Akt signaling regulates stem cell/progenitor homeostasis. In several stem cell/progenitor systems, such as hematopoietic stem cells, intestinal stems and neural progenitor cells, deletion of Pten causes greatly increased cell proliferation through Akt activation.

To determine whether PTEN-Akt signaling is involved in SHF regulation, we deleted Pten in cardiac progenitors. We found that enhanced Akt signaling promoted SHF progenitor cell proliferation through the coordination of BMP signaling and beta-catenin activity (Fig. 1).

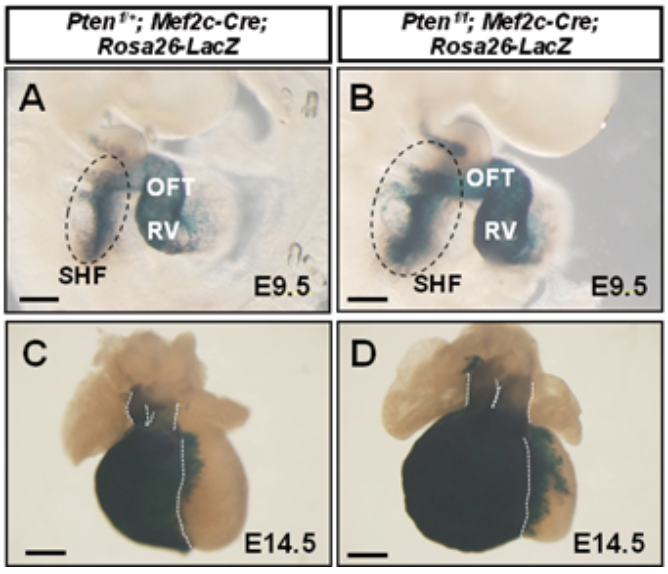

Fig. 1. Deletion of ***Pten*** in the SHF progenitors results in enlarged SHF and right ventricle.

G-quadruplexes (G4) structure in regulating heart development

G-quadruplexes (G4) are non-canonical four-stranded helical arrangements of guanine (G)-rich DNA or RNA sequences. RNA G-quadruplexes are suggested to play important roles pre-mRNA processing, RNA turnover and translation. However, the function of mRNA G-quadruplexes and their regulatory mechanisms in embryonic development remain elusive. RHAU (DHX36, G4R1) is the only known RNA helicase to resolve the G-quadruplex of 5′-UTR of the telomerase mRNA. Here, we specifically inactivated Rhau in cardiac mesoderm and progenitors, which revealed abnormal heart development leading to embryonic lethality. Gene expression profiling identified Nkx2-5, one of the earliest markers of cardiac mesoderm as a target regulated by RHAU. RHAU binds to both the 5′- and 3′-UTRs of Nkx2-5 mRNA and modulates the stability and translation of Nkx2-5 mRNA. The 5′-UTR of Nkx2-5 mRNA contains G-quadruplex that requires RHAU for effective protein translation, whilst the 3′-UTR of Nkx2-5 mRNA possesses an AU-rich element (ARE) that facilitates RHAU-mediated mRNA decay. Thus, we have uncovered the novel regulatory mechanisms of Nkx2-5 post-transcription during heart development. This is the first time to demonstrate the function of 5′-UTR G-quadruplex mediated-protein translation in organogenesis (Fig. 2).

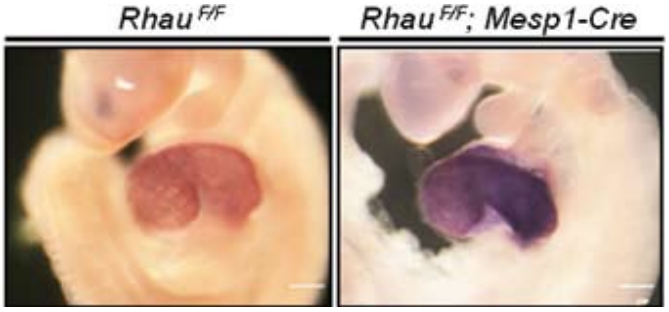

Fig.2. Deletion of Rhau in the cardiac mesoderm enhances the mRNA levels of ***Nkx2.5***.

Selected Publications

1. Wen Luo, Xia Zhao, Hengwei Jin, Lichan Tao, Jingai Zhu, Huijuan Wang, Brian A. Hemmings, and Zhongzhou Yang. (2014) Akt1 signaling coordinates BMP signaling and beta-catenin activity to regulate second heart field progenitor development. Development (Accepted)
2. Xia Zhao, Shuangshuang Lu, Junwei Nie, Xiaoshan Hu, Wen Luo, Xiangqi Wu, Hailang Liu, Qiuting Feng, Zai Chang, Yaoqiu Liu, Yunshan Cao, Haixiang Sun, Xinli Li, Yali Hu, Zhongzhou Yang. (2014) Phosphoinositide-Dependent Kinase 1 and mTORC2 Synergistically Maintain Postnatal Heart Growth and Heart Function in Mice. Mol. Cell. Biol. 34 (11):1966-75. (Spotlight article/cover)
3. Baiyin Yuan, Ping Wan, Dandan Chu, Junwei Nie,Yunshan Cao, Wen Luo, Shuangshuang Lu, Jiong Chen* and Zhongzhou Yang*. (2014) A Cardiomyocyte-Specific Wdr1 Knockout Demonstrates Essential Functional Roles for Actin Disassembly during Myocardial Growth and Maintenance in Mice. Am J Pathol. 184 (7):1967-80 (*Co-corresponding author)
4. Pei Wang, Beibei Mao, Wen Luo, Bin Wei, Wenjian Jiang, Dong Liu, Lei Song, Guangju Ji, Zhongzhou Yang,* Yong-Qiang Lai,* Zengqiang Yuan*. (2014) The alteration of Hippo/YAP signaling in the development of hypertrophic cardiomyopathy. Basic Res Cardiol.109 (5):435 (*Co-corresponding author).
5. Yijun Gao, Wenjing Zhang, Xiangkun Han, Fuming Li, Xujun Wang, Rui Wang, Zhaoyuan Fang, Xinyuan Tong, Shun Yao, Fei Li, Yan Feng, Yihua Sun, Yingyong Hou, Zhongzhou Yang, Kunliang Guan, Haiquan Chen, Lei Zhang & Hongbin Ji. (2014) YAP inhibits squamous transdifferentiation of Lkb1-deficient lung adenocarcinoma through ZEB2-dependent DNp63 repression. Nat. Commun. 5:4629.
6. Shuangshuang Lu, JunweiNie, Qing Luan, Qiuting Feng, Qi Xiao, Zai Chang, Congjia Shan, Daniel Hess, Brian A. Hemmings and Zhongzhou Yang. (2011)Phosphorylation of the Twist1-family basic helix-loop-helix transcription factors is involved in pathological cardiac remodeling. PLoS ONE 6: e19251.
7. 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.
8. 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.
9. Qiuting Feng, Ruomin Di, Fang Tao, Zai Chang, Shuangshuang Lu, Wenjing Fan, Congjia Shan, Xinli Li and Zhongzhou Yang. (2010) PDK1 regulates vascular remodeling and promotes epithelial-mesenchymal transition in cardiac development. Mol. Cell. Biol.30: 3711-3721. (Spotlight article)

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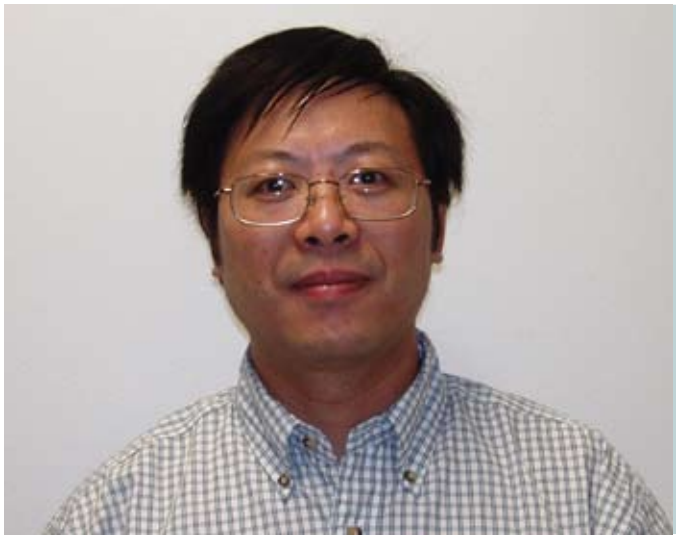

Qing Zhang, Ph.D

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 the mechanism of mitochondrial homeostasis.

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.

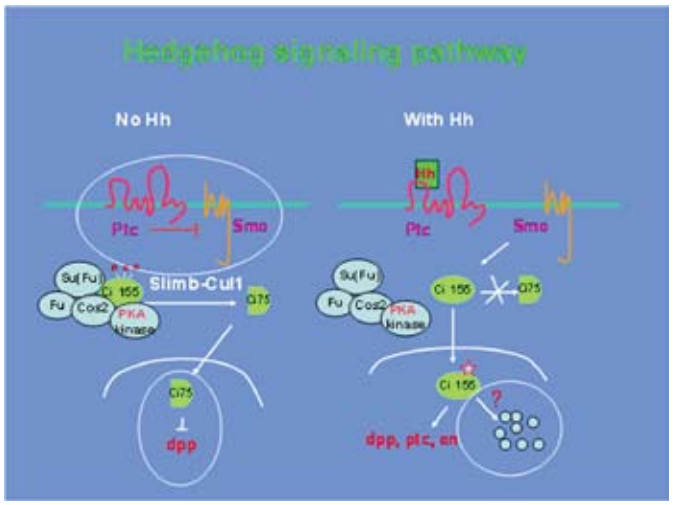

Fig1. Hedgehog signaling pathway in Drosophila.

The mechanism of mitochondrial homeostasis

Suppressor of Fused (Su(fu)) is a conserved inhibitory component of the Hh signaling pathway but how it is regulated remains poorly understood. Here we demonstrate that in Drosophila Hh signaling promotes downregulation of Su(fu) through its target protein HIB (for Hh-induced BTB protein). Interestingly, although HIB-mediated downregulation of Su(fu) depends on the E3 ubiquitin ligase Cul3, HIB does not directly regulate Su(fu) protein stability. Through an RNAi-based candidate gene screen, we identify the spliceosome factor Crooked neck (Crn) as a regulator of Su(fu) level. Epistasis analysis indicates that HIB downregulates Su(fu) through Crn. Furthermore, we provide evidence that HIB retains Crn in the nucleus, leading to reduced Su(fu) protein level. Finally, we show that SPOP, the mammalian homologue of HIB, can substitute HIB to downregulate Su(fu) level in Drosophila. Our study suggests that Hh regulates both Ci and Su(fu) level through its target HIB, thus uncovering a novel feedback mechanism that regulates Hh signal transduction. The dual function of HIB may provide a buffering mechanism to fine-tune Hh pathway activity.

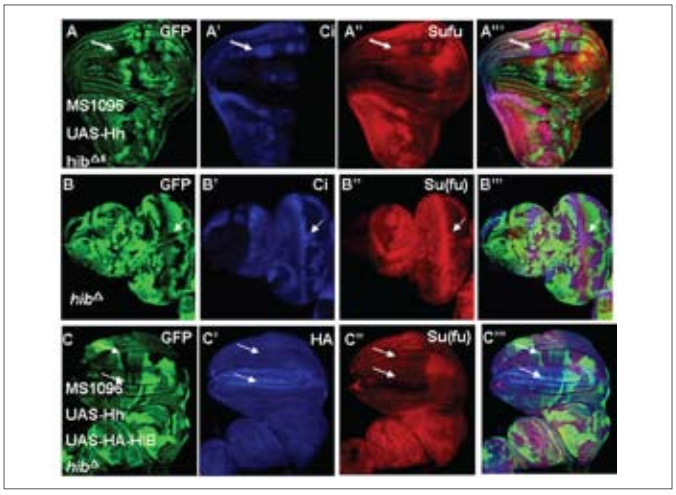

Fig 2. Hh signaling downregulates Su(fu) through Hib.

(A-A‴) Su(fu) is accumulated in hib-mutant clones when Hh is overexpressed with MS1096.

(B-B″) A eye disc immunostained to show Su(fu) (red) level increased in hib-mutant clones.

(C-C‴) show that Hib-expressing cells have diminished levels of Su(fu) which is accumulated in hib-mutant clones.

Selected Publications

1. Liu C, Zhou Z, Yao X, Chen P, Sun M, Su M, Chang C, Yan J, Jiang J, Zhang Q (2014) Hedgehog signaling downregulates suppressor of fused through the HIB/SPOP-Crn axis in Drosophila. Cell Res. 24(5):595-609.
2. 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..
3. Zhang L, Ren F, Zhang Q, Chen Y, Wang B, Jiang J (2008) The TEAD/TEF family of transcription factor scalloped mediates Hippo signaling in organ size control. Developmental Cell 14, 377-387.
4. 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.
5. 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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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

The research interests of the lab focus on investigating the molecular mechanism underlying vertebrate early development, such as formation and differentiation of germ layers, using zebrafish as a model animal.

RA plays essential roles in vertebrate embryogenesis. Its homeostasis in embryos 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*. 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 Ncor1 (nuclear receptor co-repressor) is essential for patterning the anterior–posterior axis of zebrafish hindbrain by actively repressing RA signaling (Xu et al., 2009).

Aside from its global role in embryonic pattern formation, RA signaling is essential to vertebrate mesoderm differentiation. It plays a restrictive role in primitive myelopoiesis by inhibiting 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, Ncor1 and Ncor2 play essential but distinct roles in zebrafish primitive myelopoiesis (Li et al., 2014). Other than RA signaling, the differentiation of ventral mesoderm is affected by environmental factors. Exposing zebrafish embryos with excessive sodium nitrite, a common food additive that exists widely not only in the environment but also in our body, we found that it caused developmental defects of zebrafish heart dose dependently. Comprehensive analyses revealed that excessive nitrite affects valve leaflet formation by producing too much NO signaling (Figure 1; Li et al., 2014).

Engineered endonuclease (EENs) including ZFN (zinc-finger nuclease), TALEN (transcription activator-like effector nuclease) and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated 9) are powerful tools to create genome edited animals without species limitation. Using the knock out tools, we not only produced heritable targeted inactivation of myostatin genes in yellow catfish, the first endogenous gene knockout in aquaculture fish (Dong et al., 2011, Dong et al., 2014), but also generated heritable zebrafish mutants for more than 50 genes. Especially, being a member lab of "Zebrafish All Genes KO Consortium for Chromosome 1, ZAKOC), we created 46 zebrafish mutants using CRISPR/Cas9. Although EENs are powerful tools for fish genome editing, the low efficiency of germline transmission of induced mutations and particularly knockin alleles made subsequently screening heritable offspring tedious, time-consuming and expensive. By co-microinjecting *yfp-nanos3* mRNA with Cas9 mRNA, sgRNA and single strand DNA donor, we demonstrated that founders carrying fluorescent-labeled primordial germ cells produced much higher numbers of knockin and knockout progeny (Figure 2; Dong et al., 2014).

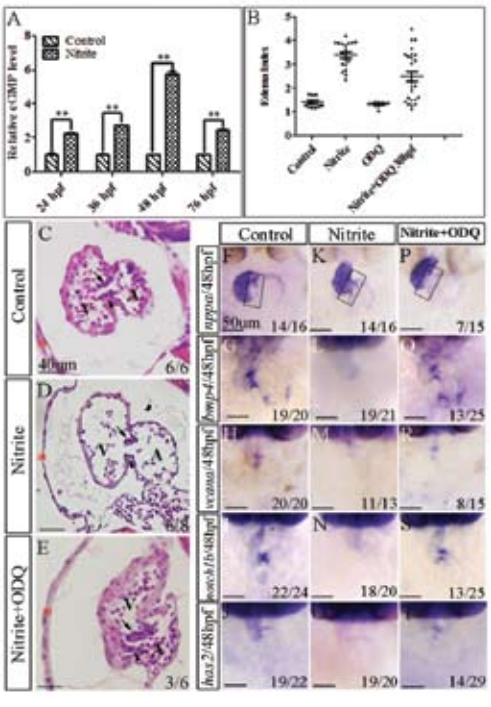

Figure 1. Inhibiting NO signaling in nitrite-exposed embryos partially rescued defective development of cardiac valve in zebrafish embryos.

(A) The cGMP level was dramatically increased in the nitrite-exposed embryos at 24, 36, 48 and 76 hpf, respectively. **: P<0.01. The values of cGMP amount in all the control embryos were normalized to 1.0, respectively. The value of cGMP amount in the nitrite-exposed embryos was the fold of the control embryos at the same developmental stage. (B) A scatter plot showing the increased EIs in the nitrite-exposed embryos were significantly reduced by microinjecting ODQ (sGC inhibitor) into nitrite-exposed embryos. Different treatments of embryos were shown in X-axis. EI (shown in Y-axis) of each embryos was shown in the plot. Average standard errors of EIs were shown in lines. (C-E) Defective histological structures of heart caused by excessive nitrite were partially rescued by microinjection of ODQ into nitrite-exposed embryos. Embryos were observed at 108 hpf. Compared to control embryos with normal pericardial membrane, myocardium and both superior and inferior valve leaflets (C), 6/8 embryos exposed to the nitrite showed thinner myocardium and defective formation of either superior or inferior leaflets (D). ODQ microinjection resulted in 3/6 of the embryos developed both superior and inferior leaflets (E). (F-T) Microinjection of ODQ partially rescued the diminished expressions of valve progenitor makers in zebrafish embryos at 48 hpf. *nppa* is not expressed in the AVC (rectangular box) of control embryos (F) but was ectopically expressed in the AVC of nitrite-exposed embryos (K). Microinjection of ODQ into nitrite-exposed embryos prevented 7 of 15 embryos from expressing nppa in AVC (P). Compared to control embryos, nitrite exposure significantly decreased or abolished expressions of *bmp4* (G, L), *vcana* (H, M), *notch1b* (I, N) and *has2* (J, O) in AVC. However, microinjection of ODQ into nitrite-exposed embryos resumed expressions of *bmp4* (Q), *vcana* (R), *notch1b* (S) and *has2* (T) in about half embryos. Red star (*): pericardial membrane; Black arrow: position of superior valve leaflet; Black arrowhead: position of inferior valve leaflet; A: atria; V: ventricle.

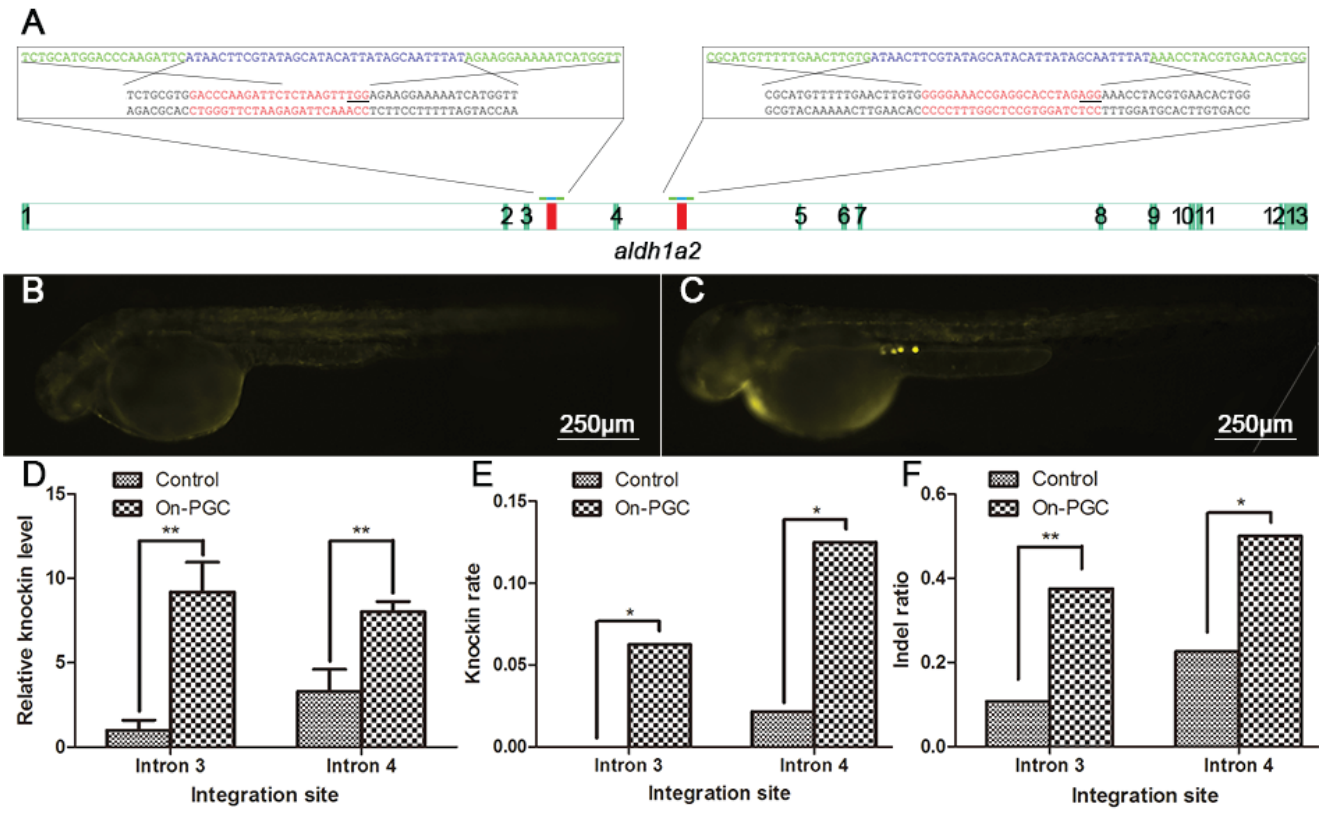

Figure. 2. Zebrafish founders carrying labeled PGCs have much more germ cells containing mloxP knockin allele and indel mutations.

(A) Schematic diagram showing knockin of mloxP in introns 3 and 4 of zebrafish *aldh1a2* through HR. Green boxes in the genomic fragment represent exons, and white boxes introns. Red boxes are sgRNA recognition sites. In the magnified view, green single stranded sequences are homology arms, and blue sequences are mloxP in the ssODN donor. In the double stranded DNA sequences represent genomic fragments at the sgRNA recognition sites. Red sequences are sgRNA recognition sites. Crosses show homologous recombination between donors and recipients. (B and C) Representative embryos with little (B) or abundant (C) YFP signal in PGCs. (D) Relative knockin level of mloxP in oocytes of on-PGC (abundant YFP signal in PGCs) and control founder zebrafish. All values are normalized to the result of knockin in intron 3 in control founder zebrafish. Data are shown in mean ± SEM (error bar). (E) The ratios of progeny carrying knockin of mloxP derived from on-PGC and control founders, respectively. (F) The ratios of progeny carrying indel mutations derived from on-PGC and control founders, respectively. Double asterisks show a difference that is statistically significant with P<0.01 (D, F). Single asterisks show a difference that is statistically significant with P<0.05 (E, F).

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2. Jingyun Li, Kui Li, Xiaohua Dong, Dong Liang, Qingshun Zhao*. 2014.Ncor1 and Ncor2 play essential but distinct roles in zebrafish primitive myelopoiesis. Developmental Dynamics, 243(12):1544-1553.
3. Junbo Li, Wenshuang Jia, Qingshun Zhao*. 2014. Nitrite affects zebrafish valvulogenesis through yielding too much NO signalling. PLoS ONE, 9(3):e92728.
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5. Ting Xu, Jing Zhao, Ping Hu, Zhangji Dong, Jingyun Li, Hongchang Zhang, Daqiang Yin*, Qingshun Zhao**. 2014. Pentachlorophenol exposure causes Warburg-like effects in zebrafish embryos at gastrulation stage. Toxicology and Applied Pharmacology, 277(2):183-91.
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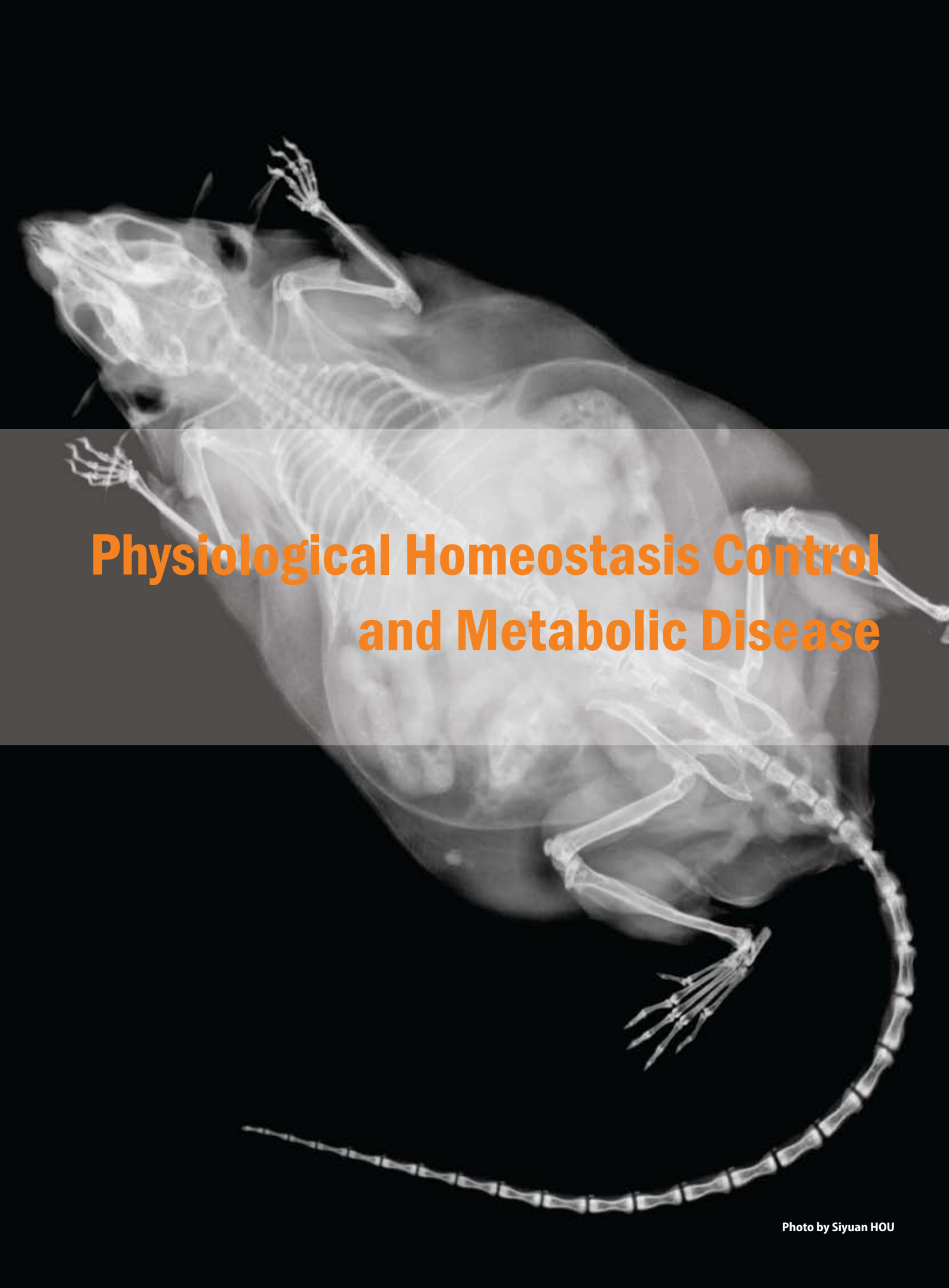
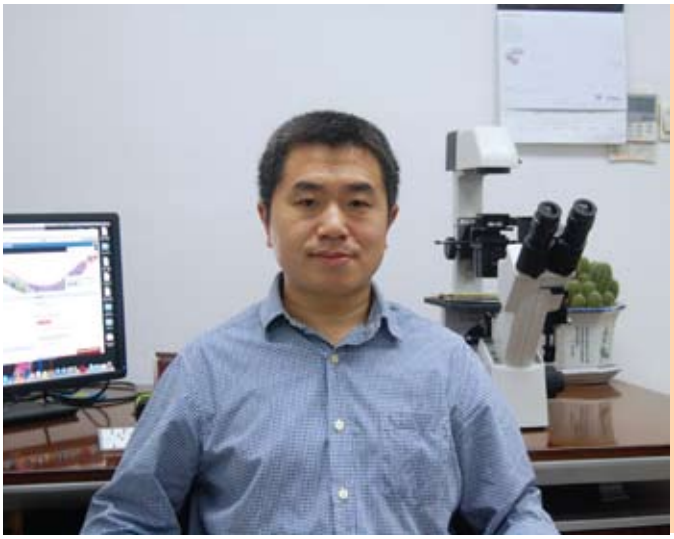


Physiological Homeostasis Control and Metabolic Disease

Photo by Siyuan HOU



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 proposed 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 in many species. Our recently published work showed that simultaneous inhibition of IGF-1 and TOR pathways via the *daf-2 rsks-1* double mutant leads to nearly 5-fold, synergistic lifespan extension. We further demonstrated that the underlying mechanisms involve positive feedback regulation of the DAF-16/FOXO transcription factor via the key energy homeostasis regulator AMPK, and the germ line tissue plays a key regulatory role in this process (Figure 1). Currently, we are using polysomal profiling coupled with RNA-Seq techniques to identify genes that are post-transcriptionally regulated in the *daf-2 rsks-1* double mutant and characterize their roles in aging (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. Recent studies have identified multiple key regulators of DR response in *C. elegans* (Figure 2). Currently, we are using combined genetic and molecular approaches to investigate how lipid metabolism as a dynamic process is involved in modulating nutrients-dependent aging process.

