ANNUAL REPORT

2016

MODEL ANIMAL RESEARCH CENTER OF NANJING UNIVERSITY

MOE KEY LABORATORY OF MODEL ANIMAL FOR DISEASE STUDY

NANJING BIOMEDICAL RESEARCH INSTITUTE OF NANJING UNIVERSITY

NATIONAL RESOURCE CENTER FOR MUTANT MICE

Director's Words

In this past year, China has been constantly bringing changes to herself, as she had been in the previous years. I cannot help but marvel at the breakthroughs in science and technology, as well as the vigorous fights against corruption in our country.

There have also been noticeable changes in institutions of higher education. There are currently around a hundred programs of excellent talents, such as Chang Jiang Scholars Program, Thousand Talent Program, the National Science Fund for Distinguished Scholars, and many more. Scholars who are on the lists are provided with more resources and a better payment. In turn, they contribute to higher institute rankings, and help the institution attract even more resources. A talent market with high liquidity has been formed, and many scholars have been working hard to become one of the talents.

At MARC, most of our scientists are only spectators of this prosperous economy. Do I worry as a "housekeeper" of MARC? Not at all. Despite the absence of the apparently lucrative titles, our scientists spared no passion in conducting scientific research. Dr. Shi revealed a new function of signal peptide assisting protein assembly, which may open up a new field for protein science and drug discovery; Dr. Zhou's, Zhao's and Zhu's labs jointly developed a SGN gene editing technology which may potentially edit genome without being limited by the sequence; Dr. Chen elucidated a novel mechanism for the formation of obesity; Dr. Zhu's lab revealed the mechanistic basis for the spontaneous tone formation of internal inner sphincter; Dr. Gan proposed a regulatory pathway underlying the signal coupling of muscle fiber switch and energy metabolism. MARC remains dynamic in its own way; titles agitate us not. We are proud to be constantly salvaging pieces of truth while sailing towards the one glimmer of light at the end of the sea, where wonders are explained and human sufferings may be lifted up like a feather.

I am looking forward to even more positive changes in the coming year. I wish all the best for Nanjing University and her scientists. May curiosity be preserved; may naivety guide us in wind and waves.

Min-Sheng Zhu

Director

CONTENTS

Research Highlight in 2016

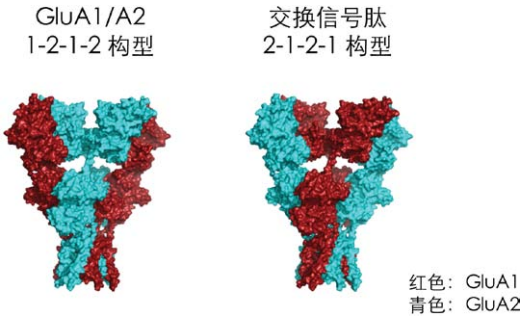
Group Yun Shi

GluA1 signal peptide determines the spatial assembly of heteromeric AMPA receptors

Xue-Yan He, Yan-Jun Li, Chakrapani Kalyanaraman, Li-Li Qiu, Chen Chen, Qi Xiao, Wen-Xue Liu, Wei Zhang,Jian-Jun Yang, Guiquan Chen, Matthew P. Jacobson, and Yun Stone Shi

Significance:

In the brain, AMPA-type glutamate receptors especially heteromeric GluA1/A2s are the major postsynaptic receptors mediating fast excitatory neurotransmission. Recently the crystal structure of GluA2 homomeric AMPARs revealed some interesting features such as that the four subunits in each AMPAR are of two different conformations. However, what the heteromeric GluA1/A2 receptors look like is unknown. In this study, we used a biochemical method called cysteine crosslinking assay to analyze the spatial architecture of GluA1/A2s. We figured out that GluA1/GluA2s have preferred spatial assembly with 1-2-1-2 architecture. To our most surprise, this spatial assembly pattern is dictated by the excisable signal peptides but not the intrinsic sequences of the subunit proteins. When the GluA1 and GluA2 signal peptides were swapped, the subunit arrangement was switched. Replacements with an unrelated GluK2 signal peptide demonstrated that GluA1 signal peptide plays a critical role in determining the spatial priority.



Highlights:

- GluA1/A2 heteromeric AMPA receptors process a preferred 1-2-1-2 spatial assembly.
- The spatial architecture of this receptor is dictated by GluA1 signal peptide.
- Our study reveals a novel function of signal peptides.