Our research focuses on the following aspects:

1. Functional genomics analysis of genes that are post-transcriptionally regulated in the super long-lived *daf-2 rsks-1* double mutant;
2. Roles of lipid metabolism in dietary restriction-induced lifespan extension;
3. Effects of microbiome on host metabolism and immune response in *C. elegans*.

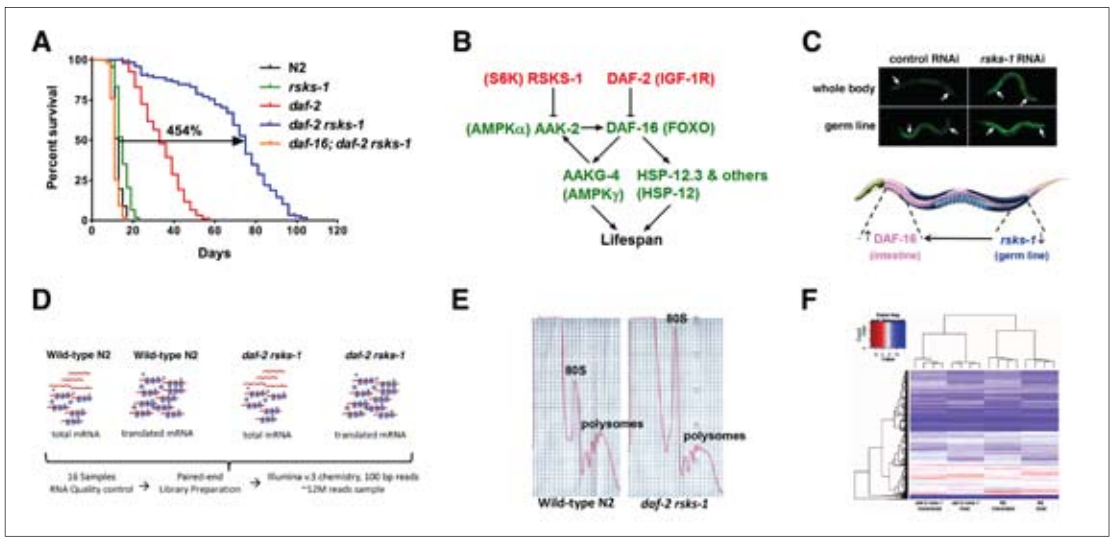

Figure 1. Functional genomics analysis of the super long-lived *daf-2 rsks-1* double mutant *in C. elegans*.

(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) AMPK-mediated positive feedback regulation of DAF-16 in *daf-2 rsks-1*. (C) Germline-specific *rsks-1* RNAi cell-non-autonomously activates DAF-16 in the intestine (arrows) as illustrated by a DAF-16 target *Pstdh-1::gfp* reporter. (D) A schematic depiction of post-transcriptional genomics analysis of the *daf-2 rsks-1* double mutant via polysomal profiling coupled with RNA-Seq. (E) Polysomal profiling of mRNAs from the wild-type N2 and *daf-2 rsks-1* double mutant. (F) Identification of genes that are regulated at the post-transcriptional levels in the *daf-2 rsks-1* double mutant.

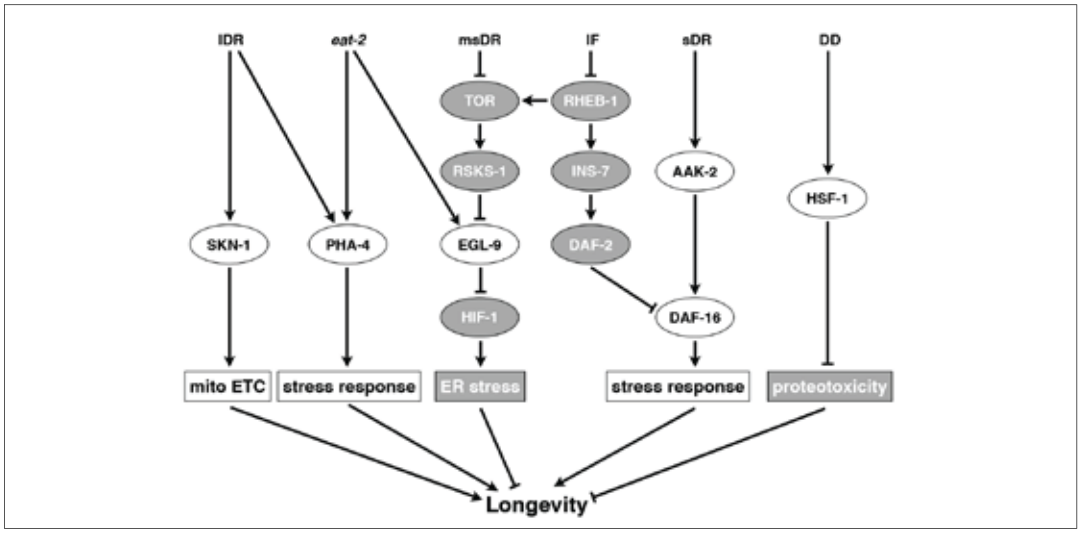

Figure 2. A genetic model depicting the modulation of lifespan by different DR regimens in *C. elegans*.

Grey, pro-aging, lifespan-shortening factors or biological processes. White, anti-aging, lifespan-extending factors or biological processes.

Selected Publications (*corresponding author)

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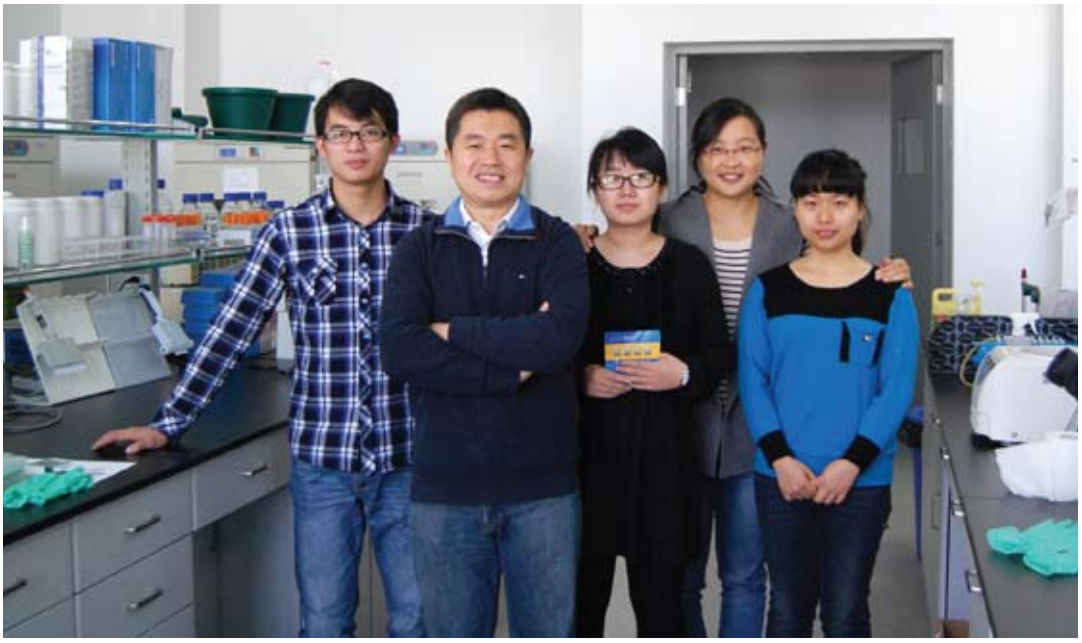

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Shuai Chen, Ph.D.

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.

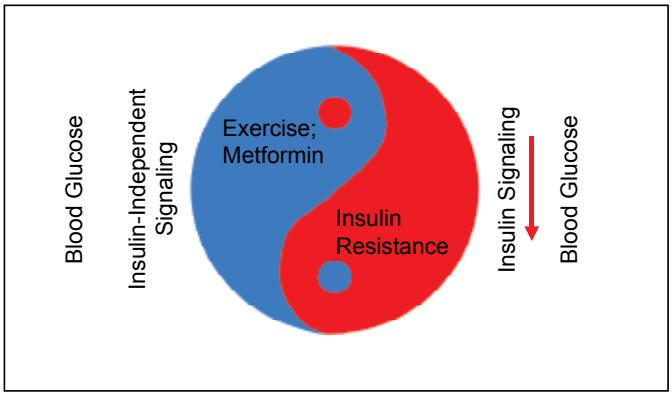

Figure 1 Insulin dependent and independent signaling in the pathogenesis of type II diabetes and its treatment.

The recent progress of my lab is as follows:

GARNL1, a major RalGAP a subunit in skeletal muscle, regulates insulin-stimulated RalA activation and GLUT4 trafficking via interaction with 14-3-3 proteins.

Insulin/PI 3-kinase/PKB (also known as Akt) signaling pathway plays an important role in regulating cardiac metabolisms that are essential for maintaining cardiac function. However, it is not well defined how PKB regulates cardiac metabolisms. In order to address this question, we took a proteomics approach and identified a number of potential PKB substrates from mouse heart in response to insulin. Some known PKB substrates such as AS160/TBC1D4 and WNK1 were found in our study, which validated our approach. Importantly, we identified a few previously unknown PKB substrates including GARNL1/RalGAPα1 that forms a RalGAP1 complex with RalGAPβ and regulates insulin-stimulated RalA activation in muscle cells. Our data show that insulin stimulates PKB-mediated Thr735 phosphorylation on GARNL1/RalGAPα1 and promotes its binding to the regulatory 14-3-3 proteins. Knock-down of GARNL1/RalGAPα1 increased, while overexpression of GARNL1/RalGAPα1Thr735Ala mutant protein decreased, the RalA activation and the RalA-dependent cell surface expression of glucose transporter GLUT4 in response to insulin in muscle cells. These findings show that GARNL1/RalGAPα1 and its phosphorylation and/or binding to 14-3-3s play a critical role in GLUT4 trafficking through RalA in muscle cells.

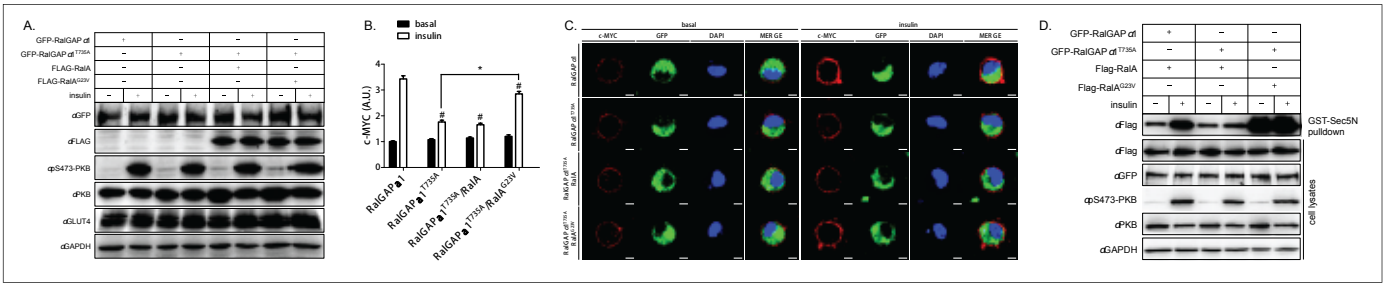

Figure 2 Inhibition of insulin-stimulated GLUT4 translocation by GARNL1/RalGAPα1T735A depended on RalA in rat L6 muscle cells

A. Overexpression of GFP-tagged GARNL1/RalGAPα1 wild-type and mutant proteins in the absence or presence of RalA wild-type or mutant proteins in L6 muscle cells. GFP-GARNL1/RalGAPα1, Flag-RalA, c-MYC-GLUT4 and PKB proteins, and phosphorylated PKB were measured in lysates (40 μg) of transfected cells that were stimulated with or without insulin. GAPDH was used as a loading control.

B. Quantitation of cell surface c-MYC-GLUT4 levels in L6 muscle cells upon overexpression of GARNL1/RalGAPα1 wild-type and mutant proteins in the absence or presence of RalA wild-type or mutant proteins. n=52, # indicates $p<0.05$ versus GFP-GARNL1/RalGAPα1 wild-type (insulin). Asterisk indicates $p<0.05$.

C. Representative images of staining of cell surface c-MYC-GLUT4 in L6 muscle cells upon overexpression of GARNL1/RalGAPα1 wild-type and mutant proteins in the absence or presence of RalA wild-type or mutant proteins. Bars indicate 5 μm in length.

D. Levels of active RalA in L6 muscle cells overexpressing GFP-tagged GARNL1/RalGAPα1 wild-type and mutant proteins together with Flag-RalA wild-type or mutant proteins. Active Flag-RalA was pulled-down from cell lysates using GST-Sec5N as a bait protein, and detected via western blot using anti-Flag antibody. GFP-GARNL1/RalGAPα1, Flag-RalA, and total and phosphorylated PKB proteins were measured in lysates (40 μg) of transfected cells with GAPDH as a loading control.

Selected Publications(* corresponding author)

1. Qiaoli Chen, Chao Quan,Bingxian Xie, Liang Chen, Shuilian Zhou, Rachel Toth, David G. Campbell, Shuangshuang Lu, Ryutaro Shirakawa, Hisanori Horiuchi, Chaojun Li, Zhongzhou Yang, Carol MacKintosh, Hongyu Wang*, Shuai Chen*. (2014) GARNL1, a major RalGAP a subunit in skeletal muscle, regulates insulin-stimulated RalA activation and GLUT4 trafficking via interaction with 14-3-3 proteins. Cell Signal 26(8): 1636-1648
2. Wang H.Y., Ducommun S., Quan C., Xie B.X., Li M., Wasserman D.H., Sakamoto K., MacKintosh C.* and Chen S.* (2013) AS160 deficiency causes whole-body insulin resistance via composite effects in multiple tissues. Biochem J. 449 (2): 479-489
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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.

Delineate the ERRγ/miR-499 networks controlling muscle fitness.

Genetically modified mouse models have demonstrated that there is a nuclear receptor-miRNA circuit that orchestrates programs controlling muscle energy metabolism and fiber type (Fig 2): 1) the PGC-1α/PPARβ/δ/ERRγ signaling can drive a trained muscle fiber program; 2) PPARβ/δ activates ERRγ which drives the miR-208b/499 circuit, thus the type I fiber program; 3) studies of human muscle confirmed that this circuit links control of muscle fiber type with energy metabolic capacity. This new information has provided important insight into the role of the nuclear receptor signaling and miRNAs network in muscle fiber type switching.

Given recent exciting evidence that the expression of the ERRγ and miR-499 links to human fitness, the nuclear receptor/miRNA regulatory circuit shows promise as a therapeutic target aimed at enhancing muscle fitness in a variety of chronic disease states that are associated with loss of muscle fitness including but not limit to obesity, diabetes, muscular disease, and aging. We are currently trying to fully define the downstream targets of the nuclear receptor-miRNA networks involved in the coordinate regulation of muscle metabolic and structural programs. We have found that in addition to the well-established role in control of muscle contractile genes, miR-499 regulates a broad array of mitochondrial metabolic genes (Fig 3).

In addition, we also explored the roles of ERRγ/miR-499 signaling in Duchenne muscular dystrophy (DMD). Our results indicate that overexpression of miR-499 in the dystrophic muscles of the mdx mice prevented the hallmarks of DMD, including muscle damage, plasma creatine kinase levels, and exercise capacity. Our data demonstrate that activation of miR-499 signaling in skeletal muscle could represent a novel strategy to prevent or treat muscular dystrophy.

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.

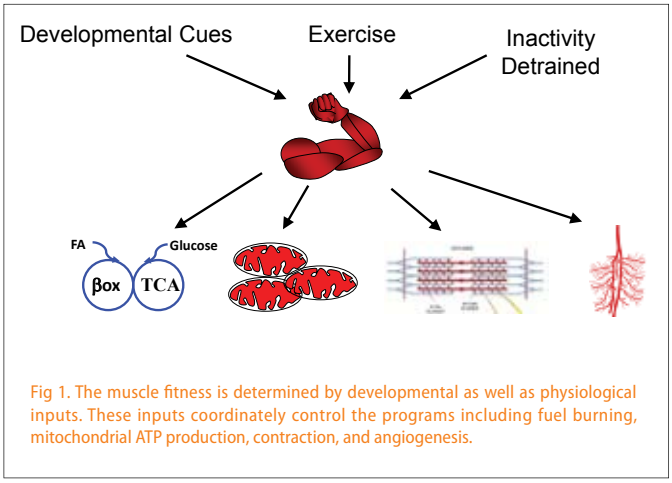

Fig 1. The muscle fitness is determined by developmental as well as physiological inputs. These inputs coordinately control the programs including fuel burning, mitochondrial ATP production, contraction, and angiogenesis.

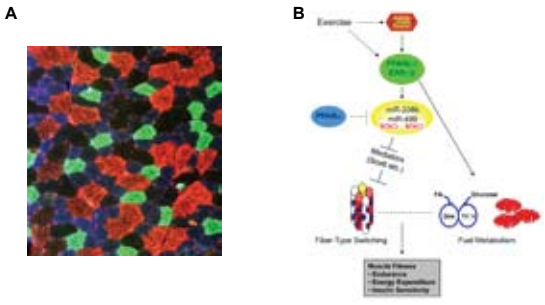

Fig 2. (A) Muscle fiber typing by immunofluorescence staining of gastroc muscle from 3 month old C57BL/6J mice. MHC1 (green), MHC2a (blue), MHC2b (red), and MHC2x (unstained). (B) Schematic depicts proposed model for nuclear receptor signaling and miRNA network in the control of muscle fiber type switching.

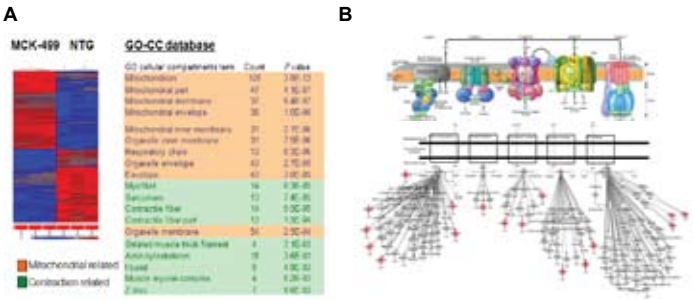

Fig 3. miR-499 regulates a broad array of mitochondrial metabolic genes.

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Metabolic homeostasis and pathogenesis

The research focus of my laboratory is continuing to shift toward the questions on tight control of the metabolic homeostasis, as well as the consequences of disruption of this regulation during pathogenesis. We approach these fundamental questions in coordinated with its complexity, by analyzing multi-organs or systems. These included defining the functions of many genes in brain, liver, adipocyte tissues, bone, gut, and immune system. We are fascinated these tissues work together in concord to balance the levels of glucose, fatty acids, and other metabolites for the physiological needs.

Recently, we have redefined the function of SCF/Kit pathway in thermogenesis with the mutant mice. The c-kit mutant mice displayed later onset of obese. These mutant mice have higher energy expenditure rate indicated by lower body temperature and cold intolerance. Additional assay confirmed that SCF/Kit pathway directly up-regulates the PGCalpha expression BAT and mitochondrial DNA synthesis(Fig.1). Interestingly, we found the expression level of both SCF and c-Kit is responsible to the high-fat diet and environmental temperature, indicating this pathway is crucial for the maintenance the normal body temperature.

Another project in our lab is to dissect the potential role of Jmjd3 in control the metabolic control neurons in the brain. We found cell specific gene targeting of Jmjd3 in RIP neuron can also lead to later onset of obese, but only in female(Fig.2). Now we know it actually control the Kisspeptin gene expression. By regulating the menstrual cycle, Kisspeptin is a key mediator for female hormone release. The relationship between estrogen and body fat accumulation is well establish in previous study. Our studies added a new piece for solving the puzzle between the CNS complicated control among CNS function, reproductive function, and metabolic homeostasis.

Understanding the metabolic 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.

The mystery of metabolic 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.

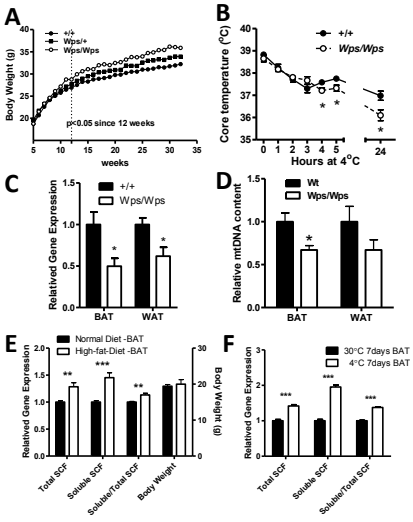

Figure 1. SCF/Kit pathway in thermogenesis

A. Growth curve of male Wt and Wps male mice on regular chow are shown. B. Body temperature of Wps mice during cold exposure was detected by an anal probe. C. The expression level of PGC1a in various tissues were determined by Real-time PCR. D. The mitochondrial DNA copy number was determined by Real-time PCR with DNA from BAT and WAT. E. 8-week-old male C57Bl/6J mice were fed with normal diet or high fat diet for 1week, mRNA levels in BAT were determined by Real-time PCR. F. 8-week-old male C57Bl/6J mice were hold in 8°c or 30°c for 1week. mRNA levels in BAT were determined by Real-time PCR.

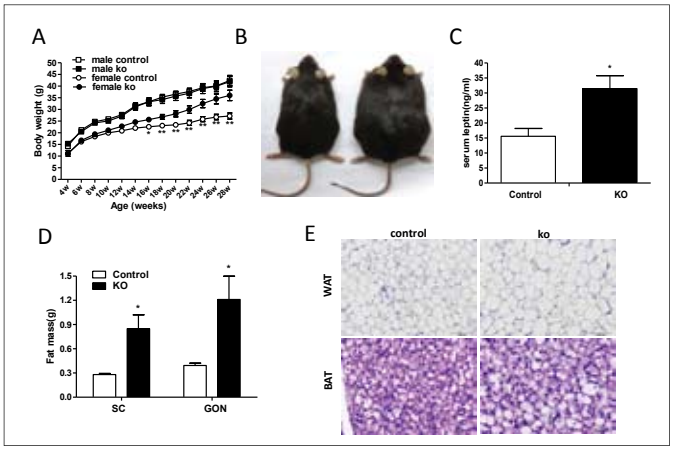

Fig.2 Female specific obesity in Rip-Cre;Jmjd3lox/lox female mice.

A, Body weight in male and female littermates fed on regular chow diet from 4 to 28 weeks of age (male n=8/n=9, female n=8/n=8). B, The gross appearance of control and knockout female mice at the age of 7 months C, Serum leptin levels in control (n=9) and knockout (n=6) mice at the age of 7 months. D, Subcutaneous (SC) and gonadal (GON) fat mass in control (n=6) and knockout (n=5) mice at the age of 4 months. E, Representative photomicrographs of WAT and BAT sections stained with hematoxylin-eosin. Scale bars, 50μm. Data are represented as mean±s.e.m. *P < 0.05, **P < 0.01, unpaired t test compared to control mice.

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Protein prenylation and metabolic disorders

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 are used to prenylate proteins with CAAX motif in their carboxyl termini. The prenylated proteins then are able to associate with membrane to initiate their function. We first identified GGPPS as a direct target gene of Egr-1, which can positively feedback to increase Egr-1 accumulation during chronic stress stimulation through enhancing Ras prenylation and membrane association (Am J Path, 2011a, 2011b; J Biol Chem 2011; EMBO J, 2011). We have constructed GGPPS floxed mice and conditionally deleted GGPPS gene in different tissues to examine its functions and its involvements in human diseases. We found that GGPPS affected protein prenylation in mouse Sertoli cell regulated Mumps infection related Orchitis formation during childhood and give rise to a long-term effect of infertility till adulthood (J Exp Med, 2013). Right now we are exploring the maintenance of metabolic homeostasis by protein farnesylation/geranylgeranylation or FPP/GGPP balance and its relationship with human disease development.

1. Cardiomyocyte protein prenylation and hypertrophy

G protein-regulated cell function is crucial for cardiomyocytes, and any deregulation of its gene expression or protein modification can lead to pathological cardiac hypertrophy. We found that GGPPS played a critical role in the postnatal heart growth through regulating the cardiomyocyte size. Cardiac-specific knockout of GGPPS in mice led to spontaneous cardiac hypertrophy beginning from 4th week, accompanied with the persistent enlargement of cardiomyocytes. Evaluation of the membrane association and hydrophobicity showed that Rheb was hyperactivated and increased mTORC1 signaling pathway after GGPPS deletion. Protein farnesylation or mTORC1 inhibition blocked GGPPS knockdown-induced mTORC1 activation and suppressed the larger neonatal rat ventricle myocyte size, demonstrating a central role of FPP/Rheb/mTORC1 axis for GGPPS deficiency-induced cardiomyocyte hypertrophy. The sustained cardiomyocyte hypertrophy progressively provoked cardiac decompensation and dysfunction, ultimately causing heart failure and adult death. Importantly, GGPPS was downregulated in the hypertrophic hearts of mice subjected to transverse aortic constriction (TAC) and in the failing human hearts. Our observations conclude that the alteration of protein prenylation promotes cardiomyocyte hypertrophic growth.

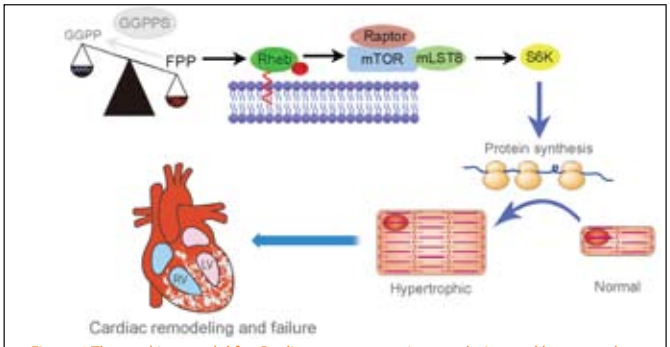

Figure 1 The working model for Cardiomyocyte protein prenylation and hypertrophy

2. Lipid-induced muscle insulin resistance is mediated by GGPPS through modulating RhoA/Rho-kinase signaling pathway

Elevated circulating free fatty acid (FFA) levels are major contributors to insulin resistance in muscle and liver, but the underlying mechanisms remain to be further elucidated. Here we show that GGPPS promotes lipid-induced muscle insulin resistance through activation of RhoA/Rho-kinase signaling pathway. GGPPS is overexpressed in skeletal muscle of *db/db* mice and mice on a high fat diet (HFD). To address the metabolic consequences of GGPPS in the skeletal muscle, we generated mice that specific delete GGPPS in skeletal muscle. Deficiency of GGPPS in skeletal muscle improved systemic insulin sensitivity and glucose homeostasis on both normal chow and high fat diet. These alterations were accompanied by activated PI3K/Akt signaling and enhanced glucose uptake in skeletal muscle. Further investigation showed that HFD increased GGPPS expression in skeletal muscle was able to enhance the geranylgeranylation of RhoA, which further induce the inhibitory phosphorylation of IRS-1 (Ser307) through increasing Rho-kinase activity. These results implicate a crucial role for GGPPS/RhoA/Rho-kinase/IRS-1 pathway in skeletal muscle that can mediate lipid-induced systemic insulin resistance in obese mice. Therefore, skeletal muscle GGPPS may provide a novel pharmacological target for the treatment obesity related type 2 diabetes.

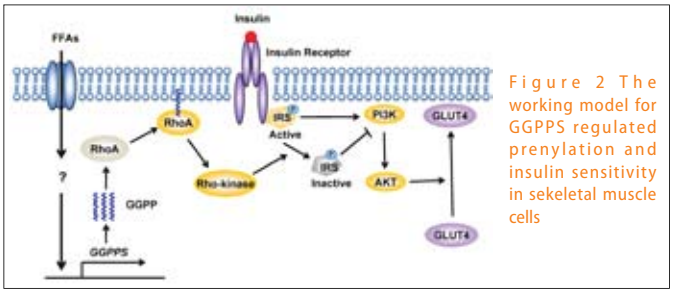

Figure 2 The working model for GGPPS regulated prenylation and insulin sensitivity in sekeletal muscle cells

3. The Rab27a prenylation in β-cell and Type 2 Diabetes Mellitus development

β-cell dysfunction associated with loss of first-phase insulin secretion is an independent predictor of type 2 diabetes mellitus (T2DM) onset. During the development of type 2 diabetes, the β-cells experience a compensatory period of excessive insulin biosynthesis and release and then subject to β-cell exhaustion and dysfunction. When we specifically delete GGPPS in pancreatic β-cell, the mice show typical T2DM β-cell dysfunction phenotype with blunted glucose-stimulated first-phase insulin secretion (GSIS) and consequential insulin secretion insufficiency. However, the islets number, size, and insulin biosynthesis have no alteration. TEM observation shows a reduced number of insulin granules adjacent to the cellular membrane, which suggested the formation of insulin granule docked pool has been blocked, while the reserve pool is not affected in GGPPS-null β-cells. Further examination reveals that the impaired geranylgeranylation of Rab27A is responsible for the insulin docked pool deficiency in GGPPS-null mice. These results suggested that GGPPS controlled Rab27A geranylgeranylation is critical for β-cell function like insulin exocytosis via regulating insulin granule docked pool formation during the development of T2DM.

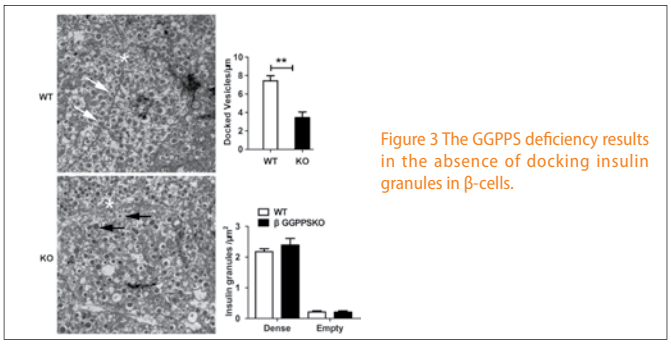

Figure 3 The GGPPS deficiency results in the absence of docking insulin granules in β-cells.

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1. Na Xu, Shan Guan, Zhong Chen, Yang Yu, Jun Xie, Fei-Yan Pan, Ning-Wei Zhao, Li Liu, Zhong-Zhou Yang, Xiang Gao, Biao Xu, and Chao-Jun Li*. The alteration of protein prenylation induces cardiomyocyte hypertrophy through Rheb/mTORC1 signaling and leads to chronic heart failure. J Pathol. 2014 doi:10.1002/path.4480
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9. Ning Shen, Tao Gong, Jian-Dong Wang, Fan-Li Meng, Long Qiao, Run-Lin Yang, Bin Xue, Fei-Yan Pan, Xiao-Jun Zhou Hua-Qun Chen, Wen Ning*, Chao-Jun Li*. Cigarette-smoke induced pulmonary inflammatory responses are mediated by egr-1/ggpps/mapk signaling. Am J Pathol., 2011, 178(1):111–119.

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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. 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. Our observations suggest 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. Interestingly, MLCK also is required for the contraction of lymphatic smooth muscle, a highly specified smooth muscle with several features of cardiac cells (Figure 1.). Force maintenance is performed by a calcium sensitization mechanism. By use of MYPT1 knockout mice, we demonstrated MYPT1 deletion causes phenotypic transition of phasic and tonic smooth muscles, and the myogenic alteration by MYPT1 deletion is enough for generation of hypertension. With thorough analysis for two line of MYPT1 T694A and T852A mutant mice, we find that MYPT1 T694 phosphorylation is essential for sustained contraction of bladder smooth muscle, whereas MYPT T852 does not (Fig.2). Our finding provide novel mechanistic insights into the specific role of MYPT1 phosphorylation on physiological and pharmacological Ca2+ sensitization.

The human body, and those of other mammals, contains up to 50 sphincters, ring-shaped structures encircling an opening or passage in hollow organs. These sphincters control the entrance of material into, or the release of contents from, these organs; mediating a variety of biological functions essential for homeostasis. All sphincters, except external anal and urinary sphincters, are made of smooth muscle cells; contraction of these cells maintains the sphincter in the closed state. It is thus fundamental to determine the molecular and cellular mechanisms by which sphincteric smooth muscle contracts at rest, i.e., basal tone formation. Internal anal sphincter (IAS) localizes at the end of the gastrointestinal tract, and has served as a prototypical model to understand basal tone genesis in sphincters. We found that the basal tone in IAS from MLCK knockout mice was essentially abolished. By directly examining Ca2+ signals and ion channel activity, we further found that Ca2+-releasing ryanodine receptors/channels (RyRs), TMEM16A Ca2+-activated Cl- (Cl¬Ca) channels, and L-type voltage dependent Ca2+ channels (VDCCs) form a module which generates a global rise in Ca2+, and that altering any one of the three channels can severely impair IAS basal tone (and to the same extent as MLCK deletion) (Figure 3). Our results demonstrate that MLCK activation by a RyR-TMEM16A ClCa channel-L-type VDCC signaling cascade in the IAS smooth muscle cells is required for basal tone formation and maintenance.

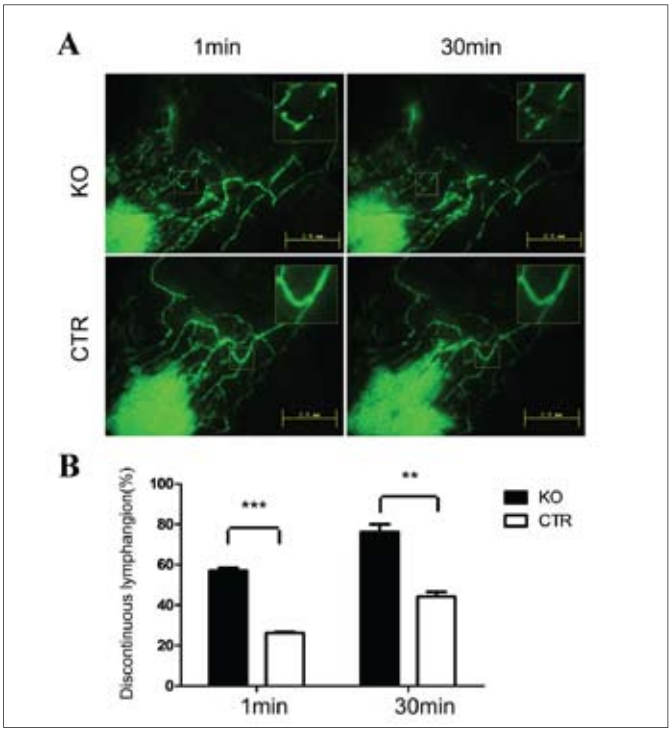

Figure 1. Visualization of lymph flow in mouse ear.

MLCKSMKO (KO) and control (CTR) mice were injected with 1μl of FITC-dextran solution at the ears. A: The visualized lymphatic vessels were photographed at different time points. The magnification indicates typical morphology of lymphagions in which mutant vessels had many discontinuous lymphangions. B: The percentage of discontinuous lymphangions were quantified. The average percentages are expressed by mean value ±SEM (n=5). **p<0.001

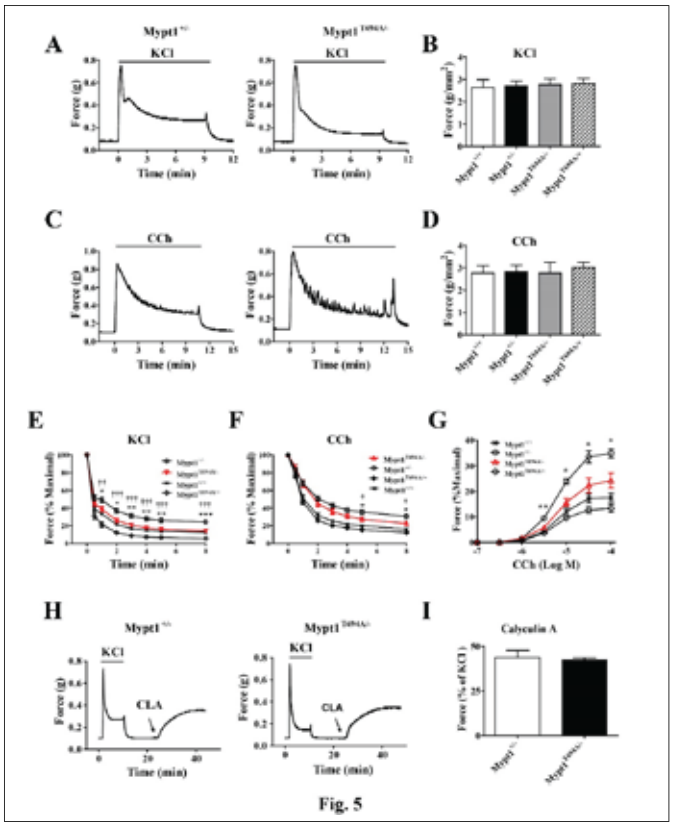

Figure 2. Bladder smooth muscle contraction of MYPT1 T694A mutants evoked by KCl and carbachol.

Bladder smooth muscle strips from E18.5 mice were isolated and subjected to force measurement responding to different stimuli.

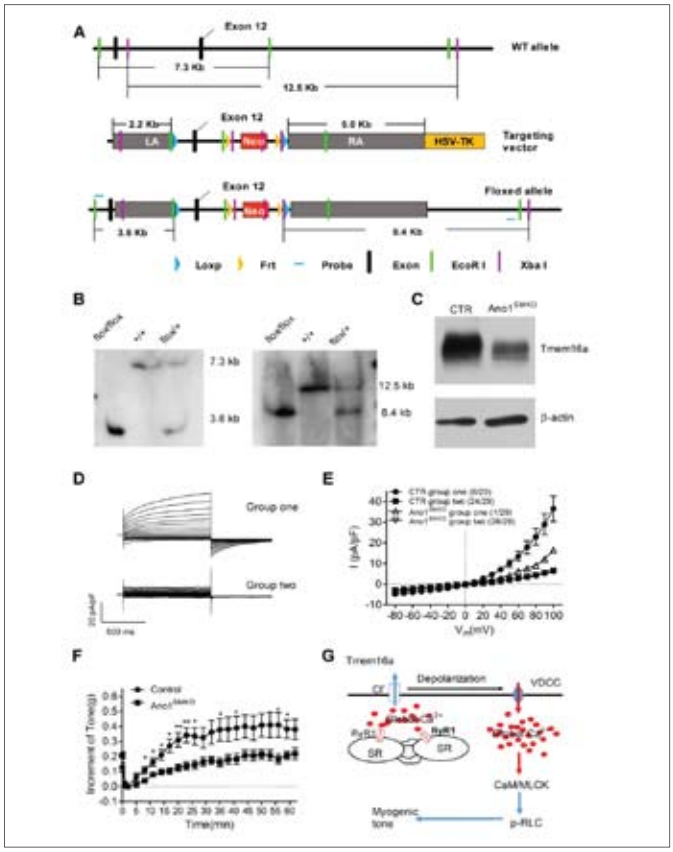

Figure 3. Basal tone is impaired in TMEM16ASMKO mice.

Selected Publications

1. Chen, C., Tao, T., Wen, C., He, W. Q., Qiao, Y. N., Gao, Y. Q., Chen, X., Wang, P., Chen, C. P., Zhao, W., Chen, H. Q., Ye, A. P., Peng, Y. J., and Zhu, M. S.* (2014) Myosin Light Chain Kinase (MLCK) Regulates Cell Migration in a Myosin Regulatory Light Chain Phosphorylation-Independent Mechanism. J. Biol. Chem. (accepted)
2. Chen, C.P., Chen, X., Qiao, Y.N., Wang, P., He, W. Q., Zhang, C.H., Zhao, W., Gao, Y.Q., Chen, C., Tao, T., Sun, J., Wang, Y., Gao, N., Kamm, K.E., Stull, J.T., and Zhu, M.S * (2014) Roles in vivo for Myosin Phosphatase Targeting Subunit-1 T694 and T852 Phosphorylation sites in Bladder Smooth Muscle. J. Physiology (accepted)
3. Qiao, Y. N., He, W. Q., Chen, C. P., Zhang, C. H., Zhao, W., Wang, P., Zhang, L., Wu, Y. Z., Yang, X., Peng, Y. J., Gao, J. M., Kamm, K. E., Stull, J. T., and Zhu, M. S.* (2014) Myosin Phosphatase Target Subunit 1 (MYPT1) Regulates the Contraction and Relaxation of Vascular Smooth Muscle and Maintains Blood Pressure. J. Biol. Chem. 289, 22512-22523
4. Wang, Y., Zhao, W., Zhang, L., Zhao, Y. N., Li, F., Zhang, Z., Dai, Y. D., Li, W. F., Qiao, Y. N., Chen, C. P., Gao, J. M., and Zhu, M. S*. (2014) Molecular and cellular basis of the regulation of lymphatic contractility and lymphatic absorption. Int. J. Biochem. Cell Biol. 53, 134-140
5. Tao, T., Chen, C., Sun, J., Peng, Y.J., and Zhu, M.S* (2014) A Bacterial Artificial Chromosome Transgenic Mouse Model for Visualization of Neurite Growth. Science China-Life Sciences (accepted).
6. Zhang, C. H., Wang, P., Chen, C.P., Zhao, W., Chen, X., Chen, C., He, W.Q., Qiao, Y.N., Tao, T., Sun, J., Peng, Y.J., Craige, S.M., Lifshitz, L.M., Keaney Jr, J.F., Fogarty, K.E., ZhuGe R.H., Zhu, M.S*. (2014). The molecular basis of the genesis of basal tone in internal anal sphincter. Nature Communication (under review)

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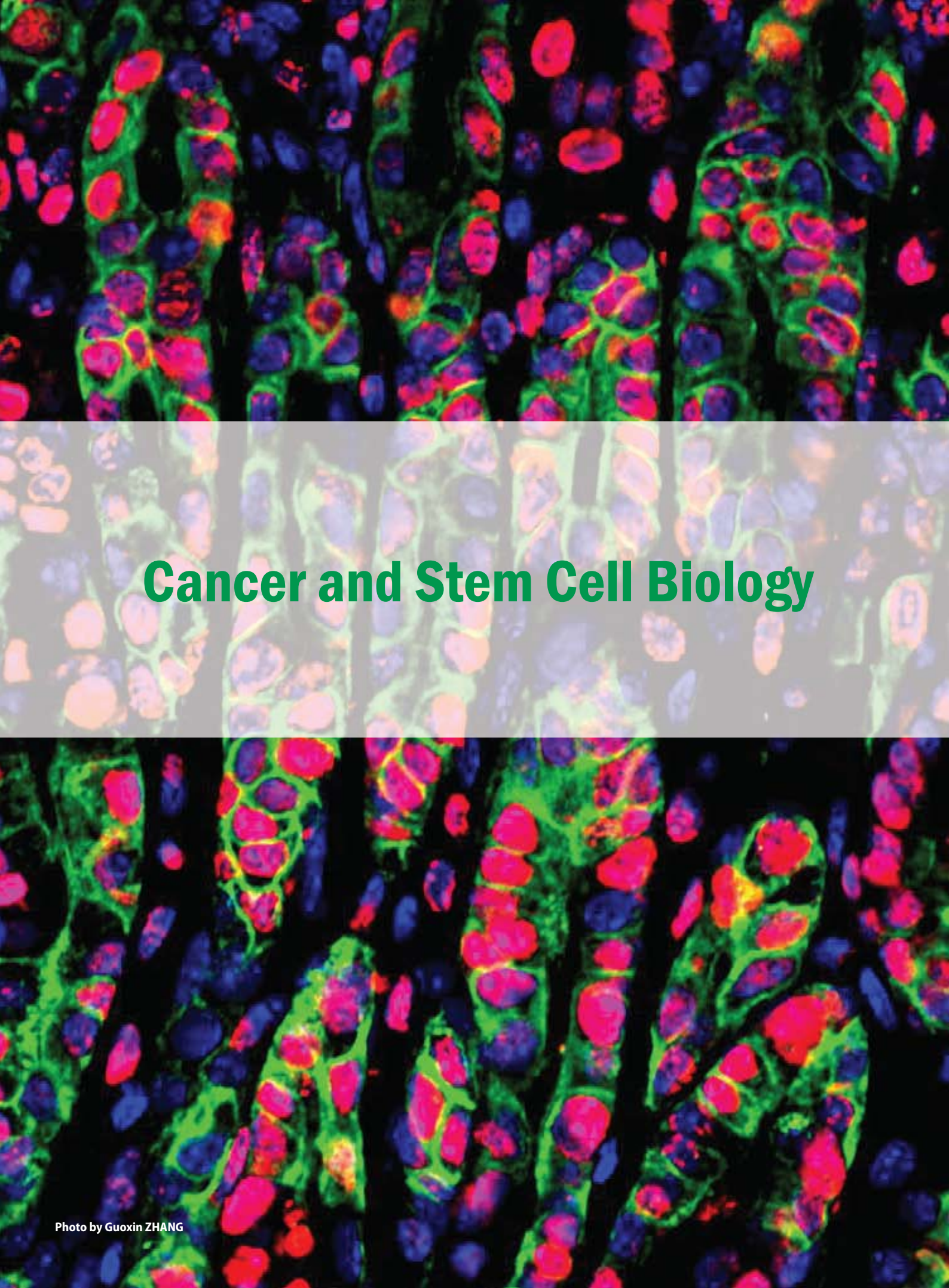
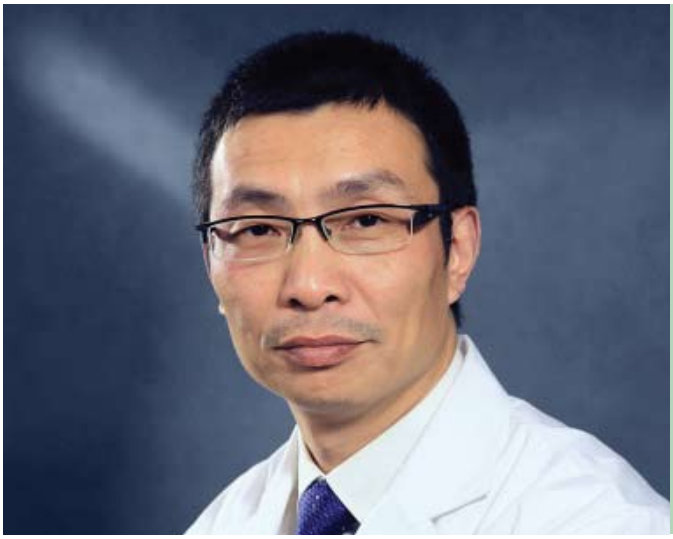


Cancer and Stem Cell Biology

Photo by Guoxin ZHANG



Qing Jiang, Ph.D.

Our Lab'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 2014. Our Lab'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 2014.

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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 in 2014 showed that UQCC gene was a candidate gene associated with DDH occurrence.(Figure. 1) Replication studies also demonstrated a missense mutation in TXNDC3 gene as a risk factor for DDH. (Figure. 2)

Popu-lation	Case					Control					P value for allele frequency	Odds ratio (95% CI)
	Genotype				Allele G frequency	Genotype				Allele G frequency		
	AA	AG	GG	Sum		AA	AG	GG	Sum			
Set A	260	109	17	386	0.19	285	226	47	558	0.29	$4.82*10^{-7}$	1.77(1.41-2.21)
Set B	426	293	36	755	0.24	500	371	73	944	0.27	0.0338	1.18(1.01-1.38)
Set A*+ Set B*	686	402	53	1141	0.22	785	597	120	1502	0.28	$3.63*10^{-6}$	1.35(1.19-1.53)

Figure. 1 Association of rs6060373 of the UQCC gene with DDH

Popul ation	Case					Control					P value for allele frequency	Odds ratio (95% CI)
	Genotype				Allele T frequency	Genotype				Allele T frequency		
	TT	TC	CC	Sum		TT	TC	CC	Sum			
Set A	62	185	138	385	0.4	118	298	142	558	0.48	0.00077	0.731(0.607-0.880)
Set B	98	276	225	599	0.4	297	734	454	1485	0.46	0.0017	0.804(0.711-0.922)
Set A + Set B	160	461	363	984	0.4	415	1032	596	2043	0.46	$1.53*10^{-5}$	0.786(0.705-0.877)

Figure 2. Association of rs10250905 of the TXNDC3 gene with DDH

2. DVT related studies

Due to the trauma caused by operation, patients with lower extremity venous thrombosis occurred at a higher rate, the complication of pulmonary artery embolism will directly threaten the life of patients. In 2014 we found that the related biological markers may bring a breakthrough for the diagnosis and treatment of operation after the trauma of venous thrombosis in lower extremity deep vein thrombosis. We also demonstrated the incidence of ankylosing spondylitis and osteoarthritis patients after joint replacement DVT (Figure. 4)and denied p- selectin and associated DVT after operation. (Figure. 5) We explored the risk factors of thrombosis incidence after arthroscopic knee surgery, and confirmed that the complexity of surgery and the age of the patientwere associated with arthroscopic surgery related thrombosis. (Figure. 6) Further analysis by meta-analysis clarified the effect of low molecular weight heparin in the prevention of postoperative arthroscopy thrombosis, guiding the clinical prevention of thrombosis with reference value. (Figure. 7)

miRNA	Control(n=14)	Cases(n=14)	Fold change	P value
	Fmol/L	Fmol/L		
miR-582	931.1(840.4)	3164.4(1509.9)	3.4	0.0000628*
miR-195	4.0(2.2)	51.0(26.9)	12.8	0.0000265*
miR-532	103.7(56.6)	477.3(205.6)	4.6	0.0000332*

Figure 3 DVT & miRNA

Incidence of DVT in both groups						
Disease	DVT	Cont ml	Total	%	κ2	P
AS	8	46	54	14.8	0.89	>0.05
OA	17	78	95	17.9		

Figure 4 AS OA & DVT

DVT versus p-selection					
Subject	DVT=1		DVT=2		P value
	Mean	S.D.	Mean	S.D.	
p-selection(pre)	2.43	1.5	2.14	0.95	0.67
p-selection(post)	2.6	1.58	2.26	1.04	0.45
Amount of change of p-selection	-0.17	0.57	-0.12	0.33	0.98
P value were obtained by using Mean-Whitney test					

Figure 5 P-Selectin & DVT

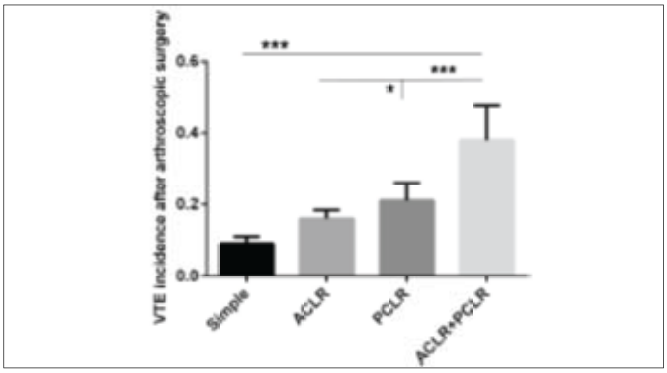

Figure 6 Arthroscopic surgery & DVT

Pooled Absolute Risk Reduction in the Development of DVT and Proximal DVT with LMWH from RCT5					
Prophylaxis	Combined Enrollment	Total DVT		Proximal DVT	
		Prevalence(95% CT),%	ARR%	Prevalence(95% CT),%	ARR%
Placebo/control	914	11.3(2.9-35.6)	9.6	11.3(2.9-35.6)	1.2
LMWH	1356	1.7(1.2-2.6)		1.7(1.2-2.6)	
ARR, absolute risk reduction;CI,confidence interval;DVT,deep venous thrombosis;LMWH,low-molecular weight heparin					

Figure 7 LMWH & DVT

3. Drugs & materials 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. In 2014, we showed the matrix of bone and articular cartilage matrix for removal of antigen processing preparation can be used to repair articular cartilage lesions. We started to get 3D printer for the preparation of repair materials. Using biological materials to repair articular cartilage lesion is an important research direction of intervention therapy of degenerative osteoarthritis, the project research results will bring revolutionary breakthrough in the preparation of new materials.