Group Shuai Chen

Disruption of the AMPK–TBC1D1 nexus increases lipogenic gene expression and causes obesity in mice via promoting IGF1 secretion

Liang Chen[1,5], Qiaoli Chen[1,5], Bingxian Xie[1,5], Chao Quan[1], Yang Sheng[1], Sangsang Zhu[1], Ping Rong[1], Shuilian Zhou[1], Kei Sakamoto[2], Carol MacKintosh[3], Hong Yu Wang[1,4*] and Shuai Chen[1,4*]

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Abstract:

TBC1D1 is a Rab GTPase activating protein that is phosphorylated on Ser231 by the AMP-activated protein kinase (AMPK) in response to intracellular energy stress. However, the in vivo role and importance of this phosphorylation event remains unknown. To address this question, we generated a mouse model harboring a TBC1D1^{Ser231Ala} knockin mutation and found that the knockin mice developed obesity on a normal chow diet. Mechanistically, TBC1D1 is located on IGF1 storage vesicles, and the knockin mutation increases endocrinal and paracrinal/autocrinal IGF1 secretion in a Rab8a-dependent manner. Hypersecretion of IGF1 causes increased expression of lipogenic genes via activating the protein kinase B (PKB, also known as Akt)–mammalian target of rapamycin (mTOR) pathway in adipose tissues, which contributes to the development of obesity, diabetes and hepatic steatosis as the knockin mice age. Collectively, these findings demonstrate that the AMPK–TBC1D1 signaling nexus interacts with the PKB–mTOR pathway via IGF1 secretion, which consequently controls expression of lipogenic genes in the adipose tissue. These findings also have implications for drug discovery to combat obesity.

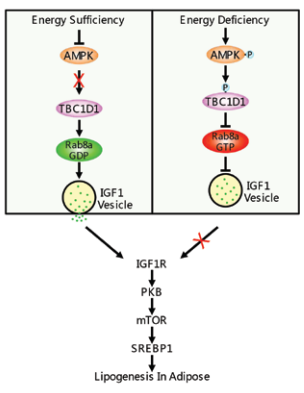


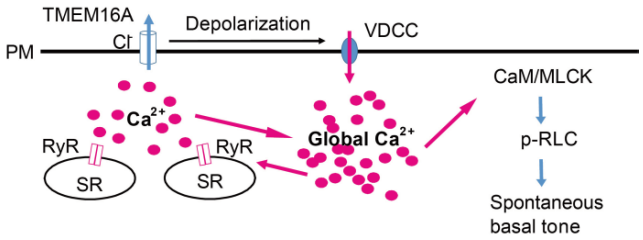
Figure Legend: A diagramatic model illustrating that the AMPK-TBC1D1-Rab8a signaling axis links energy status to IGF1 secretion and controls lipogenesis in the adipose

Group Minsheng Zhu

The molecular basis of the genesis of basal tone in internal anal sphincter

Cheng-Hai Zhang *, Pei Wang *, Dong-Hai Liu, Cai-Ping Chen, Wei Zhao, Xin Chen, Chen Chen, Wei-Qi He, Yan-Ning Qiao, Tao Tao, Jie Sun, Ya-Jing Peng, Ping Lu, Kaizhi Zheng, Siobhan M. Craige, Lawrence M. Lifshitz, John F. Keaney Jr, Kevin E. Fogarty, Ronghua ZhuGe * & Min-Sheng Zhu *

Smooth muscle sphincters exhibit basal tone and control passage of contents through organs such as the gastrointestinal tract; loss of this tone leads to disorders such as fecal incontinence. However, the molecular mechanisms underlying this tone remain unknown. Here we show that deletion of myosin light chain kinases (MLCK) in the smooth muscle cells from internal anal sphincter (IAS-SMCs) abolishes basal tone, impairing defecation. Pharmacological regulation of ryanodine receptors (RyRs), L-type voltage-dependent Ca^{2+} channels (VDCCs), or TMEM16A Ca^{2+}-activated Cl- channels significantly changes global cytosolic Ca^{2+} concentration ([Ca^{2+}]i) and the tone. TMEM16A deletion in IAS-SMCs abolishes the effects of modulators for TMEM16A or VDCCs on a RyR-mediated rise in global [Ca^{2+}]i and impairs the tone and defecation. Hence MLCK activation in IAS-SMCs caused by a global rise in [Ca^{2+}]i via a RyR-TMEM16A-VDCC signaling module sets the basal tone. Targeting this module may lead to new treatments for diseases like fecal incontinence.



Group Chaojun Li

PP2Acα Positively Regulates Mice Liver Regeneration Termination through AKT/GSK3β/Cyclin D1 Pathway

Shan-Shan Lai[1], Dan-Dan Zhao[1], Peng Cao[2,3], Ke Lu[1], Ou-Yang Luo[1], Wei-Bo Chen[1,4], Jia Liu[1], En-Ze Jiang[1], Zi-Han Yu[1], Gina Lee[5], Jing Li[5], De-Cai Yu[4], Xiao-Jun Xu[6], Min-Sheng Zhu[1], Xiang Gao[1,*], Chao-Jun Li[1,*], Bin Xue[1,*]

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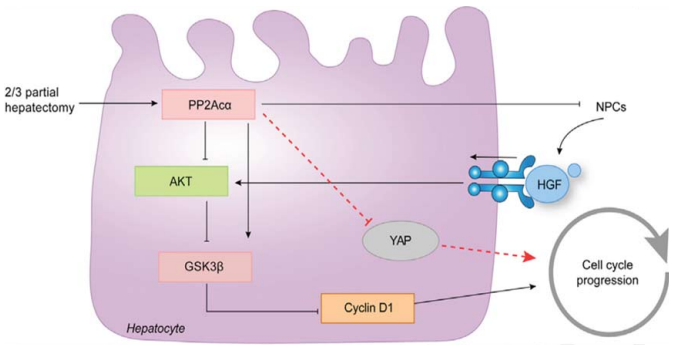
Graphical Abstract:



Working model of PP2Acα regulating the liver regeneration termination

Background:

Liver injury triggers a highly organized and ordered liver regeneration (LR) process. Once regeneration is complete, a stop signal ensures that the regenerated liver is an appropriate functional size. The inhibitors and stop signals that regulate LR are unknown, and only limited information is available about these mechanisms.