In 2014 we showed KGN intra-articular injection in the knee joint cartilage repair model was helpful histologically, suggesting that KGN can effectively promote the repair of cartilage tissue. (Figure. 8) We also studied, effects of cell sheet on bone and cartilage transplantation whereby we found that cell sheet can significantly enhance the connection with the surrounding cartilage graft. (Figure. 9) At present, we have purchased a 3D printer, which will be incorporated into cartilage repair work.

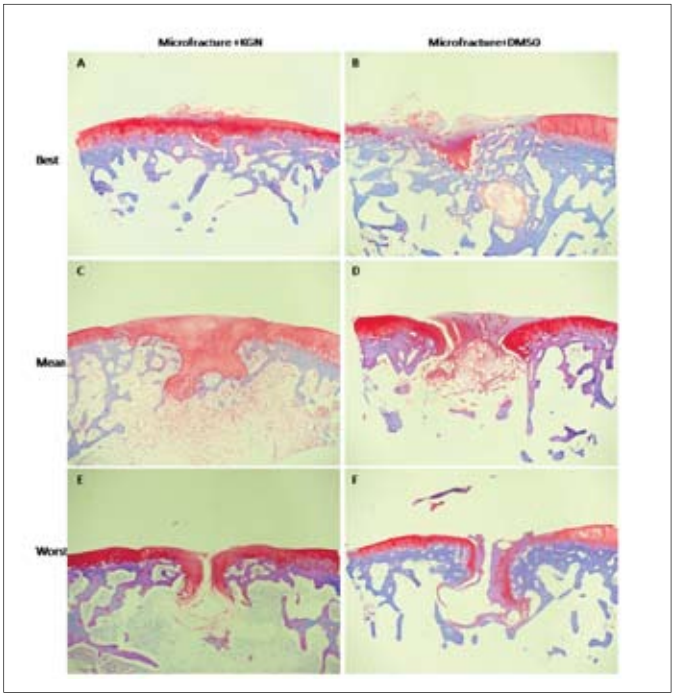

Figure 8 KGN & Cartilage Repair 2

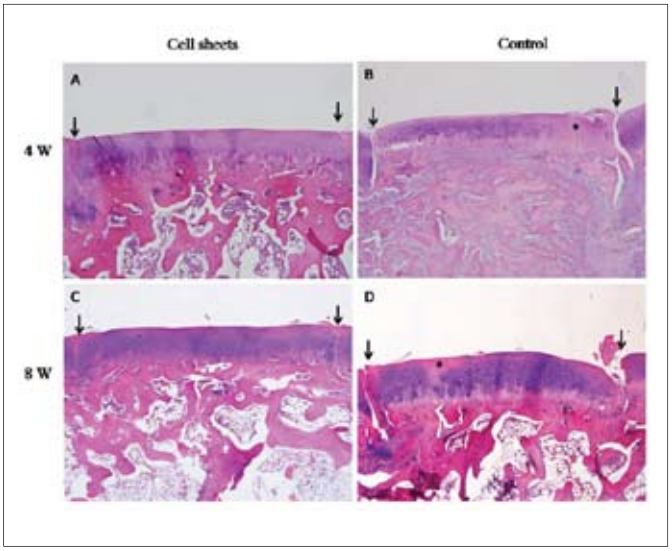

Figure 9 Cell sheet & Cartilage Repair

4. SOST in bone and joint degeneration

We aim to elucidate the role and mechanism of SOST in articular cartilage lesions and pathological bone. To date, pharmacological treatment of osteoarthritis has no definitive effect,our current study is likely to reveal the role and mechanisms of SOST in the occurrence and development of osteoarthritis, thus providing a new theoretical method for treatment of osteoarthritis In 2014, we discovered that SOST might be involved in the regulation of pathological ectopic bone formation. (Figure. 10) Our results also show that the regulation of SOST expression by inflammatory cytokines (e.g. IL-1) and hypoxia. These results suggest that, in different oxygen environments SOST may be regulated by a variety of inflammatory factors in two aspects, the formation of cartilage ossification progress in pathological ossification and osteoarthritis.

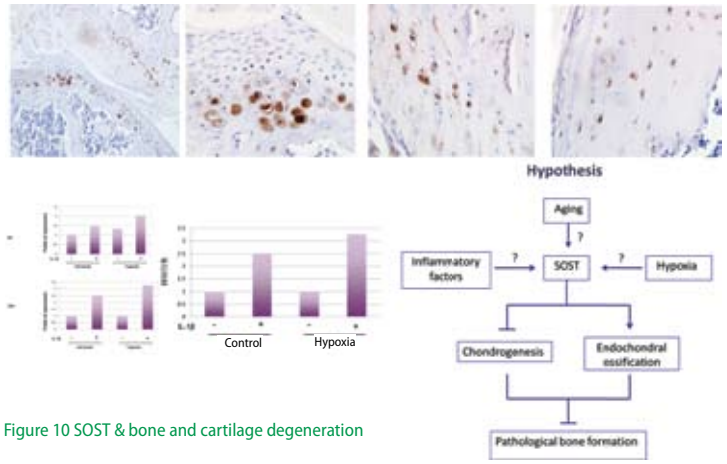

Figure 10 SOST & bone and cartilage degeneration

5. International collaboration

In 2014, we have cooperated with the University of Western Australia and RIKEN to conduct studies about bone metabolism related disease. Right now, we mainly focuses on osteoporosis related researches including treatment measures and relevant molecular mechanisms.

Selected Publications

1.Qin J, Liang H, Shi D, Dai J, Xu Z, Chen D, Chen X, Jiang Q. A panel of microRNAs as a new biomarkers for the detection of deep vein thrombosis. J Thromb Thrombolysis. 2014 Sep 23. [Epub ahead of print]

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4. Shi D, Xu X, Song K, Xu Z, Dai J, Chen D, Jiang Q. Comparison of venous thromboembolism after total hip arthroplasty between ankylosing spondylitis and osteoarthritis. Biomed Res Int. 2014;2014:712895.

5. Sun Y, Jiang Q.Authors' reply. Arthroscopy. 2014 Jul;30(7):780.

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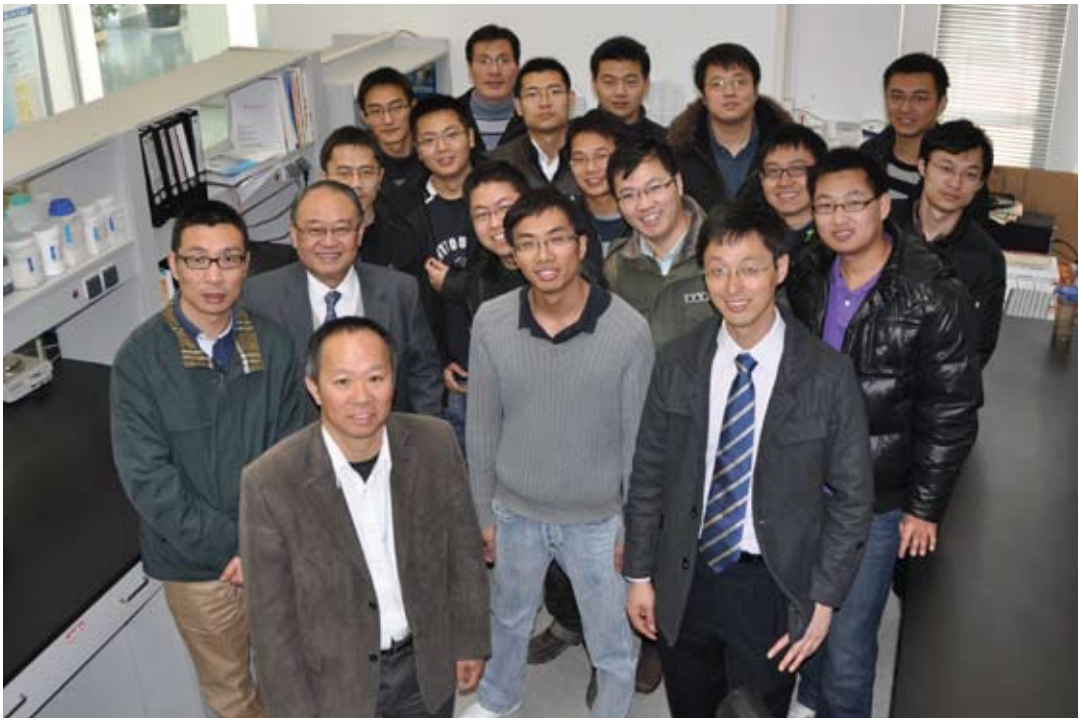
7. Sun Y, Chen D, Xu Z, Shi D, Dai J, Qin J, Jiang Q. Incidence of symptomatic and asymptomatic venous thromboembolism after elective knee arthroscopic surgery: a retrospective study with routinely applied venography. Arthroscopy. 2014 Jul;30(7):818-22.

8. Shi D, Sun W, Xu X, Hao Z, Dai J, Xu Z, Chen D, Teng H, Jiang Q. A replication study for the association of rs726252 in PAPPA2 with developmental dysplasia of the hip in Chinese Han population. Biomed Res Int. 2014;2014:979520. doi: 10.1155/2014/979520. Epub 2014 Feb 3.

9. Yang X, Li M, Chen D, Shi D, Zhou Z, Zhu B, Jiang Q. Diffusion tensor imaging for anatomical and quantitative evaluation of the anterior cruciate ligament and ACL grafts: a preliminary study. J Comput Assist Tomogr. 2014 Jul-Aug;38(4):489-94.

10. Sun Y, Chen D, Xu Z, Shi D, Dai J, Qin J, Qin J, Jiang Q. Deep venous thrombosis after knee arthroscopy: a systematic review and meta-analysis. Arthroscopy. 2014 Mar;30(3):406-12.



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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.

p53 is extremely important for stress response and tumor suppression. The knowledge of its function and associated regulatory mechanisms is invaluable in our understanding of malignant transformation far beyond the molecule itself. Our past and present work mainly focused on the regulation and functionality of p53 using a variety of in vivo mouse models. p53 protein is undetectable in normal tissues. With the newly established BAC transgenic p53 reporter mice, we revealed a previously unrecognized prevailingly high expression pattern of endogenous p53 in the proliferating compartments during mouse development, postnatal growth, tissue homeostasis, regeneration and tumorigenesis (Figure 1). Further, we provided evidence suggesting that this distinct expression pattern is governed by proliferative signals acting on the cis-elements in both the p53 promoter and 3' UTR. Importantly, p53 protein is selectively activated in the proliferating cells and tissues upon stress, suggesting a causal link of p53 basal expression with its function. The posttranslational control of p53 stability is mainly mediated by Mdm2, an E3 ubiquitin ligase. In studying the negative regulation of Mdm2 on p53, we found that Mdm2 deletion in mice led to premature follicle depletion and ovarian failure, indicating its crucial role for the survival of oocytes during folliculogenesis (Figure 2). Our results further suggested that when compared with somatic cells, the tight regulation of p53 by Mdm2 in oocytes resulted in a diminished p53 response upon exposure to the chemotherapeutic drugs.

Tumor microenvironment has been increasingly recognized to play critical roles in tumor progression, maintenance and metastasis. Chronic inflammation is one of the major players mediating the tumor promoting effects of the microenvironment. In probing the role of p53 in counteracting inflammation and suppressing tumor formation in spontaneous tumor models, we found that p53 deficiency accelerated adenoma formation in Apc^{min} mice and p53 activation suppressed adenoma growth and colitis-associated tumor invasion. These and other evidence support a significant role of tumor suppressors in modulating the tumor microenvironment, which in turn have impacts on tumorigenesis.

In addition, we recently aimed to develop 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.

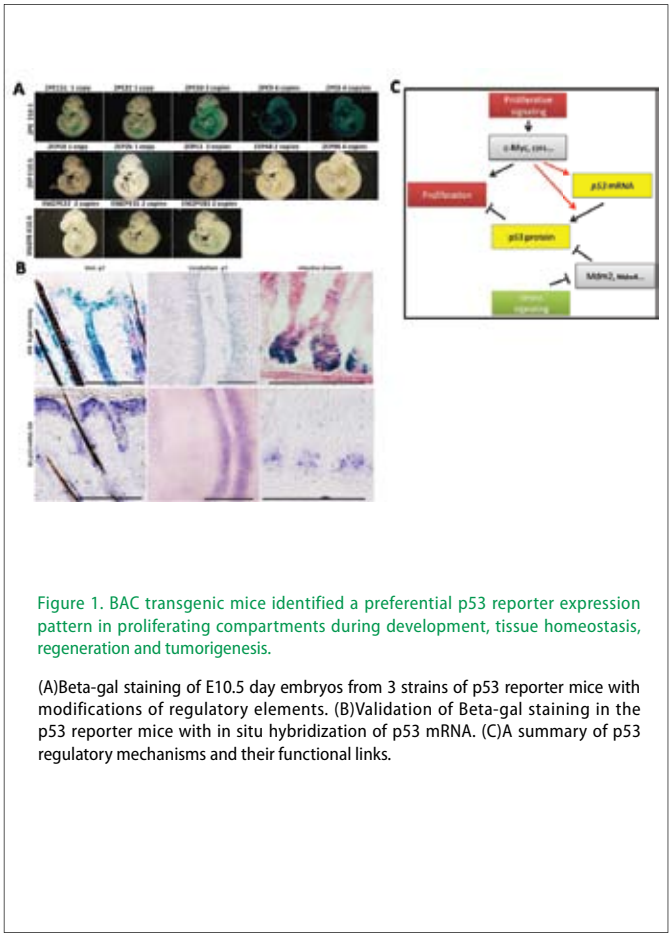

Figure 1. BAC transgenic mice identified a preferential p53 reporter expression pattern in proliferating compartments during development, tissue homeostasis, regeneration and tumorigenesis.

(A)Beta-gal staining of E10.5 day embryos from 3 strains of p53 reporter mice with modifications of regulatory elements. (B)Validation of Beta-gal staining in the p53 reporter mice with in situ hybridization of p53 mRNA. (C)A summary of p53 regulatory mechanisms and their functional links.

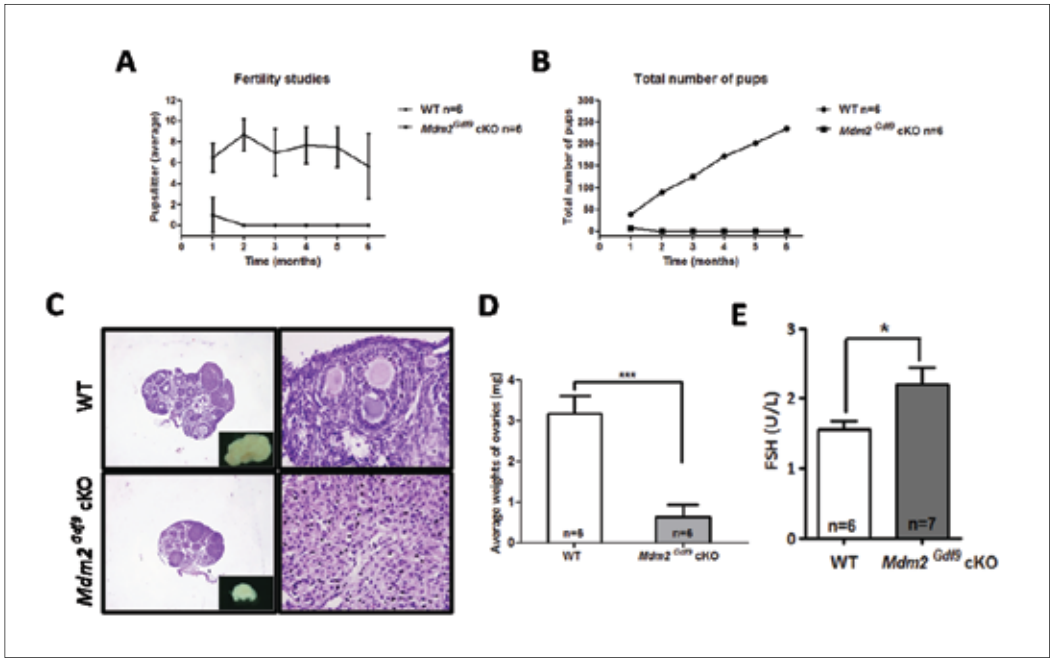

Figure 2. Mdm2 deletion in mice mediated by Gdf9-Cre resulted in female infertility.

(A, B) Fertility studies of Mdm2 cKO females. (A) Average number of pups per litter. (B) The cumulative number of pups over a 6-month period (n=6). (C) Ovaries from 6-week-old Mdm2 cKO mice showed all follicular structures were diminished, with a few corpora lutea remaining. (D) Average weights of ovaries obtained from 6-week-old Mdm2 cKO and WT mice. (E) FSH Levels in sera of young adult (3-4 months old) Mdm2 cKO and WT mice.

Selected publications

1. Wang J, Zhu HH, Chu M, Liu Y, Zhang C, Liu G, Yang X, Yang R, Gao WQ. (2014) Symmetrical and asymmetrical division analysis provides evidence for a hierarchy of prostate epithelial cell lineages. Nat Commun. 5:4758.
2. 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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Our immune system is evolved to defend against invading pathogens as well as other types of exogenous and endogenous insults. On the other hand, the deficiency, or more frequently, the mal-functioning of the immune system contributes to a plethora of disease states. Using innate immune regulation elicited by viral pathogens and tumors as major models, we look to gain more insights regarding the regulation of the immune system. Ultimately, we hope that our bench discoveries can in some way impact management of human diseases.

1. The metabolic 'division' of innate immunity

As one the most fundamental regulatory systems, the cellular energy metabolism is believed to be a pivotal component of innate immune activation (see Fig. 1A as a general example). However, the underlying mechanisms for such cross-talks are poorly understood. Using both cell culture and mouse models, we began to explore this emerging field. In an initial study, we found that viral stimulation can selectively promote glycolytic flux in macrophages (see Fig. 1B for glycolysis introduction). The increased glycolysis is due to virus-induced expression of several glycolytic enzymes. Moreover, glycolytic activation promotes macrophage functions such as cytokine production and phagocytic activity (Fig. 1C, 1D). Though we are still in the process of understanding the link between glycolysis and macrophages' anti-viral activities, this work has demonstrated a cross-talk mechanism linking metabolism and immune function.

In the meantime, we are analyzing other macrophage phenotypes under altered cellular metabolism. In addition, we seek to establish animal models to probe the role of immune cell metabolism in disease states independent of exogenous pathogens. In collaboration with tumor biologists such as Dr. Jun Yan at MARC, we have initiated a direction determining whether metabolic regulation in macrophages also modulates their behaviors in tumor microenvironment. In this regard, both implanted and spontaneous tumor models are under test.

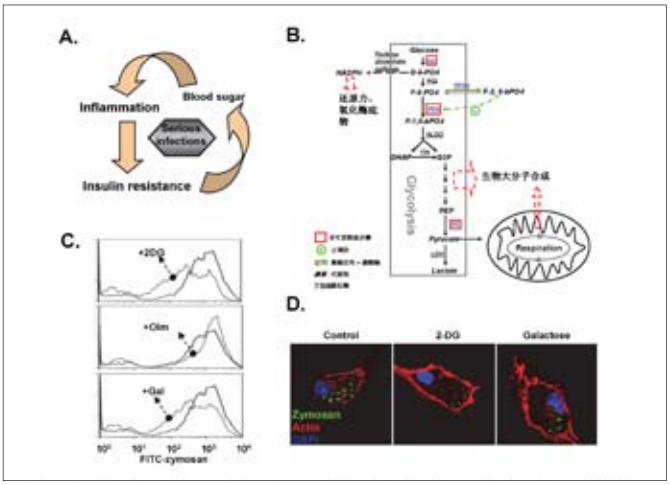

Figure 1: Cellular metabolism regulates immune cell function.

(A) An immunity-centered hypothesis for the adaptive value of inflammation-associated insulin resistance, where the key assumption being up-regulation of blood glucose may help promote immunity against pathogens. (B) Glycolysis and associated pathways. (C) Glycolytic (2-DG, galactose), but not OXPHOS (oligomycin) inhibitors reduced macrophage phagocytosis determined by flow cytometry. (D) Results similar to (C) were observed using con-focal microscopy.

2. Transcriptional and epigenetic regulation of inflammation

Innate immune activation triggers robust transcription of pro-inflammatory cytokines in a cell type-specific manner, representing an excellent system to study the mechanisms whereby the epigenome shapes transcription responses. Using macrophage as a model, we are interested in examining how lineage-specific transcription factors (TFs) of macrophages define the epigenome and how inflammation-associated TFs integrate the epigenome information to impact transcriptional readouts. As both lineage-specific and inflammation-associated TFs function via their corresponding cis-elements, an efficient and convenient method to carry out element-specific perturbation was in demand. In collaboration with Dr. Xingxu Huang at MARC, we recently established a CRISPR-based, highly effective and non-mutational method to specifically interrogate cis-element function in human cells (Fig. 2A, B, C, D). We are in the process of using this novel element-targeting method to investigate mechanisms underlying macrophage lineage development and inflammation.

Additionally, we have taken advantage of several conditional knockout mouse models of epigenetic regulators (in collaboration with Dr. Jun Yan) to probe the function of such regulators in controlling the magnitude and duration of inflammation.

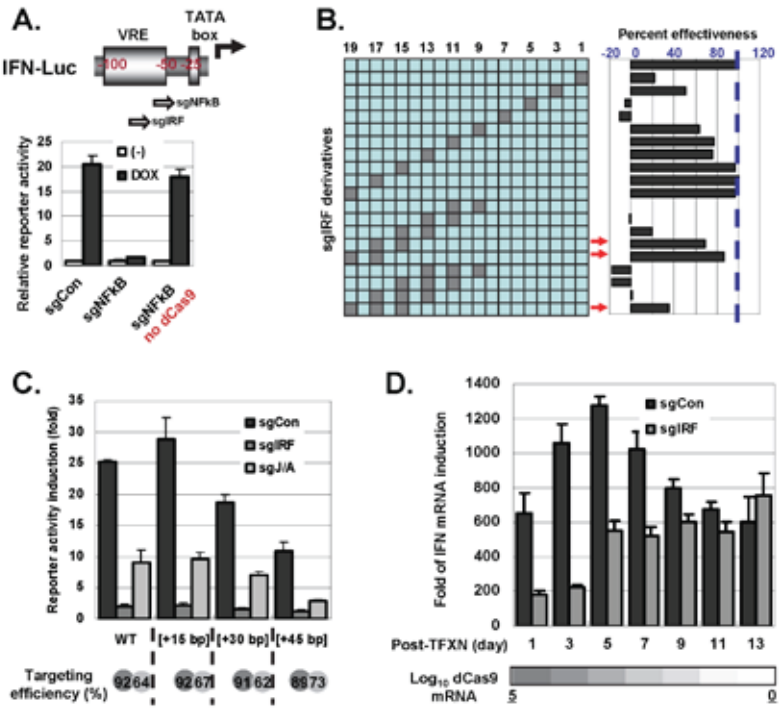

Figure 2: A novel CRISPR-based method to model cis-element loss-of-function.

(A) dCas9/sgRNA complex suppresses the activity of a cis-element in IFNβ-luciferase reporter. (B) Positional mismatch in a sgRNA against the IRF element of IFNβ was performed to determine the specificity of dCas9/sgRNA-mediated element targeting. (C) dCas9/sgRNA is sufficient to inhibit cis-element activity, independent of interference with general transcription. (D) dCas9/sgRNA can target endogenous cis-elements, where the effectiveness correlates to the duration of dCas9 expression.

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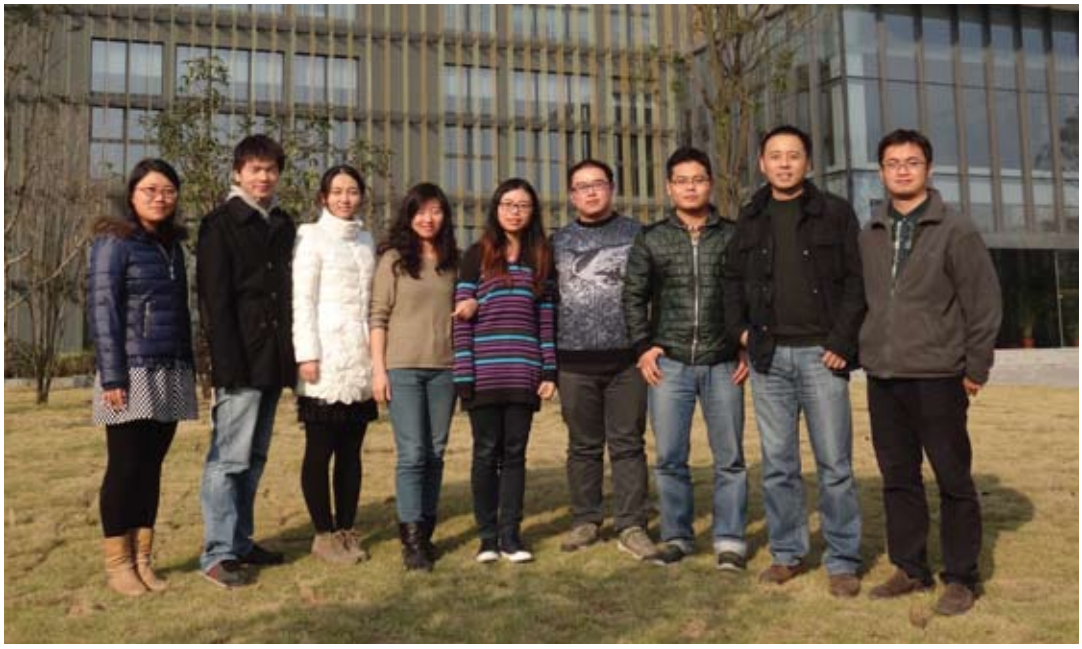

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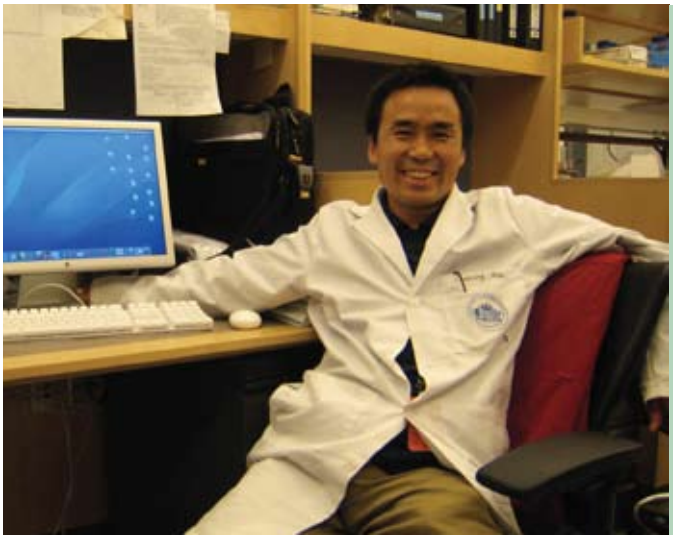

Jinzhong Qin, Ph.D.

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 underlying molecular mechanisms that regulate the pluripotent state is still elusive. Our previous studies demonstrated that L3mbtl2, an mbt family member, is critical for early embryo development as well as pluripotency maintenance in embryonic stem (ES) cells. Deletion of L3mbtl2 results in embryonic lethality with failure of gastrulation and accordingly this correlates with compromised proliferation and abnormal differentiation of L3mbtl2-deficient ES cells. In ESCs, L3mbtl2 establishes an atypical PRC1 complex that includes Oct4, G9A and several components of the E2F6 and NuRD repressor complexes. Accordingly, 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 central goal of our group is to comprehensively establish the role of L3mbtl2-containing atypical PRC1 in stem cells, embryonic development, and cancer and to characterize 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. We have generated different L3mbtl2 mutants (see figures below) and we are currently investigating the role of posttranslational modifications such as SUMOylation in L3mbtl2-mediated maintenance of self-renewal of ES cells.

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.

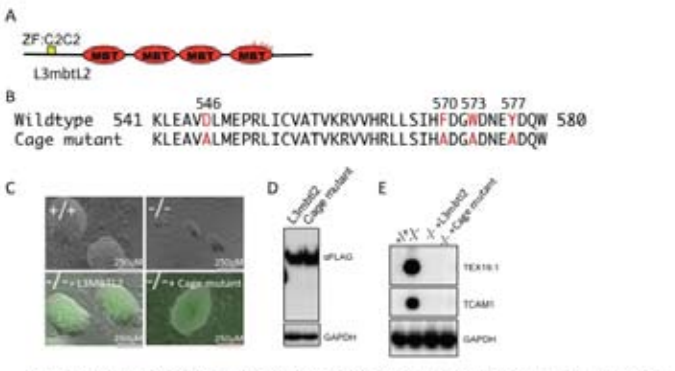

Figure 1. Mutant with four point mutations in the aromatic cage rescued similar to wild-type L3mbtl2. A. Illustration of L3mbtl2 domain organization. B. L3mbtl2 aromatic cage mutant. C. Aromatic cage mutant kept the ability to rescue colony growth. D. Western blot analysis confirmed protein expression of the mutant. E. The mutant was able to silence target gene expression.

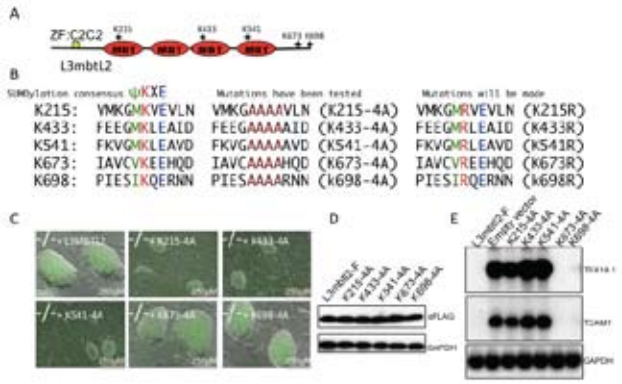

Figure 2. Crosstalk between summoylation and L3mbtl2 in ES cells. A. Cartoon depicting zinc finger and MBT domains in L3mbtl2 (including five consensus SUMOylation sites). B. Generation of L3mbtl2 putative summoylation mutants. C. Ability of these mutants in colony growth rescue. D. Western blot analysis confirmed protein expression of these mutants. E. Northern blot analysis demonstrated the potential of these mutants in target gene expression.

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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 inflammation and development of new clinical immunoassay techniques.

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PPARγ is a major transcription factor that controls a large variety of genes implicated in differentiation of several cell types, regulation of inflammation, maintenance of metabolic homeostasis. The evidence for the use of PPARγ agonists in the treatment of metabolic syndrome indicates that pharmacological activation of PPARγ is the practical therapeutic option for the amendment of metabolic disorders including metabolic inflammation, insulin resistance and tumor etc. Herein, we are focusing on the mechanisms underling the post-translational modifications (phosphorylation, nitrosylation, acetylation) of PPARγ and uncovering the related functions mediated by modified PPARγ in regulating metabolic inflammation and tumor-associated inflammation. We are also interested in the pharmacological ac

The Role of PPARγ-Ser 186 Phosphorylation in Regulation of Insulin Sensitivity and Metabolic Inflammation

We have found a new phosphorylation mode of PPARγ and further explore the physiological and pathological significance of this molecular modification. We have found that PPARγ-Ser186 phosphorylation regulates the production of adipokine and other related factors and reduces the insulin sensitivity, which could be reversed by Metformin and Rosiglitazone. We further discover that PPARγ-Ser186 phosphorylation mediates the polarization and activation of macrophages infiltrated in adipose tissue, hereby leading to the development of metabolic inflammation. Collectively, we think that the PKCα/PPARγ acts as a functional axis that may play an important role in regulating metabolic disorders (Fig.1)

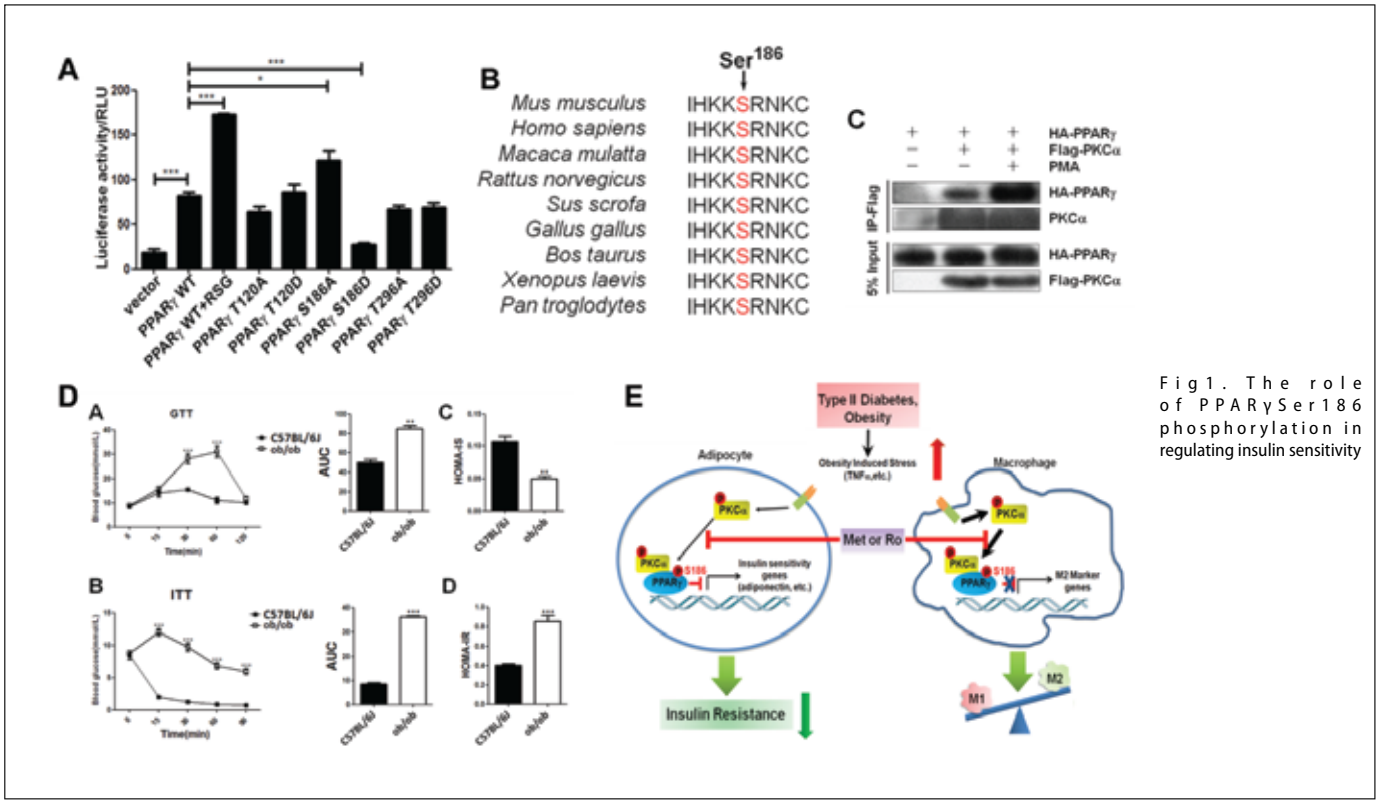

Fig1. The role of PPARγSer186 phosphorylation in regulating insulin sensitivity

The pharmacological actions of certain functional molecules by targeting PPARγ

We had processed the screening of functional ligands of PPARγ by using several chemical libraries. Consequently, we have caught several natural products acting as modulator or ligand of PPARγ that display their efficacy of regulating PPARγ transcriptional activity. Apigenin and two derivatives of Oleanane Triterpenoids are more effective comparing with some TZD class of drugs. Especially, apigenin attenuated high-fat induced inflammation, meanwhile alleviated hepatic, muscular steatosis in obese mice significantly. Apigenin decreases the infiltration of macrophages into adipose tissue and restore the macrophage M1/M2 status. Apigenin regulated the phenotype of macrophages via binding to and activating PPARγ and then inhibits NF-κB /p65 translocating into nucleus (Fig 2).

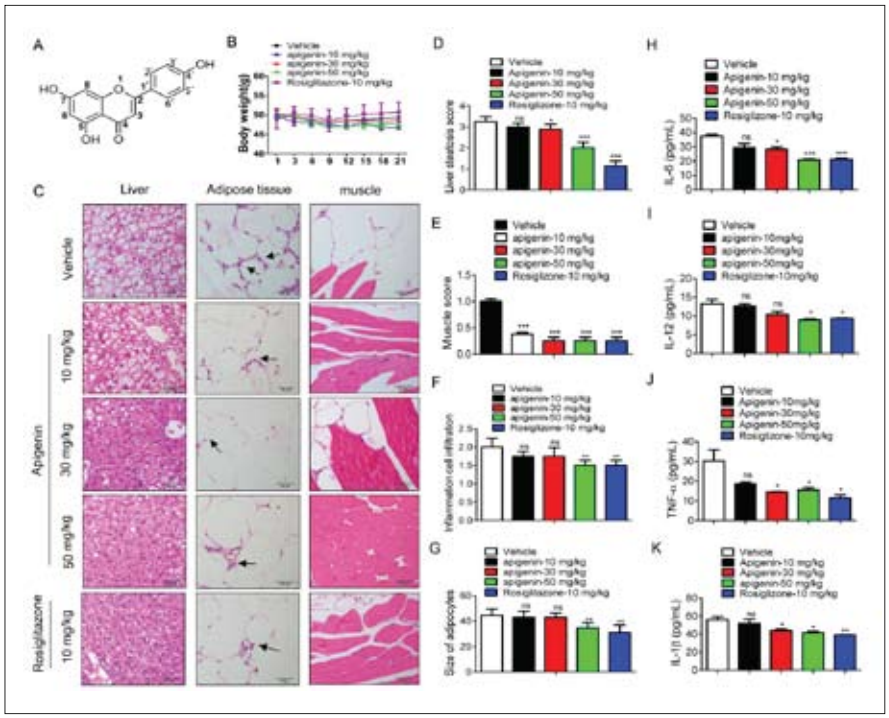

Fig 2. Apigenin attenuated metabolic inflammation

The novel technique for detection of nucleic acid

We build up a new system for detecting nucleic acid based on immunoassay. mRNA of inspecting gene is treated as the target without amplification. This new technique can quantify the viral load accurately, and overcome the disadvantages of PCR, LAMP, etc, which rely on the amplification of the target sequence that causes defects with high positive rate.

This new technology of nucleic acid immunodetection, according to the basic principles of ELISA, is used to detect DNA/RNA in origin. Now we have completed the sensitive detection of the short RNA oligomers synthetically, the long RNA transcribed in vitro, and the total RNA of tumor cells or clinical samples. Comparing the relative detection method, QiaGen HC2, not only the sensitivity but also the cost of this method reduces the one over ten (Fig.3).

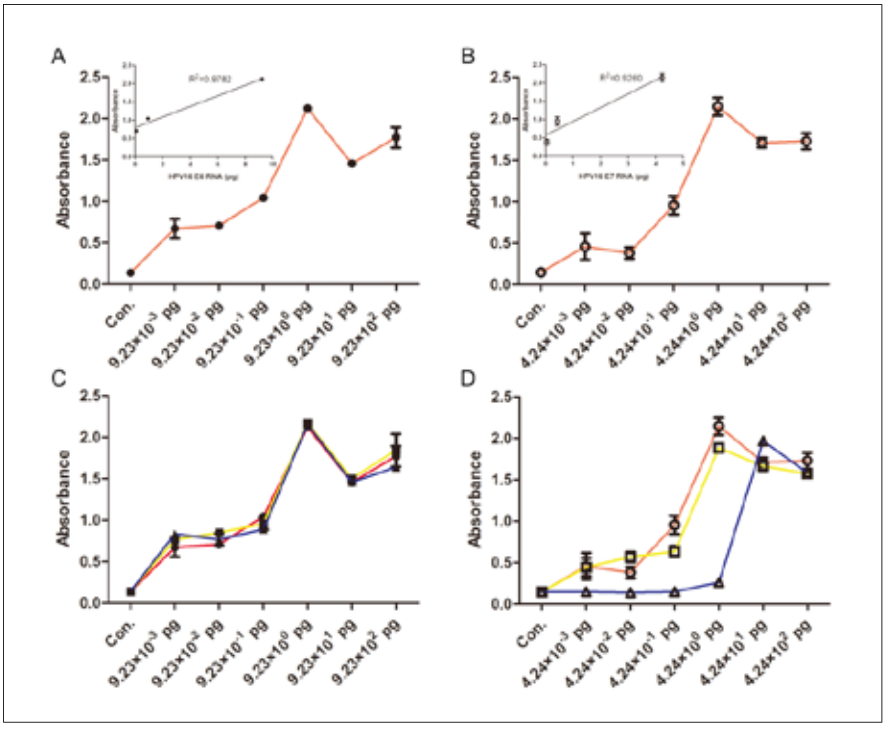

Fig.3 Sensitivity analysis of this immunoassay to detect in vitro transcription products of HPV16 E6(A) and E7 (B), and different probe systems in detection of HPV16 E6 (C) and E7 (D) RNA

Selected publications: (*corresponding author)

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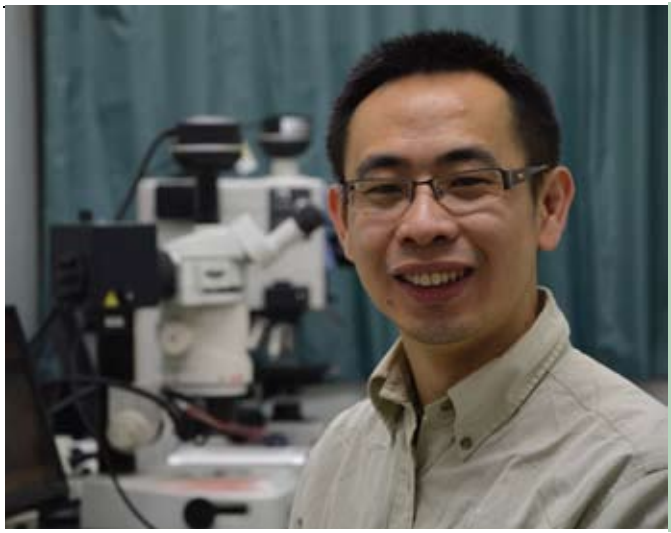

Jun Yan, Ph.D.

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 in 2008 moved to Columbia University in New York as Associate Research Scientist. In late 2009, Jun joined the Model Animal Research Center of Nanjing University as Associate Professor and Principal Investigator.

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Cancer Epigenetics

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, which have been dissected by whole-genome sequencing. Notably, besides frequent altered genes, such as TP53, 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.

Recent progresses in the lab:

As a small population in solid tumor, cancer stem cells are resistant to conventional chemotherapeutic agents. 1) We are the first to identify that an frequently amplified/overexpressed oncogene SRC-3 in bladder cancer directly interacts and coactivates hypoxia-inducible factor 1α (HIF1α), eventually inducing Warburg effect in cancer cells (Fig. 1) for the maintenance of CSC. Our data indicate that blocking of glycolytic pathway can inhibit the tumorigenecity of SRC-3 overexpressing bladder cancer cells (Zhao W, et al. 2014). We are now characterizing the epigenetic alterations involved in CSC maintenance under hypoxic microenvironment. (2) Histone H3K27 is an important site for post-translational modification. We found that oncoprotein EZH2, a histone methyl-transferase for H3K27, positively regulates cancer cell metastasis and cancer cell stemness, through repressing TIMP-3 (Hou Z, et al. 2013) and tumor suppressor miRNAs (submitted). Consistently, we found that EZH2 expression predicts the response for cisplatin chemotherapy in lung cancer patients(Xu C, et al, 2014), further supporting that EZH2 is involved in cancer recurrence. Interestingly, we identified that a novel long noncoding RNA--Sox2ot, is frequently amplified in lung cancer. EZH2 functions as a downstream target of Sox2ot for cancer cell proliferation through G2/M phase (Hou Z, et al. 2014; Fig. 2). Based on our data above, we tested the idea whether EZH2 is a potential therapeutic target. By using Chinese herbal extract, we proved that honokiol suppresses EZH2 expression/activity, and significantly inhibits cancer cell stemness and invasion (submitted). Taken together, EZH2 plays an essential role for cancer cell aggressiveness and is a promising druggable target for cancer. (3) Based on recent exome sequencing data, we identified a group of epigenetic modifiers involved in carcinogenesis. We are striving to establish and characterize these novel mouse cancer models, which recapitulat human cancer development (Fig. 3). These mouse models will provide excellent platforms for pre-clinical study in near future.

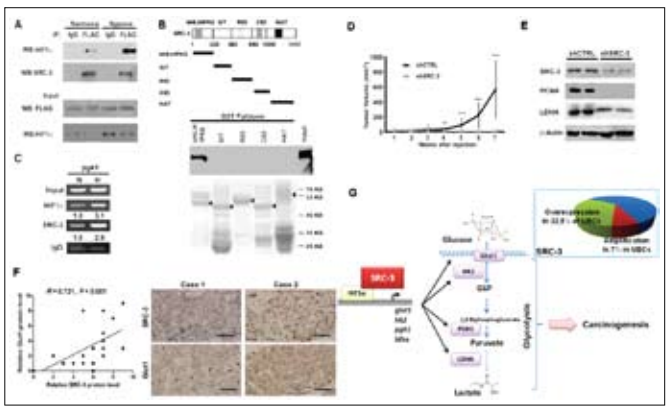

Fig 1. SRC-3 promotes Warburg effect in cancer cells through interaction with HIF1α.

A) CoIP assay.

B) Pull-down assay.

C) ChIP assay.

D) Xenograft assay.

E) Glycolytic protein LDHA is reduced in SRC-3 knockdown tumors.

F) Clinical relevance.

G) Cartoon for SRC-3 induced glycolysis in urinary bladder cancer(UBC) cells.

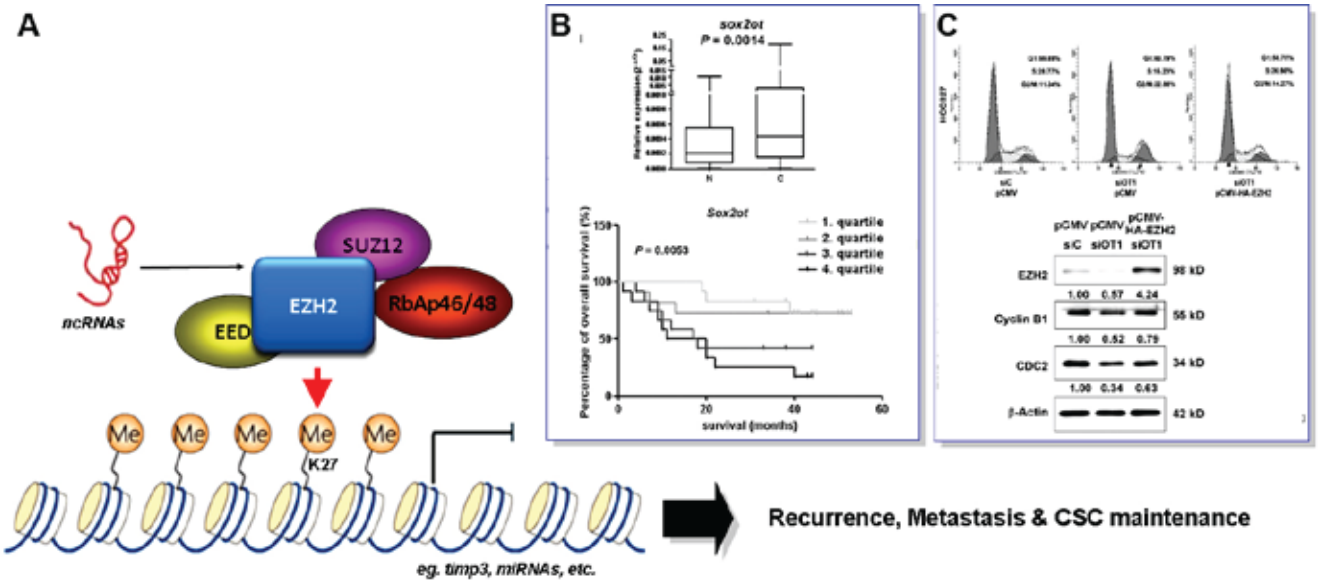

Fig 2. The essential role of EZH2 in cancer.

A) EZH2 network in cancer cells.

B) Overexpression of Sox2ot predicts clinical outcome.

C) EZH2 overexpression partially rescues Sox2ot depletion-induced G2/M arrest.

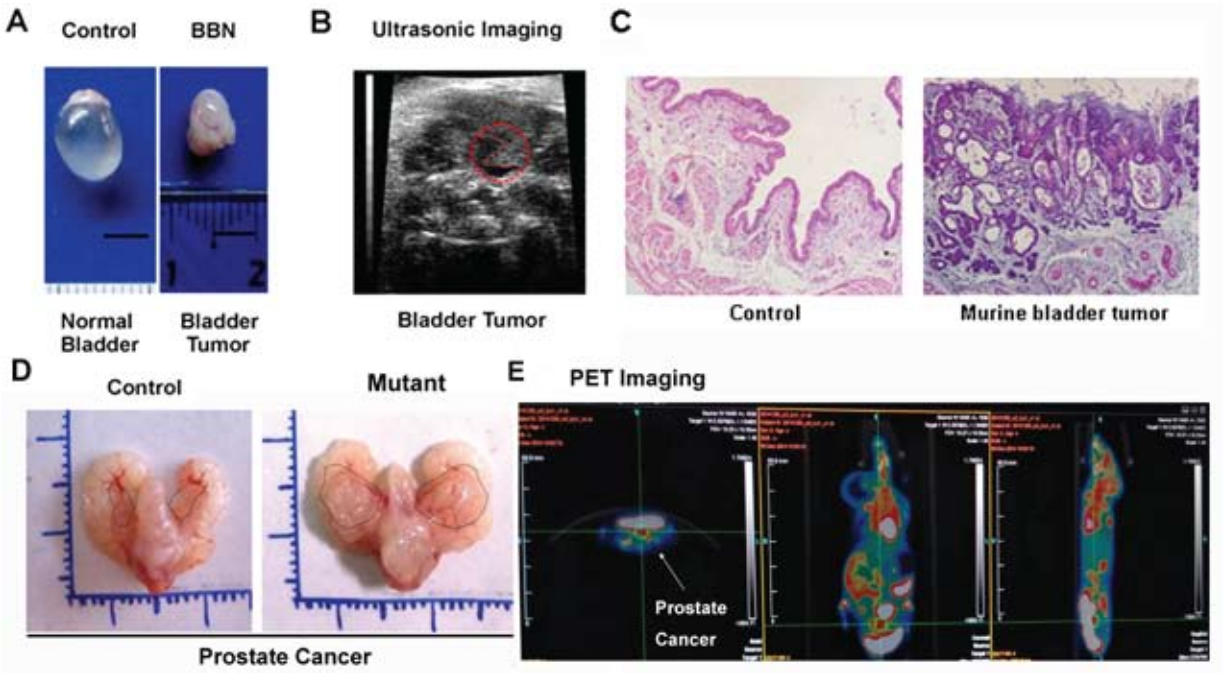

Fig 3. Bladder cancer mouse model.

A) Photograph of mouse bladder.

B) In vivo US imaging.

C) Histologic analysis of normal bladder and bladder tumor.

D) Photograph of mouse urogenital system showing prostate cancer lesion, labelled by dashline circle.

E) 18FDG-PET imaging of mutant mouse with prostate cancer by transverse, coronal and sagittal sections. Arrow indicates prostate cancer.

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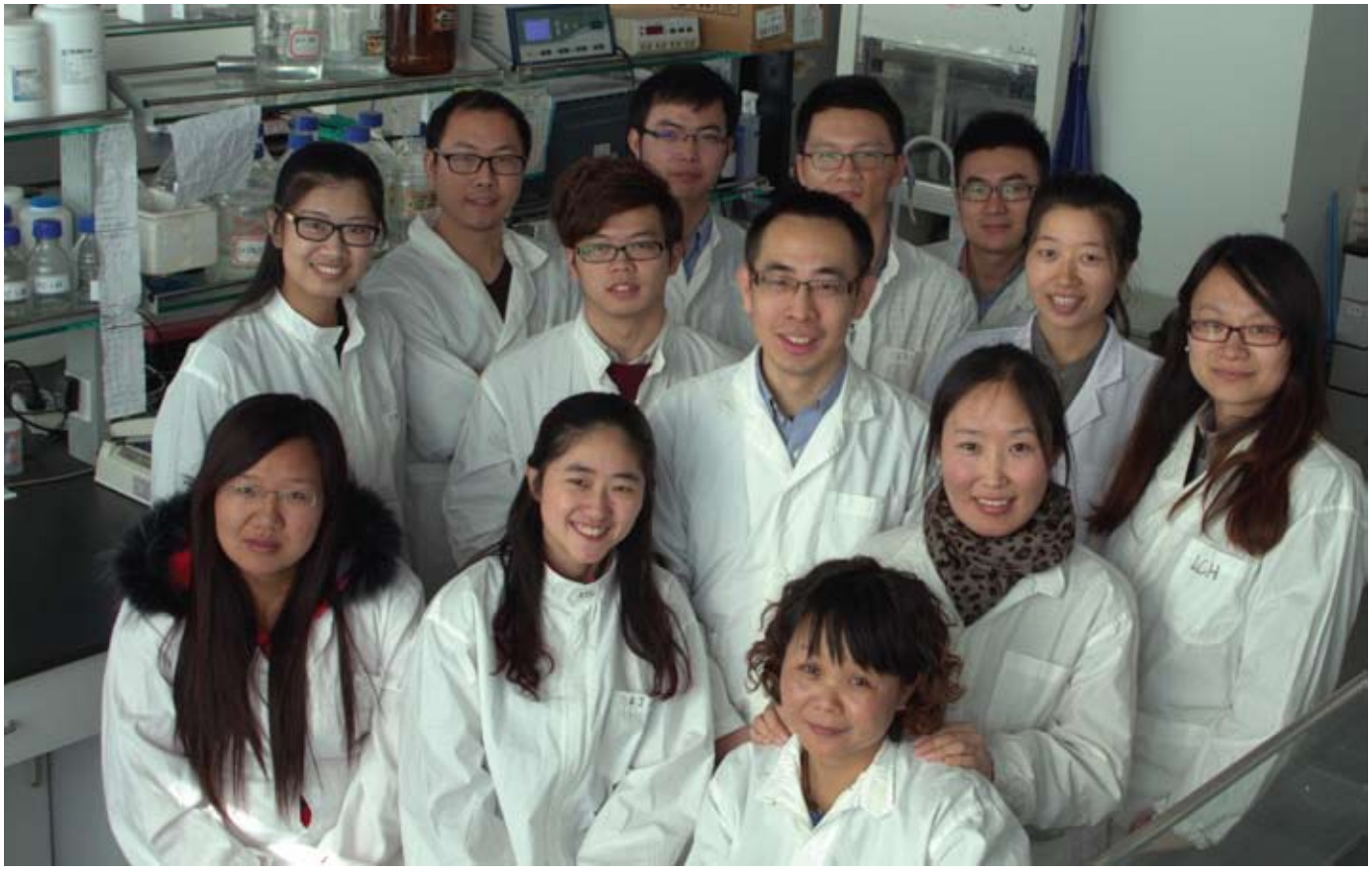

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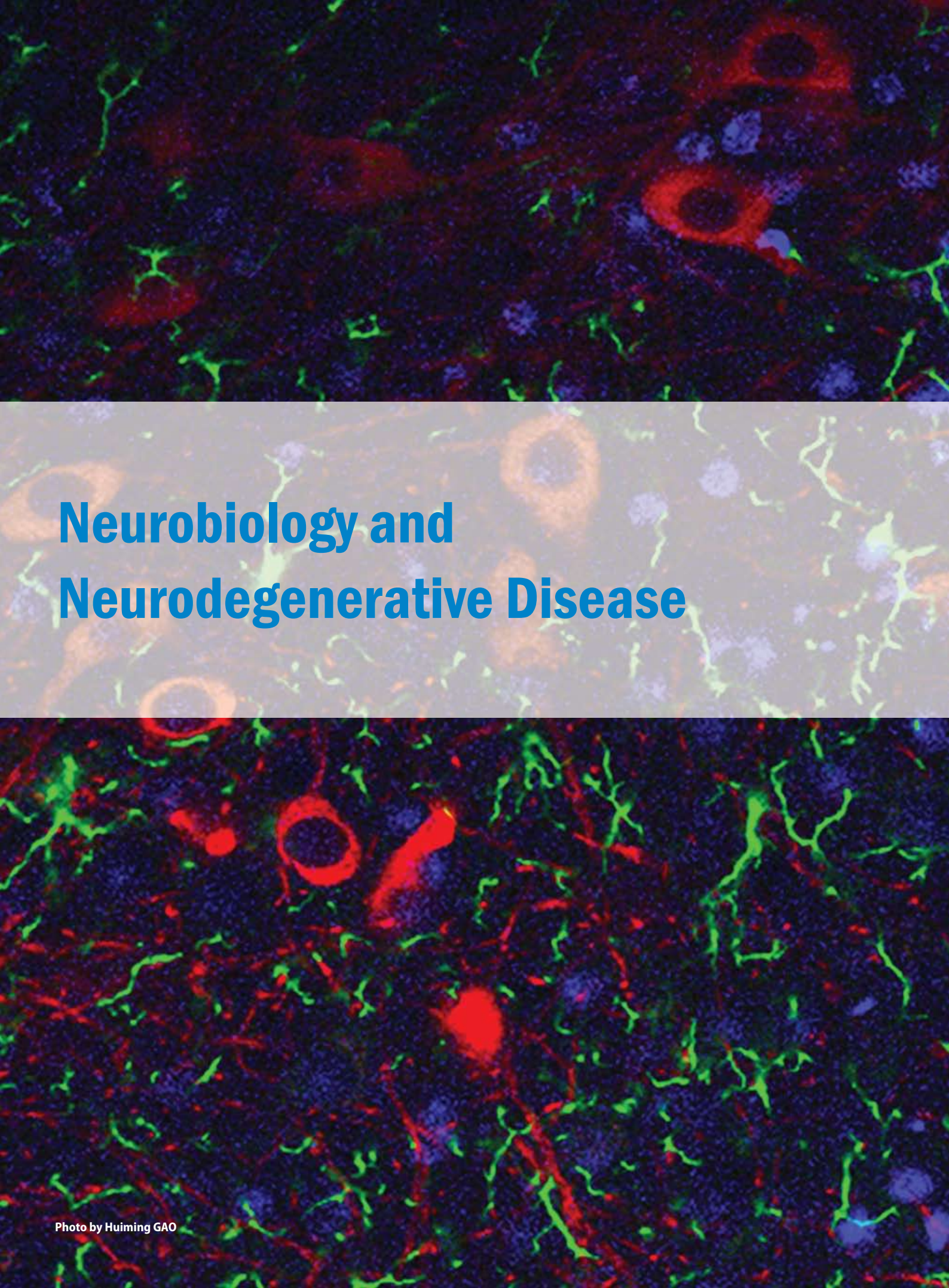

Neurobiology and Neurodegenerative Disease

Photo by Huiming GAO

Guiquan Chen, Ph.D.

Guiquan obtained his PhD in Neuroscience at the University of Edinburgh in Scotland and then conducted his postdoctoral research at Harvard Medical School in Boston. He joined the MARC of Nanjing University as a Principle Investigator in 2011. His long-term research goal is to understand molecular mechanisms by which the γ–secretase complex affects neuronal survival. His lab uses a combination of mouse genetics, molecular biology, cellular and behavioral neuroscience techniques to address this question. Elucidation of molecular mechanisms for age-related neurodegeneration may help identify novel therapeutic targets for the prevention and the treatment of neurodegenerative diseases.

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Molecular mechanisms for neurodegeneration

Alzheimer's disease (AD) is the most common form of dementia and is associated with age-dependent neuron loss. However, mechanisms underlying neurodegeneration are still poorly understood. Neurogenesis is defined as a process of generating functional neurons from neural stem cells in the brain of mammals. The pool of neural progenitor cells (NPCs) and the proliferative potential of NPCs are markedly diminished in neurodegenerative diseases.

Several important studies have recently demonstrated that the microRNA network plays a critical role in AD (Hébert et al., 2008, 2009, 2010). We generated a viable mature neuron-specific Dicer cKO mouse. We found that 6-month Dicer cKO displayed remarkable cortical atrophy. We examined NeuN, a marker for mature neurons. Immunohistochemistry (IHC) showed massive reductions on NeuN immuno-reactivity in the cortex of cKO mice at 6 months (Fig. 1A). Western analysis also showed significant reduction on NeuN levels in cKO mice at 6 months (Fig. 1B). We used BrdU to label proliferating NPCs. We found that BrdU+ cells exhibited no detectable difference in the SGZ of control and Dicer cKO (Fig. 1C) at 2.5 months. At 6 months, BrdU+ cells were hardly seen in the SGZ of Dicer cKO (Fig. 1C). The average number of BrdU+ cells showed a significant genotype effect ($p<0.01$) at 6 months, suggesting that the proliferating ability of NPCs is impaired.

Neuroinflammation can cause impairment of adult neurogenesis. We studied neuroinflammatory responses by examining GFAP, a marker for astrocytes. We found that immuno-reactivity of GFAP was increased in the brain of Dicer cKO at 2.5 months (Fig. 2A) but tremendously increased at 6 months (Fig. 2B), indicating severe neuroinflammation (Fig. 2C). To study synaptic structure in cKO mice, we examined pre-synaptic marker, SVP38 and post-synaptic marker, PSD95. We found no changes on immuno-reactivity of SVP38 in Dicer cKOs at 2.5 months but dramatic reductions at 6 months (Fig. 3A). Western analysis confirmed changes on SVP38 (Fig. 3B). PSD95 levels were also decreased at 6 months (Fig. 3B), indicating age-dependent synaptic degeneration.

Current focus of the lab is using various mouse models to study molecular mechanisms responsible for neurodegeneration. A big effort has also been made to investigate whether neuron death could be stopped or prevented using both pharmacological and genetic methods.

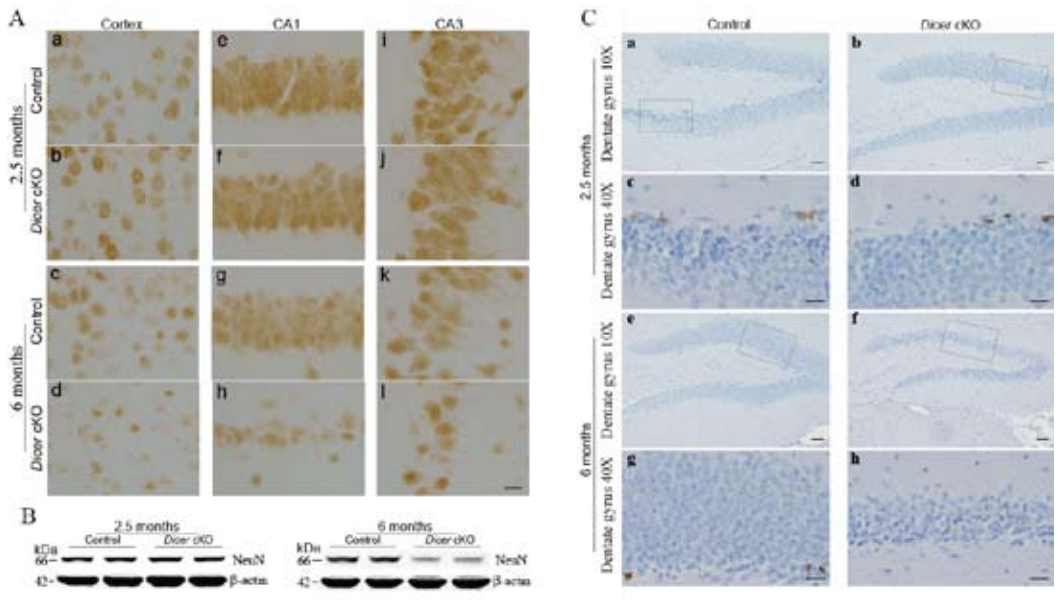

Figure 1. Age-related neurodegeneration and neurogenesis impairment in Dicer conditional knockout (cKO) mice.

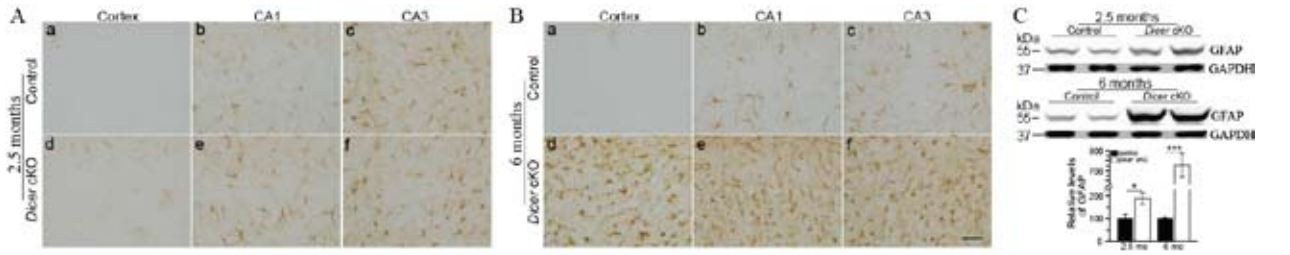

Figure 2. Progressive neuroinflammation in Dicer cKO mice.

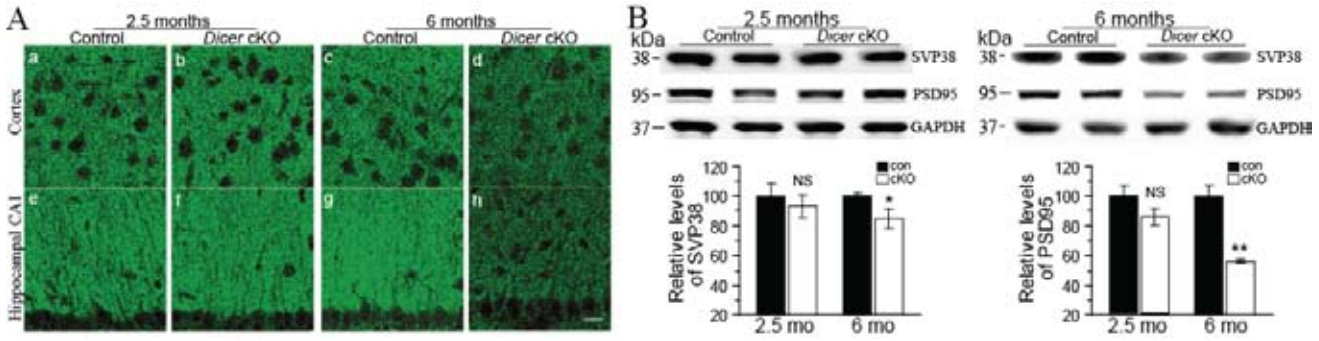

Figure 3. Age-dependent synaptic degeneration in Dicer cKO mice.

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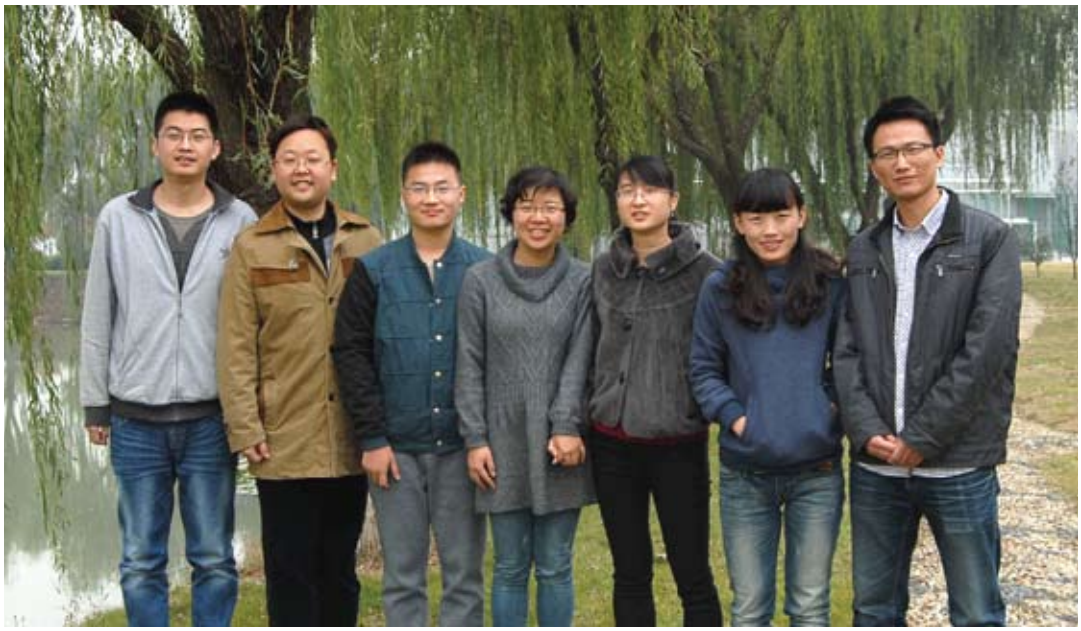

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Neuroinflammation and neurodegeneration

Chronic, irreversible degeneration of neurons in the brain causes progressive memory loss in Alzheimer's disease (AD) and movement impairment (e.g. tremor and rigidity) in Parkinson's disease (PD). At present, no drug can stop such neurodegeneration. Importantly, what drives the decades-long progression of neurodegeneration in neurodegenerative diseases remains unknown. Many previous studies including ours have revealed significant contribution of brain inflammation (neuroinflammation) to the development of 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.

Neuroinflammation is a self-defensive attempt by the brain to remove harmful stimuli and to initiate the healing process. The neuroinflammatory response must be tightly regulated and actively terminated to prevent unnecessary tissue destruction. Persistent injurious stimuli (e.g., toxins, pathogens, and autoimmunogens) and failed resolution of acute neuroinflammation can flip a protective immune response to chronic destruction to brain tissues. As a potent anti-inflammatory cytokine, interleukin-10 (IL-10) plays an important role in resolving inflammation and maintaining immune homeostasis in various tissues including the brain. However, the precise mechanism orchestrating the resolution of acute neuroinflammation and mechanisms governing IL-10 induction remain largely undetermined. We recently found that IL-10 knockout mice were more sensitive to PD-producing neurotoxin MPTP than wildtype mice, suggesting an important role of IL-10-mediated neuroinflammation in neurodegenerative disease. We next investigate regulatory mechanism by which early-released pro-inflammatory factors from microglia influence later IL-10 production. We identified negative regulation of IL-10 induction by earlier-released TNF-α and PGE2 from LPS-elicited microglia. Further studies showed that negative regulation of IL-10 production by TNF-α is mediated by PGE2. Mechanistic studies indicated that EP2 receptor-mediating G protein-independent β-arrestin signalings are responsible for PGE2-induced suppression of IL-10 production.

Our studies have identified a negative regulatory role of PGE2 on IL-10 induction in brain microglia and demonstrated that such negative regulation is mediated by EP2-β-arrestin-dependent pathway, thereby elucidating a novel mechanistic basis for neuroinflammation resolution by PGE2 and IL-10. Our findings suggest that pharmacological regulation of neuroinflammation resolution through targeting EP2-β-arrestin-dependent signaling cascade may mitigate neuroinflammation-mediated neurodegeneration and may become a promising therapeutic strategy for neurodegenerative diseases. 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.

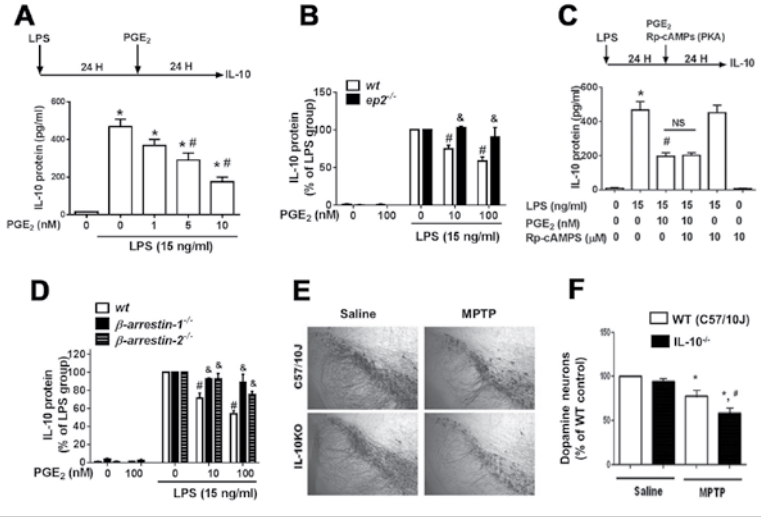

Figure 1. PGE2 inhibits IL-10 production via EP2-mediated β-arrestin signaling in neuroinflammatory condition.

(A) PGE2 negatively regulates LPS-induced IL-10 production in a dose-dependent manner. (B) PGE2-elicited suppression of IL-10 induction was eliminated by genetic disruption of the PGE2 receptor EP2. (C) Inhibition of cAMP-dependent signal transduction failed to affect PGE2-mediated inhibition of IL-10 production, suggesting that a G protein-independent pathway was involved. (D) Deficiency in β-arrestin-1 or β-arrestin-2 abolished PGE2-elicited suppression of IL-10 production. (E, F) IL-10 knockout mice were more sensitive to PD-producing neurotoxin MPTP than wildtype mice, as shown by more neuronal loss in the substantia nigra of MPTP-injected IL-10 knockout mice than wildtype mice.

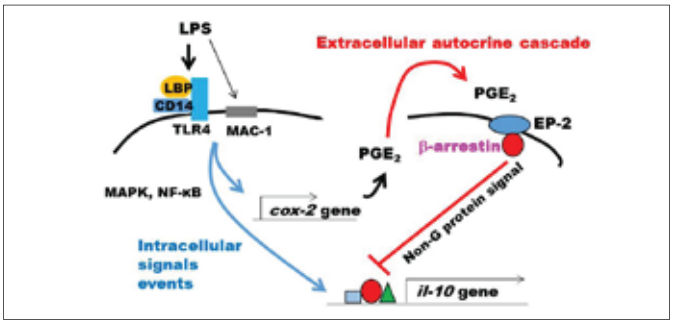

Figure 2. Regulatory mechanism of early pro-inflammatory cascade on IL-10 expression in the neuroinflammatory condition.

In time frame of neuroinflammatory cascade, IL-10 expression is later than most proinflammatory factors such as TNF-α, COX-2, and PGE2. Direct administration of pro-inflammatory factors failed to initiate IL-10 production in the absence of LPS in microglia. Thus, it is plausible that IL-10 induction was dependent on intrinsic signal events such as MAPK and NF-κB pathways, but not in need of early-released pro-inflammatory factors. Conversely, deficiency in cox-2 gene significantly decreased PGE2 production but increased IL-10 production. PGE2 inhibited LPS-induced IL-10 production via EP2-mediating G protein-independent β-arrestin signaling pathway.

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1. Chu CH, Chen SH, Wang Q, Langenbach R, Li H, Zeldin D, Chen SL, Wang S, Gao H-M*, Lu RB*, Hong JS (2014) PGE2 Inhibits IL-10 Production via EP2-Mediated β-Arrestin Signaling in Neuroinflammatory Condition. Molecular Neurobiology. 2014 Sep 14. [Epub ahead of print]
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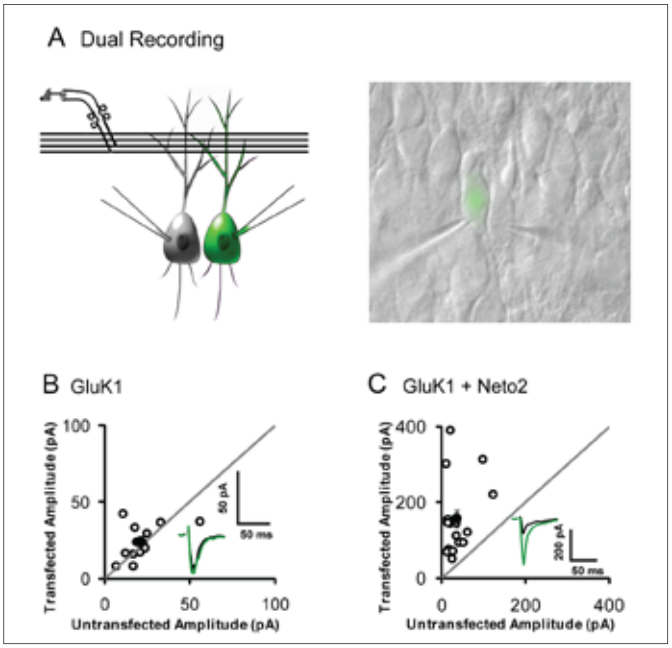
The Fundamental Mechanisms of Neural Plasticity

The central neural system (CNS) is a complex network, in which the appropriate functions depend on the information exchange among neurons through a specified structure named synapse. The neurotransmitters released from presynaptic neurons bind to postsynaptic receptors, enhancing or inhibiting postsynaptic activity. Meanwhile, postsynaptic neurons also release certain regulatory information and modulate presynaptic activity, allowing the feedback to occur. Many of high neuronal functions result from the changes of neurotransmission, or so called plasticity. Dysfunction of synaptic plasticity is the major causes of neurodegeneration 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 determined by 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.



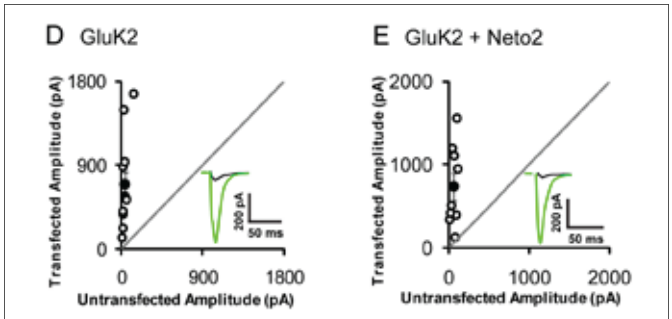


Figure 1. Synaptic response mediated by GluK1 and GluK2 expressed in CA1 neurons. A.Dual recording. Left: a carton model of dual recording system. Simutaneous recording was made on one green experimental neuron and an adjacent control neuron. A stimulating electrode was used to generate common input 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.

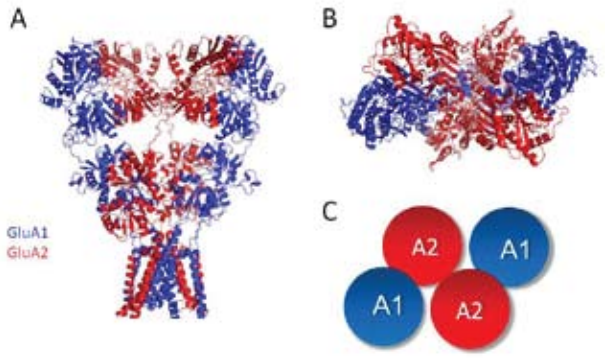


Figure 2. the Model of Heteromeric AMPA receptors. A non-reducing western assay was designed to examine the assembly and stoichiometry of GluA1/A2 heteromeric AMPA receptors. The conclusion was depicted with following models. A. The structural model of GluA1/A2. In the model the 2 GluA1s and 2 GluA2s form a functional assembly of AMPA receptor. B. Top-view of GluA1/A2. C. The sketch model of GluA1/A2 showing the channels are in 1-2-1-2 architecture but not 2-1-2-1 assembly.

Selected publications

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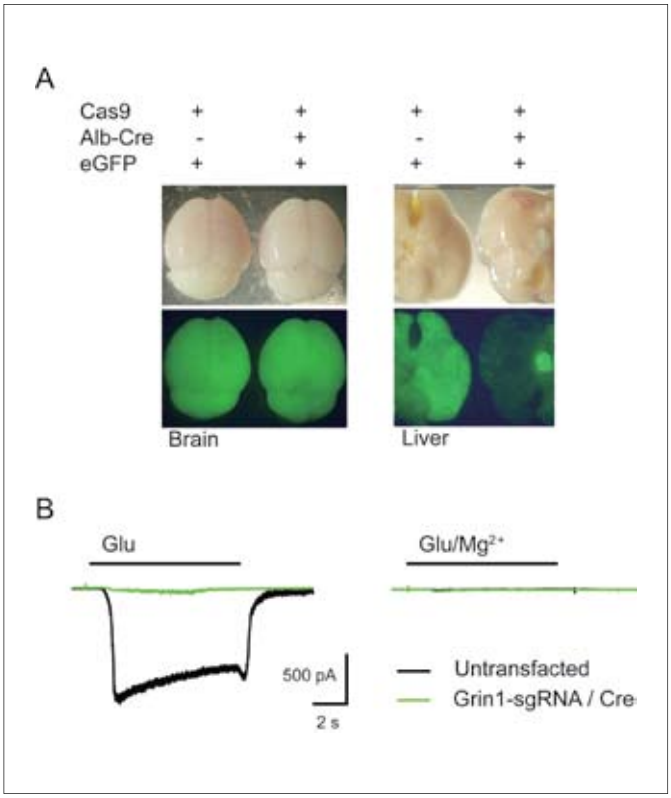

Figure 3. Characterization of a Cas9 transgenic mouse.

A. Characterization of Cas9 efficiency in liver. GFP-sgRNA-Cas9 mice was crossed with eGFP transgenic mice, and then was crossed with Abl-Cre line, where Cas9 will be induced by the Cre recombinase in liver cells. The GFP expression was not different in brain. The GFP expression was significantly reduced in liver. B. Characterization of Cas9 efficiency in neuron cells. In primary culture of cerebellar granule neurons, expression of Cre recombinase and Grin1-sgRNAs removed the NMDA receptor mediated glutamate currents. Right, the glutamate currents was diminished by the transfection of Cre recombinase and Grin1-sgRNAs. Administration of Mg2+ blocked the glutamate currents, indicating the currents was mediated by NMDA receptors.

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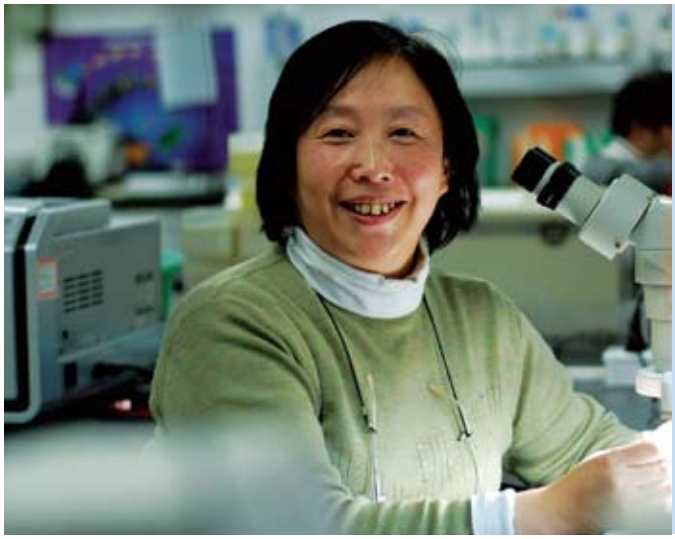

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Circadian rhythms are endogenous rhythms in physiology or behavior with a period length near 24 hours in all genetically studied organisms. In most cases, these rhythms are generated by endogenous processes referred to as circadian oscillators. These 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 interlocking 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, cell cycle progression, feeding behaviour etc. (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.

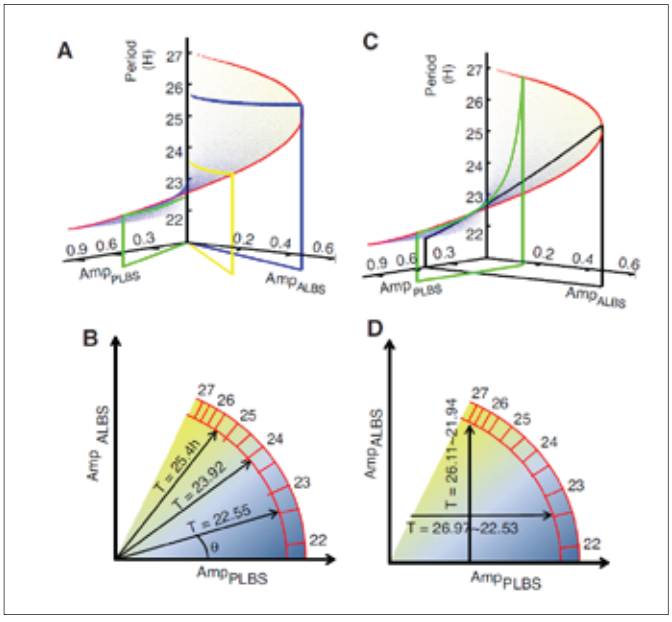

Figure 1 An intensity ratio of interlocking loops regulates circadian period length

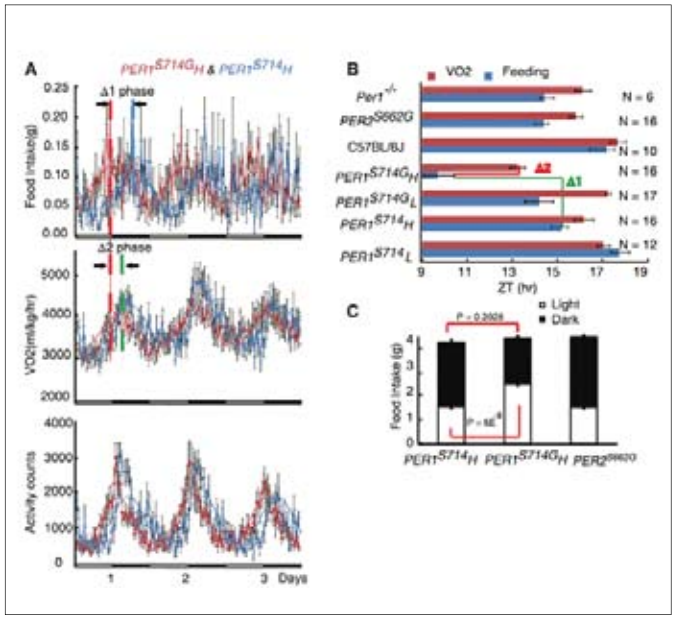

Figure 2 PER1 and PER2 regulates feeding and Sleep behavior separately

Selected publications

1. Jie Yan, Guangsen Shi, Zhihui Zhang, Xi Wu, Zhiwei Liu, Lijuan Xing, Zhipeng Qu, Zhen Dong, Ling Yang and Ying Xu (2014) An intensity ratio of interlocking loops regulates circadian period length. Nucleic Acid Research, 42(16):10278-87.
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3. Xi Wu, Binbin Wang, Zhen Dong, Sirui Zhou, Zhiwei Liu, Guangsen Shi, Yunxia Cao, Ying Xu (2013)A NANOS3 mutation linked to protein degradation causes premature ovarian insufficiency. Cell Death & Disease, 4:e825.
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7. Weiwei Tao, Siyu, Guangsen Shi, Jinhu, Ying Xu and Chang Liu (2011) SWItch/Sucrose NonFermentable (SWI/SNF) Complex Subunit BAF60a Integrates Hepatic Circadian Clock and Energy Metabolism. Hepatology, 54(4):1410-20.
8. Xi Wu, Zhiwei Liu, Guangsen Shi, Lijuan Xing, Xiaohan Wang , Xiwen Gu , hipeng Qu Zhen Dong, Jing Xiong, Xiang Gao, Chenyu Zhang, &Ying Xu (2011) The Circadian Clock Influences Heart Performance. Journal of Biological Rhythms, 26 (5): 402-411.
9. Xiaohan Wang, Xiang Gao & Ying Xu 2011 MAGED1: Molecular Insights and Clinical Implications. Annals of Medicine, 43:347-355.
10. Xiaohan Wang, Jing Tang, Lijuan Xing, Guangsen Shi, Haibin Ruan, Xiwen Gu, Zhiwei Liu, Xi Wu, Xiang Gao and Ying Xu (2010) Interaction of MAGED1 with nuclear receptors affects circadian clock function. The EMBO Journal 29:1389-1400.

Group members

Principal investigator

Ying Xu

Graduate Students

Zhen Dong
Zhipeng Qu
Yang An
Pancheng Xie
Zhihui Zhang
Dongchuan Liu

Former graduate students

Xiwen Gu
Xiaohan Wang
Xi Wu
Zhiwei Liu
Lijun Xing
Guangsen Shi

National Resource Center For Mutant Mice (NRCMM)

NRCMM are recognized as a leading mammalian genetics research center focusing on mouse genetics research and a unique resource for the scientific community NRCMM have developed advanced and reliable platform of mouse genome modification such as transgene, gene knock-out and knock-in and ENU chemical mutagenesis, etc.

NRCMM is now harboring 1500 mouse strains, we have distributed ~150,000 genetically engineered mice including cardiovascular diseases, tumor, metabolic diseases, immunodeficiency and neurodegenerating diseases models to research institutes and laboratories. We also help import and export mouse strains in China.

NRCMM established good relationship and long-term cooperation with Eli Lilly, GSK, Pfizer, UC Davis and the Beatson Institute for Cancer Research, the University of Western Australia, MRC, Schepens Eye Research Institute, Harvard Medical School.

- The **only** national resource center for mutant mice authorized by the Ministry of Science and Technology of China
- The **first** research center certificated by AAALAC International Institution and keep the **longest** certification so far in China
- 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

HISTORY 2001 2006 2009 2010 2011 2012 2013 2014

2001 Foundation
2006 AAALAC International
2009 AAALAC International
2010 National resource center for mutant mice
2011 IMPC
2012 AAALAC International
2013 CAS9
2014 New facilities to enable
:

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

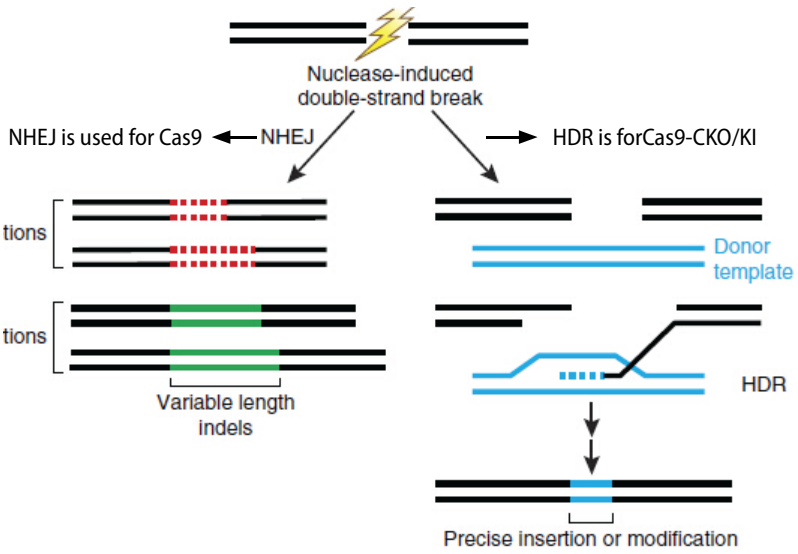
AAALAC International
NRCMM was certificated by AAALAC International with full accreditation for the third time

New facilities to initiate
NRCMM enabled building of 30000 square meters with 60000 mouse cage, in order to better serve the scientific research and experiment

1.2015 Highlights

The CRISPR-Cas9 method provides an efficient and facile strategy to create loss-of-function gene mutations which are the 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) Shortly cycle: skip ES cell targeted step,4-6 months;

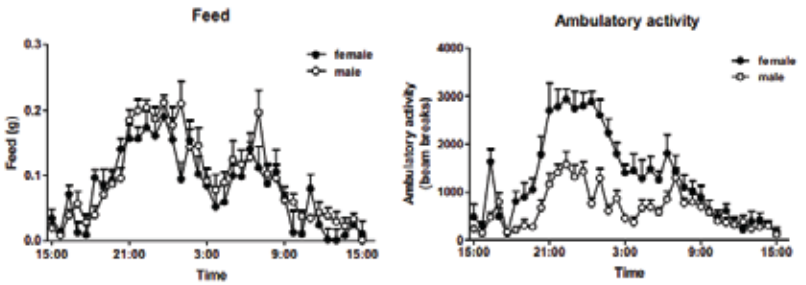
2) Simple: one step nuclear micro-injection;

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

4) No species limited;

Cooperation organization:

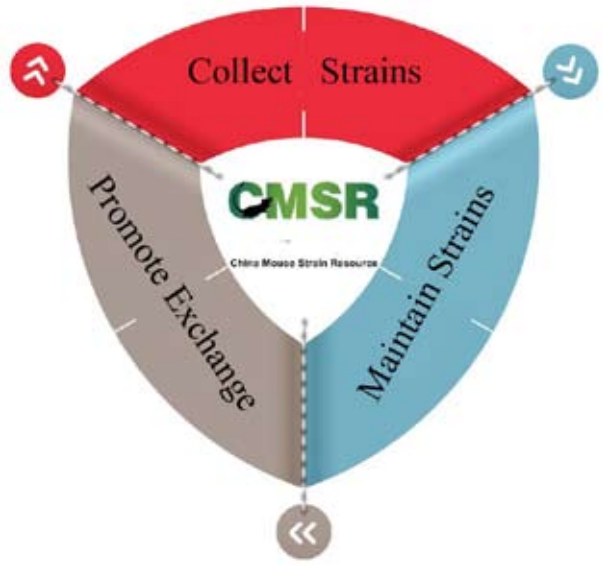
Calorimetry



Columbus Instruments 16 channel

Indirect calorimetry provides detailed information on the energy metabolism of mutant mice. Energy expenditure is evaluated through indirect calorimetry by measuring oxygen consumption with an open flow respirometric system. CO2 and O2 sensors measure the difference in CO2 and O2 concentrations in air volumes flowing through control or animal cages. The amount of oxygen consumed over a given period of time can thus be calculated, as far as the air flow through the cage is known. Data are expressed as ml O2h-1animal-1. The system also monitors CO2 production, therefore, the respiratory exchange ratio (RER) and heat production can be calculated. An activity and food and water intake monitoring system can also be integrated into the set up in order to investigate circadian pattern and behaviour.

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.

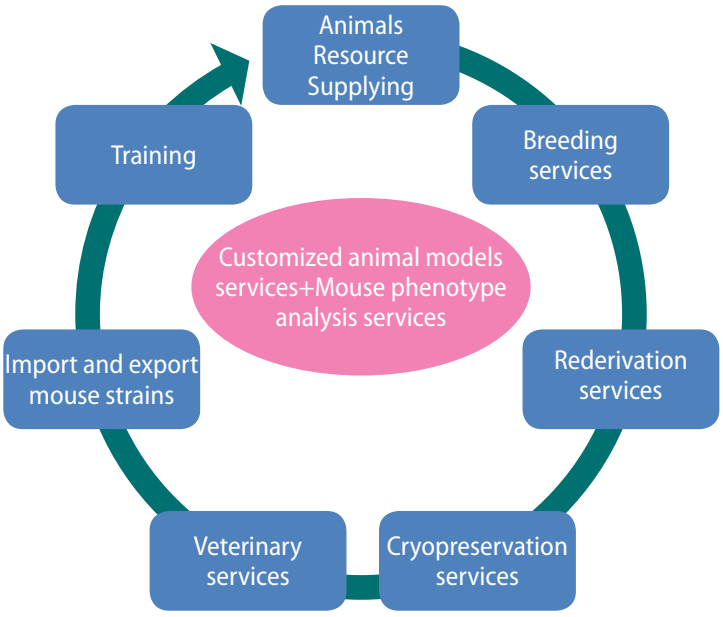
- Ensure equal access to valuable research resources in China
- Promote the sharing and collaborations of model mouse resources
- Dedicate to avoiding repetitive import and production of mouse models based on the assertions of proper intellectual property rights
- Collect and organize available mouse 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

The first sharing alliance meeting was helding on Jun 18th, 2014. At this meeting, the members reached an agreement on the alliance statutes, got the meeting resolutions, and elected MARC (NRCMM) to be unit director, the experimental animal center of Tsinghua University to be vice unit director, Pro. Xiang Gao to be the first chairman

3.Service System of MARC

1.One-Stop Service Provided by Us



North China regional manager:Pingfan Wu
Email:wupf@nicemice.cn wupf@nbri-nju.com
Tel:18951655807

Central China area manager: Mina Wu
Email:wumn@nicemice.cn wumn@nbri-nju.com
Tel:18951655803

East China area manager:Lu Ying
Email:yinglu@nicemice.cn yinglu@nbri-nju.com
Tel:15365191065

South China area manager: Baoli Wei
Email:weibl@nicemice.cn weibl@nbri-nju.com
Tel:13725331344

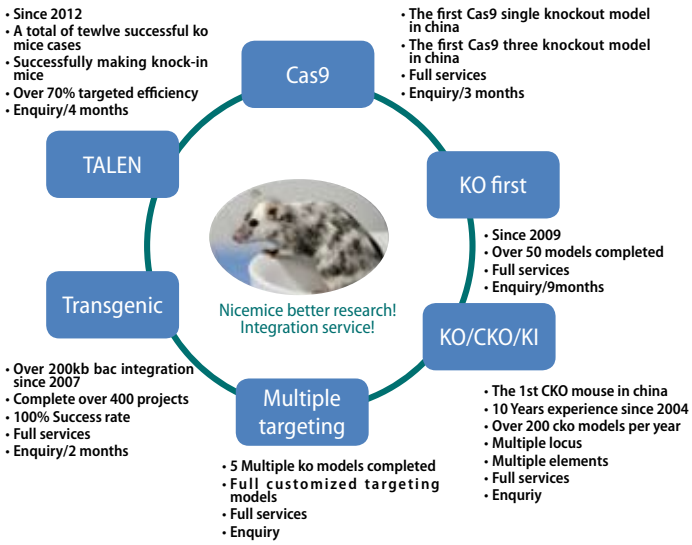
The mice sales manager:Xin Liu
Email:liuxin@nicemice.cn liuxin@nbri-nju.com
Tel:18951991387

Marc Mice ™
Better Science

2.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.



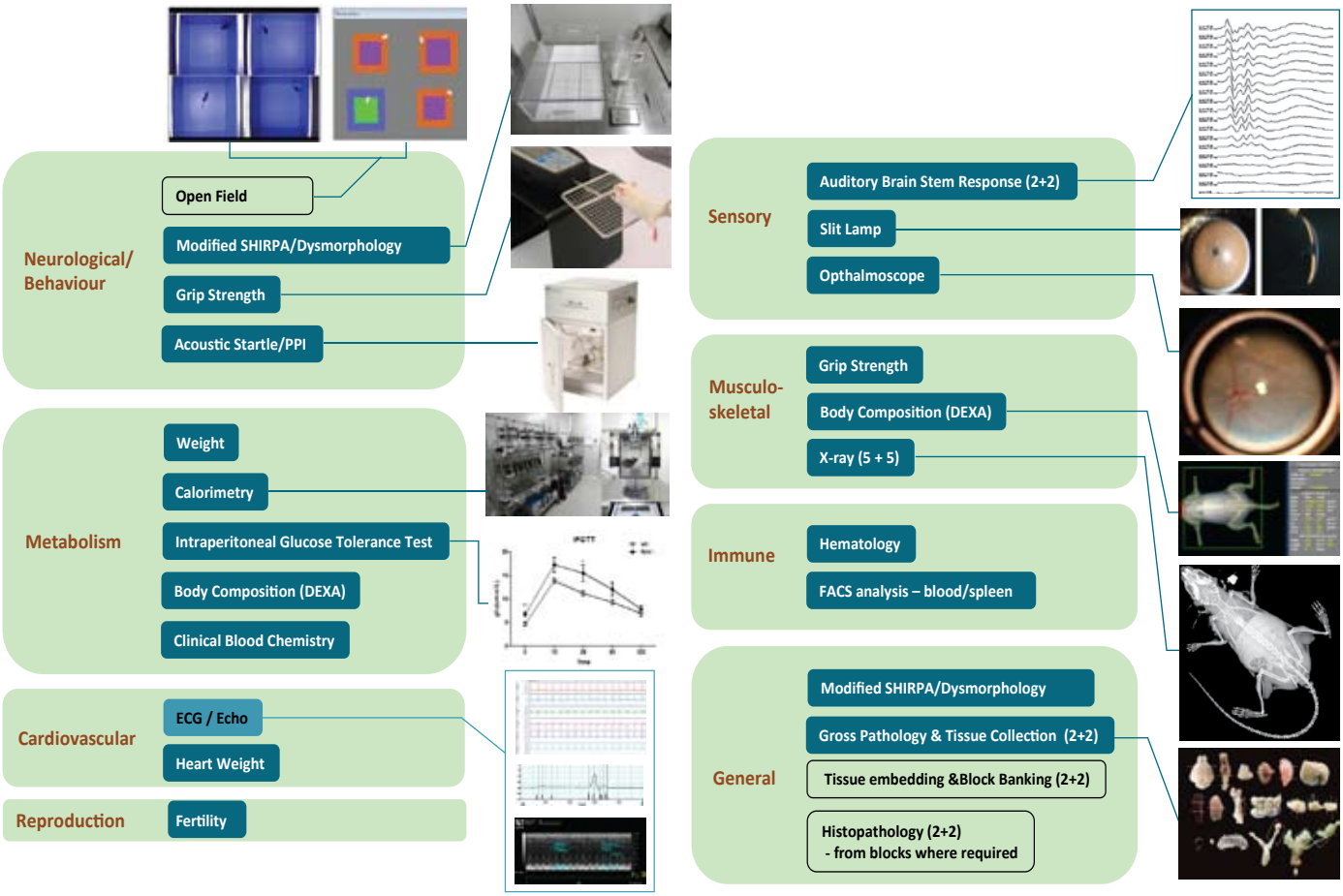
3.International Standardization Phenotypic Analysis Platform

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.



4.Supplying Spf Laboratory Mouse Strains

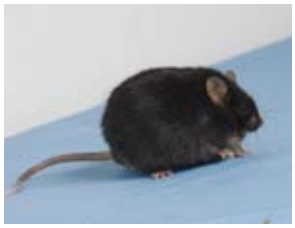
NRCMM holds hundreds strains of inbreed strains, mutant strains, disease models (including cardiovascular disease, adiposity, diabetes mellitus, immunodeficiency, alzheimer disease, tumor, etc.). We also provide many "tool mice" such as tissue-specific CRE transgenic mice and fluorescent protein report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

Throughout the year of 2014, MARC has accomplished an output of around 50,000 model mice including commercial purchases and exporting service.At the same time, we have produced 66 independent intellectual property rights of the strains and received 45 donations lines added to our Resource Centre.

Star mice

B6.Cg-*Lep*^{ob}/JNju

Type II Diabetic mouse model



B6.BKS(D)-*Lepr*^{db}/JNju

Type II Diabetic mouse model

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

Type II diabetic mice model

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

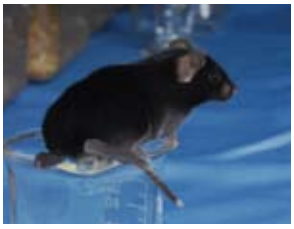
Atherosclerosis

Immune deficiency mouse model

NOD-*Prkdc*^{tm26cas9d52}Il2rg^{tm26cas9d22}Nju

NOD.CB17-*Prkdc*^{scid}/JNju

CByJ.Cg-*Foxn1*^{nu}/JNju



B6.129S7-*Rag1*^{tm1Mom}/JNju

5.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, diseases, and natural disasters such as earthquakes and fire.

In 2014, NRCMM have finished approximately 500 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 a short lifespan or breeding windows.

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

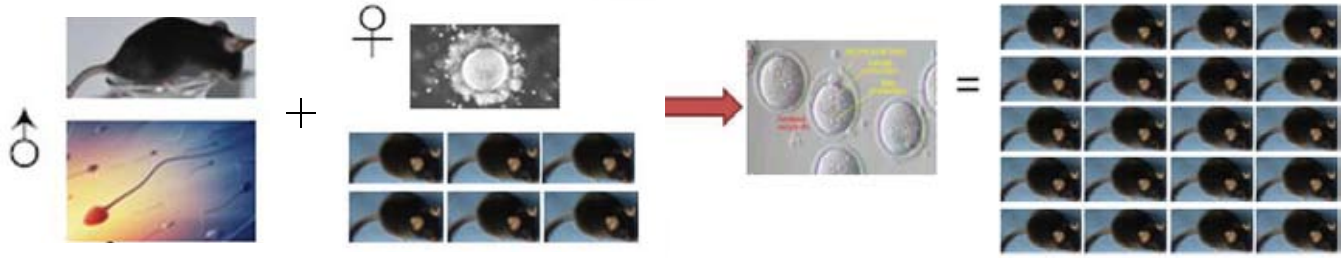
2) Embryo or sperm cryopreservation and recovery to ensure the safety of your valuable research models.

3) Mice health test: A platform to test the health 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%.

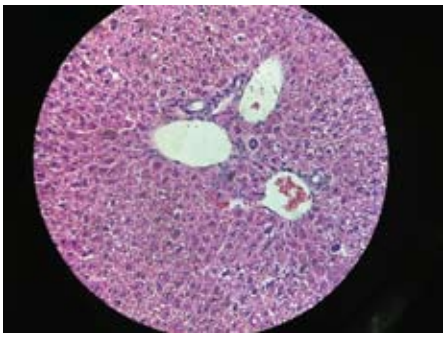
Process: Order—Design &Confirmation—Sign agreement—Payment—Operation—Shipment &Result report

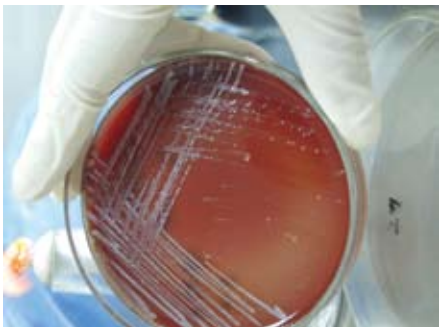


6.Laboratory Animal Pathogen Detection And Veterinary Services

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 in 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 Contact: Yanghui Xin Email: yanghx@nicemice.cn





7.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, tissues etc.

We import frozen materials, from different depositories like KOMP, MMRRC, EUCOMM, EMMA, from different countries such as USA, Britain, Germay, France, Australia. We also export mouse embryos, tissues to USA, Britain, Canada, Belgium and other countries.

We are always committed to providing perfect and professional services to both domestic and foreign customers.

8.Training Services

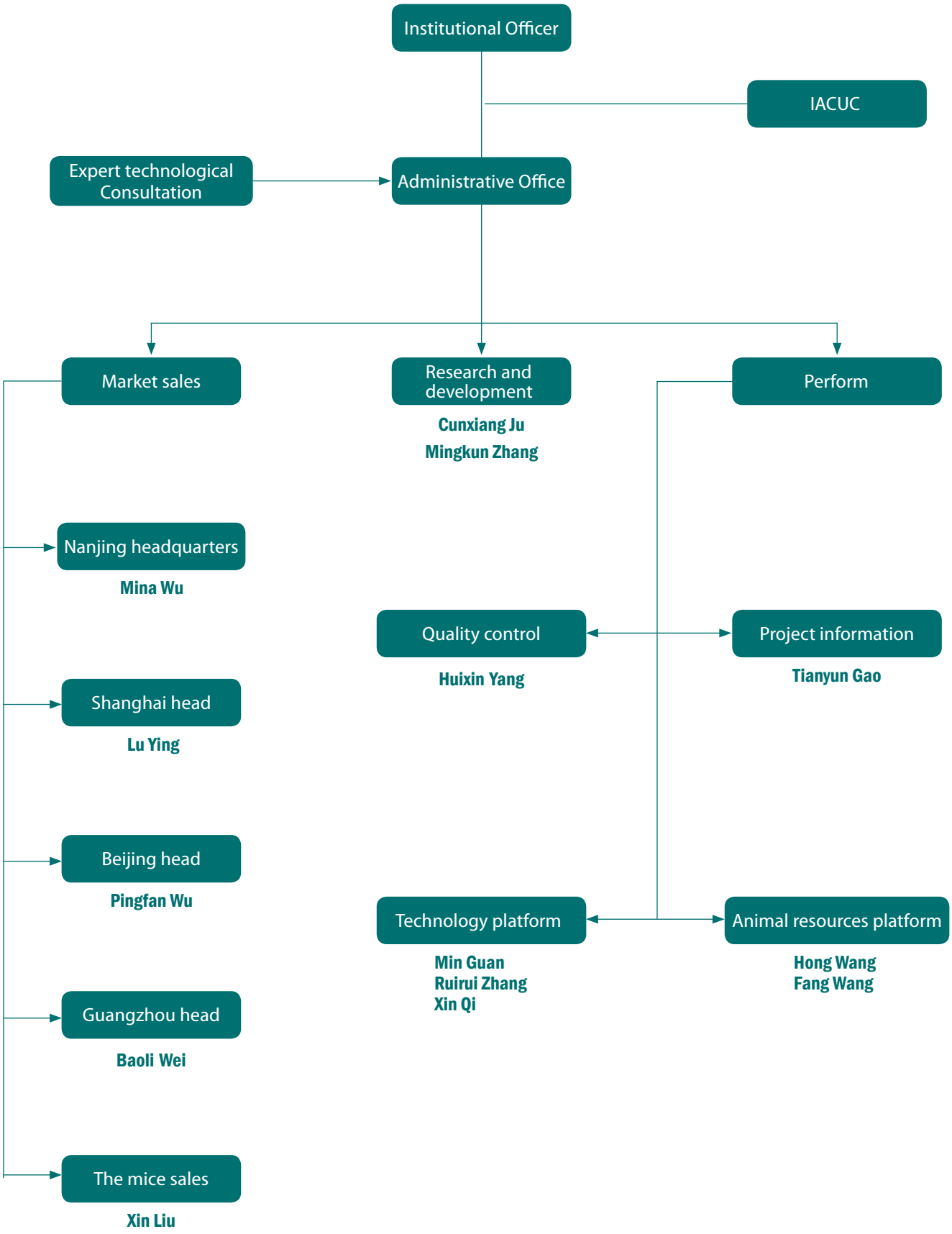
NRCMM provides training for technicians and students on skills of species breeding and management, construction of engineering target mice, etc.

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

Detailed training class time, please see website: www.nbri-nju.com





Institutional Officer
IACUC
Expert technological Consultation
Administrative Office
Market sales
Research and development
Cunxiang Ju
Mingkun Zhang
Perform
Nanjing headquarters
Mina Wu
Quality control
Huixin Yang
Project information
Tianyun Gao
Shanghai head
Lu Ying
Beijing head
Pingfan Wu
Technology platform
Min Guan
Ruirui Zhang
Xin Qi
Animal resources platform
Hong Wang
Fang Wang
Guangzhou head
Baoli Wei
The mice sales
Xin Liu

Publications in 2014

1	Huang Z, Ruan H, Zhang Z, Chen W, Lin Z, Zeng H, Gao X (2014) Mutation in the first Ig-like domain of Kit leads to JAK2 activation and myeloproliferation in mice. The American journal of pathology 184: 122-132.
2	Yuan B, Wan P, Chu D, Nie J, Cao Y, Luo W, Lu S, Chen J, Yang Z (2014) A cardiomyocyte-specific Wdr1 knockout demonstrates essential functional roles for actin disassembly during myocardial growth and maintenance in mice. The American journal of pathology 184: 1967-1980.
3	Wang P, Mao BB, Luo W, Wei B, Jiang WJ, Liu D, Song L, Ji GJ, Yang ZZ, Lai YQ, Yuan ZQ (2014) The alteration of Hippo/YAP signaling in the development of hypertrophic cardiomyopathy. Basic Research in Cardiology 109: 435.
4	Feng XJ, Qin HH, Shi Q, Zhang Y, Zhou FF, Wu HC, Ding S, Niu ZY, Lu Y, Shen PP (2014) Chrysin attenuates inflammation by regulating M1/M2 status via activating PPAR gamma. Biochemical Pharmacology 89: 503-514.
5	Dai XY, Wang C, Dai J, Shi DQ, Xu ZH, Chen DY, Teng HJ, Jiang Q (2014) Association of Single Nucleotide Polymorphisms in Estrogen Receptor Alpha Gene with Susceptibility to Knee Osteoarthritis: A Case-Control Study in a Chinese Han Population. Biomed Research International: 151457.
6	Shi DQ, Sun W, Xu XQ, Hao Z, Dai J, Xu ZH, Chen DY, Teng HJ, Jiang Q (2014) A Replication Study for the Association of rs726252 in PAPPA2 with Developmental Dysplasia of the Hip in Chinese Han Population. Biomed Research International: 979520.
7	Shi DQ, Xu XQ, Song K, Xu ZH, Dai J, Chen DY, Jiang Q (2014) Comparison of Venous Thromboembolism after Total Hip Arthroplasty between Ankylosing Spondylitis and Osteoarthritis. Biomed Research International: 712895.
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10	Niu YY, Shen B, Cui YQ, Chen YC, Wang JY, Wang L, Kang Y, Zhao XY, Si W, Li W, Xiang AP, Zhou JK, Guo XJ, Bi Y, Si CY, Hu B, Dong GY, Wang H, Zhou ZM, Li TQ, Tan T, Pu XQ, Wang F, Ji SH, Zhou Q, Huang XX, Ji WZ, Sha JH (2014) Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos. Cell 156: 836-843.
11	Wang L, Zhang J, Duan J, Gao X, Zhu W, Lu X, Yang L, Li G, Ci W, Li W, Zhou Q, Aluru N, Tang F, He C, Huang X, Liu J (2014) Programming and Inheritance of Parental DNA Methylomes in Mammals. Cell 157: 979-991.
12	Liu ZW, Huang ML, Wu X, Shi GS, Xing LJ, Dong Z, Qu ZP, Yan J, Yang L, Panda S, Xu Y (2014) PER1 Phosphorylation Specifies Feeding Rhythm in Mice. Cell Reports 7: 1509-1520.
13	Liu C, Zhou Z, Yao X, Chen P, Sun M, Su M, Chang C, Yan J, Jiang J, Zhang Q (2014) Hedgehog signaling downregulates suppressor of fused through the HIB/SPOP-Crn axis in Drosophila. Cell Research 24: 595-609.
14	Ma YW, Zhang X, Shen B, Lu YD, Chen W, Ma J, Bai L, Huang XX, Zhang LF (2014) Generating rats with conditional alleles using CRISPR/Cas9. Cell Research 24: 122-125.
15	Chen QL, Quan C, Xie BX, Chen L, Zhou SL, Toth R, Campbell DG, Lu SS, Shirakawa R, Horiuchi H, Li CJ, Yang ZZ, MacKintosh C, Wang HY, Chen S (2014) GARNL1, a major RalGAP alpha subunit in skeletal muscle, regulates insulin-stimulated RalA activation and GLUT4 trafficking via interaction with 14-3-3 proteins. Cellular Signalling 26: 1636-1648.
16	Lu Y, Zhang Y, Li L, Feng XJ, Ding S, Zheng W, Li JX, Shen PP (2014) TAB1: A Target of Triptolide in Macrophages. Chemistry & Biology 21: 246-256.
17	Li J, Li K, Dong X, Liang D, Zhao Q (2014) Ncor1 and ncor2 play essential but distinct roles in Zebrafish primitive myelopoiesis. Developmental Dynamics: [Epub ahead of print].
18	Pang XJ, Shu YX, Niu ZY, Zheng W, Wu HC, Lu Y, Shen PP (2014) PPAR gamma 1 phosphorylation enhances proliferation and drug resistance in human fibrosarcoma cells. Experimental Cell Research 322: 30-38.

19	Ma YW, Ma J, Zhang X, Chen W, Yu L, Lu YD, Bai L, Shen B, Huang XX, Zhang LF (2014) Generation of eGFP and Cre knockin rats by CRISPR/Cas9. FEBS Journal 281: 3779-3790.
20	Zhou JK, Wang JY, Shen B, Chen L, Su Y, Yang J, Zhang WS, Tian XM, Huang XX (2014) Dual sgRNAs facilitate CRISPR/Cas9-mediated mouse genome targeting. FEBS Journal 281: 1717-1725.
21	Chang CJ, Zhao W, Xie BX, Deng YM, Han T, Cui YY, Dai YD, Zhang Z, Gao JM, Guo HQ, Yan J (2014) Pao Pereira Extract Suppresses Castration-Resistant Prostate Cancer Cell Growth, Survival, and Invasion Through Inhibition of NF kappa B Signaling. Integrative Cancer Therapies 13: 249-258.
22	Dong Z, Dong X, Jia W, Cao S, Zhao Q (2014) Improving the efficiency for generation of genome-edited zebrafish by labeling primordial germ cells. International Journal of Biochemistry & Cell Biology: [Epub ahead of print].
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Seminar

	Date	Speaker	Title	Unit
1	2014/11/25	Hui Zong	Know your enemies to win the war: dirty tricks of cancer cells revealed by mouse genetic mosaic model	University of Virginia
2	2014/11/19	Chengyong Shen	Neuromusuclar Synapse and Muscular Dystrophy	Georgia Regents University
3	2014/11/7	Fang He	Fragile X tremor / ataxia syndrome	University of Michigan
4	2014/11/1	Martin M. Matzuk	TGF-beta signaling and microRNA in reproduction and ovarian cancer	Baylor College of Medicine
5	2014/10/27	Grahame Hardie	Canonical and non-canonical regulation of AMP-activated protein kinase	Dundee University, UK
6	2014/10/21	Dazhi Wang	micro RNAs in the heart	Harvard University
7	2014/9/24	Haojie Huang	Androgen receptor activity abnormality in castration resistant prostate cancer defined by functional enhancer RNAs	Mayo Clinic USA
8	2014/8/14	Chenleng Cai	A Voyage to Understand Mammalian Heart Development and Regeneration	Icahn School of Medicine at Mount Sinai
9	2014/7/25	Wenchao Song	Complement, Inflammation and Vascular Injury Diseases	University of Pennsylvania, USA
10	2014/7/18	Fuwen Wei	Giant Panda and its protection	Institute of Zoology, Chinese Academy of Sciences(CAS)
11	2014/7/17	Dong Liang	The role of mesodermal integrin alpha 5 during cardiovascular development	Center for Translational Medicine, Thomas Jefferson University
12	2014/7/16	Jingqiang Wang	The Msx1 homeoprotein recruits histone methyltransferases to repressed target genes during development	Fudan University
13	2014/7/10	Jun Lu	microRNA-mediated mechanisms in normal and malignant hematopoiesis	Yale University
14	2014/7/8	Shuhan Sun	LncRNA and hepatoma carcinoma cell	2nd-Military Medical University
15	2014/7/3	She Chen	Regulation of microtube stability & organization by mammalian PAR3 in specifying neuronal polarity	Fudan University
16	2014/6/12	Weiqiang Gao	Hot topic on drug development	Shanghai Jiao Tong University
17	2014/6/10	Liming Sun	Necrosis, A Kinase Initiated Death Pathway	Biochem&Cell Institute,Shanghai, CAS
18	2014/6/4	Linfei Luo	Repairment of Organ	South-western University
19	2014/5/30	Xiaojun Huang	Leukemia in China	Peking University
20	2014/5/29	Wei Wang	A statergy of drug screening based a whole cell	Nanjing University

21	2014/5/19	Yuhong Fan	Novel regulatory roles of linker histones in stem cells and cancer	School of Biology of Georgia Institute of Technology
22	2014/5/15	Minzhu Xie	Haplotype algorithm	Hunan Normal University
24	2014/5/8	Anming Meng	Maternal materials and development	Tsinghua University
25	2014/4-10	Yong Liu	Metabolic homeostasis and metabolic diseases	The Institute for Nutritional Sciences SIBS, Chinese Academy of Sciences
27	2014-4-8	Ying He	Seeking novel component/mechanism of biological rhythmicity maintenance	Univ. of California
28	2014-4-2	Karine Belguise	The emerging functions of novel PKC theta in breast cancer progression	Universite Toulouse , FRANCE
29	2014-4-2	Lixin Wei	The roles of hepatic progenitor cells and inflammatory injury in liver carcinogenesis	Second Military Medical University
30	2014-4-1	Xiaobo Wang	Forces in collective cell behaviors	Universite Toulouse , FRANCE
31	2014-3-24	Yimin Zou	Growth steering by conserved cell polarity signaling pathways	University of California, San Diego
32	2014-3-19	Xin Sun	Mouse Models of Congenital Lung Diseases	University of Wisconsin Madison
33	2014-3-6	Xiao Lei	Stem cell, trangene , and application fo Talen and CRISPR/CAS9	Zhejiang University
34	2014-2-28	Xinjun Zhang	Novel Mechanisms for Modulating Wnt Signaling	Boston Children's Hospital and Harvard Medical School
35	2014-2-19	Yuheng Fan	The molecular basis of mammalian female germ cell maintenance	Zhejiang University
36	2014-5-10	Yingming Liang,	Lat deletion in naive T cells results in lymphoproliferative disorder	Xinxiang Medical University
37	2014-2-11	Wei Fang	Department of Cell Biology, HHMI Duke University Medical Center	Duke University Medical Center,
38	2014-1-28	Chao Tong	Genetic dissection of autophagy pathway	Zhejiang University
39	2014-1-13	Yanfang Fu	Efficient Genome Targeting using CRISPR/Cas9 nucleases	Harvard Medical School

Courses and Teachers

The MARC, as an institute of the University of Nanjing, is home to approximately 150 PhD students. They carry out their dissertation studies under the supervision of MARC group leaders. In addition, MARC group leaders present lectures and laboratory courses at universities in China, in particular, at Nanjing University, and in other countries. In 2014, the following lecture series and courses were given by MARC staff at Nanjing University (unless indicated otherwise).

Bioinformatics:
Mingshen ZHU

Cell Biology and Molecular Biology
Shuai CHEN
Di CHEN
Chaojun LI

Cell signaling
Geng LIU
Jianghuai LIU
Jun YAN
Chaojun LI
Zhongzhou YANG

Genetics
Xingxu HUANG
Qing ZHANG

Doctoral qualification exam I&II
All PIs in MARC

Frontier of Cell Biology
Yeguang CHEN (Tsinghua University)
Weiqiang GAO (Shanghai Jiaotong University)
Linfei LUO (South-Western University)
Yong LIU (Institute of Nutrition, Shanghai, CAS)
Anming MENG (Tsinghua University)

Mechanism of Development
Ying CAO
Jiong CHEN
Zhongzhou YANG
Qingshun ZHAO

Medical Genetics (Shanghai Jiaotong University)
Xiang GAO

MARC seminar in Genetics
All PIs in MARC

MARC seminar in Developmental Biology
All PIs in MARC

Life, Evolution and Health
Xiang GAO

The Third RIKEN BRC/Nanjing University MARC International Summer Intensive Course of the Mouse
Xiang GAO
Ying XU
Shuai CHEN
Zhongzhou YANG
Jing ZHAO
Xiaohui WU (Fudan University)

PhD Theses

MARC students successfully defended the following PhD theses in 2014

PhD Theses:

Group Ying CAO

Qing CAO

Role and regulation of Klf4 gene during Xenopus early embryogenesis

Group Jiong CHEN

Jing WU

Cofilin-Mediated Actin Dynamics is Essential for Actin Bundles Formation and Maintenance

Jun LUO

The MAGUK family protein Dlg5 regulates apical complexes and follicular epithelial polarity in Drosophila

Group Xiang GAO

Siyuan HOU

In Vivo Functional Analysis of Long Conserved Non-coding Sequences

Shujun JIANG

The role of systemic inflammation in type diabetes resolution after duodenum-jejunum gastric bypass

Li XIAN

Explore the role of catalytic subunit of PP2A in the liver metabolism

Group Geng LIU

Chenxi ZHANG

Role of MDM2-p53 Pathway in Follicular Survival and Development

Group Chaojun LI

Na XU

The function of protein prenylation in the postnatal heart remodeling in the mouse

Group Xingxu HUANG

Xinxing GAO

G4 DNA structure resolvase RHAU regulates spermatogonia differenciation

Bing SHEN

Gene Editing by CRISPR/Cas9

Juan XU

The role of AIP1 in the regulation of early germ cell migration and spermatogonial stem cell niche

Jun ZHANG

Dissecting function of DNA methylation in mammals: from genome-wide mapping to site-specific editing

Group Ying XU

Guangseng SHI

The roles of Fbxl3 in regulating circadian clock

Xi WU

Function of Nanos3 in Germ Cell Development

Group Jun YAN

Wei ZHAO

SRC-3 fuels bladder cancer through remodulation of cancer metabolism

Group Zhongzhou YANG

Qi XIAO

Heterogeneity of the cardiomyocyte population in heart development and regeneration

Group Qing ZHANG

Chen LIU

Hedgehog signaling downregulates Suppressor of Fused through the HIB/SPOP-Crn axis in Drosophila

Group Qingshun ZHAO

Zhangji DONG

Genome editing in yellow catfish and zebrafish using engineered endonucleases

Group Mingshen ZHU

Caiping CHEN

Constitutive Regulation of Force Maintenance by MYPT1 Phosphorylation in Smooth Muscle

2014 Annual Conference of MARC

The 2014 MARC Annual Conference was held in Jiangsu University (Zhenjiang, Jiangsu province) from November 2nd to 4th. This meeting was organized by Drs. Qing Zhang and Jianghuai Liu's laboratories. More than 194 scientists and students from MARC, Jiangsu University, Nanjing General Hospital of Nanjing Military Command attended the conference, which was held in the Jiangsu University Conference Center. Dr. Zhongzhou Yang, director of MARC and chairman of the conference, summarized the research and education progress of MARC in the past year at the opening ceremony. In the following sessions, PIs and student representatives presented latest research 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 2014 annual report of NRCMM. About 75 posters were presented by senior students to exhibit their research works. In the Teacher-Student Interaction session, interested issues and topics were discussed.

Zan Huang from Dr. Xiang Gao's laboratory, was awarded the 2014 Student of MARC for his excellent research on the stem cell factor/Kit signalling pathway that regulates mitochondrial function and energy expenditure. Jun Zhang from Xingxu Huang's laboratory and was the runner-up for the award.

Fifteen students received 2014 Outstanding Poster Prize.

2014 Summer Camp

Graduate students are always our treasure at MARC; therefore we treat the recruitment and training of students as a top priority. This year, we held the 5th Summer Camp from July 7–11. Fifty-five excellent undergraduates were selected from a pool of 298 applicants from 69 universities nationwide.

We have organized specific sessions to promote interaction between undergraduate students and our faculty members/graduate students. 8 faculty members gave lectures on the current progress in biomedical research, ranging from circadian rhythms, cell migration to heart regeneration and neurodegeneration.

To enhance students, interest in the experiment, the summer camp also included an experimental session, in which students used model animals including mouse, Drosophila melanogaster, zebra fish and Caenorhabditis elegans to perform interesting experiments.

At nights, we held academic salons, which enabled faculty members to closely interact with summer camp students. Moreover, 2 of our outstanding graduate students, Zan Huang and Juan Xu, talked tothe Summer Camp students about on their own research and living experience at MARC. The purpose of the Summer Camp is to train and attract students for future biomedical research involving model animals both at MARC and at other institutes in China.

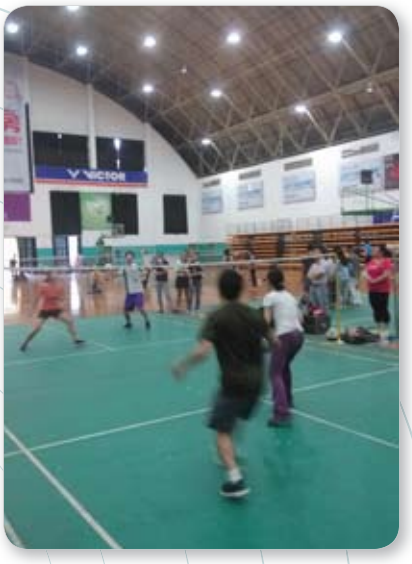
2014 Students Union

Currently, there are 150 students, out of a total 200 members in MARC, and they form a critical component of MARC. The students in MARC not only are immersed in science but also are cultivated by a culture promoting humanity, critical thinking, and social well-being.

Students were recruited all over the country competitively. Financial support is generous when compared to the national average standards for graduate students, and it is provided by both MARC and Chinese government. .

The student academic seminar was held weekly to stimulate their academic interests and enthusiasm. This year, invited speakers were not only from science background, renowned speakers from literature, music and social science backgrounds were also invited , and they include Grammy Award winner Rhonda Larson, distinguished French literature translator Prof. Jun XU and experienced sociologist Prof. Xiaohong ZHOU.

In addition to mental and intellectual exercise, the students are also keen to exercise their bodies by holding regular table tennis, Badminton, basketball and soccer matches.



Administration and Services









Animal facility
Zhao Jing et al see Page

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Shiman WANG
Yugang WU
Renping XU
Chunmei ZHANG
Pinfang ZHOU

Chief Operating Officer and Deputy Director
Jing ZHAO
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Facility management
Hui ZHENG

Finance and controlling
Haiyan PAN
He WAN

Housekeeping
Chunping CHEN
Renxia DAI
Lingling GU
Juhua LI
Jiping SHEN
Zhaofeng TIAN
Mei WANG
Lianmei WANG*
Xiuxin XU
Guoye ZHANG
Mingrong ZHANG
Guoxiang ZHENG

Human resources
Yijun HUAI

Informatics
Jian NI
Jie XIA*

Intellectual property
Xing LIU

Mechanical & electrical workshop
Lin CHEN
Mingming CHEN
Hengxin QIU
Jianwang GUO
Rongjun QIAN
Bing WANG
Ronglin WANG
Hongbo ZHANG

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Tianye CONG
Wenjing FAN
Ke GAN
Yijun HUAI
Xing LIU
Qian SUN
Lingling ZHU

PhD program
Xing LIU

Radiation safety
Hui ZHENG

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Model Animal Research Centre of Nanjing University
12 Xuefu Road Nanjing 210061 China
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