Significance:

We found that the catalytic subunit of PP2A was markedly up-regulated during the late stage of LR. To further investigate the function of PP2Acα in LR, we generated liver-specific PP2Acα-knockout mice and performed PH on PP2Acα-/- mice. PP2Acα-/- mice showed prolonged LR termination, an increased liver size compared to the original mass and lower levels of serum ALT and AST compared with control mice. In these mice, cyclin D1 protein levels, but not mRNA levels, were increased. Mechanistically, AKT activated by the loss of PP2Acα inhibited glycogen synthase kinase 3β (GSK3β) activity, which led to the accumulation of cyclin D1 protein and accelerated hepatocyte proliferation at the termination stage. Treatment with the PI3K inhibitor wortmannin at the termination stage was sufficient to inhibit cyclin D1 accumulation and hepatocyte proliferation.

Perspective:

We describe a new molecular mechanism for the regulation of LR termination, which will enrich the understanding of the molecular mechanism of liver regeneration termination control. As a critical factor for the termination of LR, PP2Acα may provide guidance for the treatment of chronic liver diseases, liver transplantation, and liver malignancy.

Student of the Year 2016

Zhipeng Qu

Zhipeng Qu received his Bachelor's degree of Biological Science and Technology in 2010 from School of Life Science, Northwest University of China. He joined Dr. Ying Xu's lab at the year of 2009 to study the molecular mechanism of the circadian clock in mice.

For the past seven years, his work focused on the regulation of bimodal behavioral rhythms in mammals and the mechanism of methuosis. Firstly, he and his colleagues found that ZBTB20-mediated PROKR2 signaling is critical for the evening behavioral rhythms. Depletion of ZBTB20 in nerve system resulted in the loss of early evening activity, but the increase of morning activity. Overexpression of PROKR2 in suprachiasmatic nucleus could partly restore evening activity defect of mice lacking ZBTB20. Moreover, through phenotypic screening and structure-activity relationship analysis, he and his colleagues obtained a potent methuosis-inducing compound (SL-V50). Then, they identified VIM as the specific target protein of this compound. Indeed, treatment with SL-V50 inhibited the polymerization of VIM protein in vitro, and significantly reduced the tetramers of VIM^{WT}, but not of VIM^{Y276F} expressed in cultured cells.

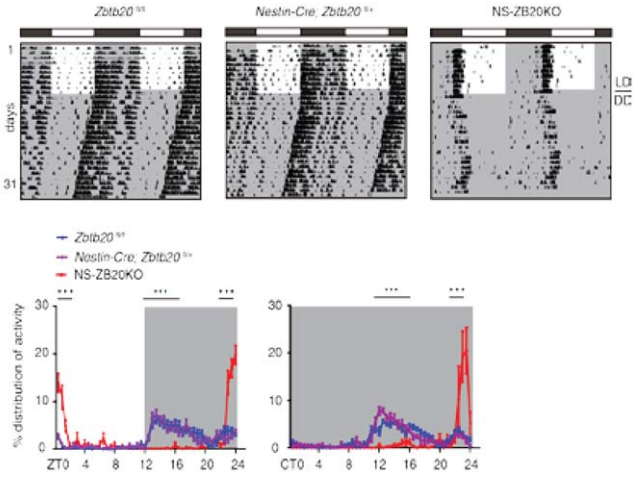


Figure 1. Loss of ZBTB20 alters evening activity and morning activity.

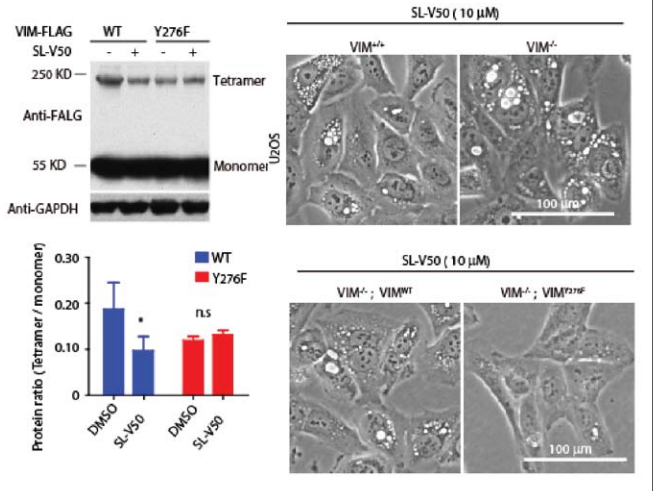


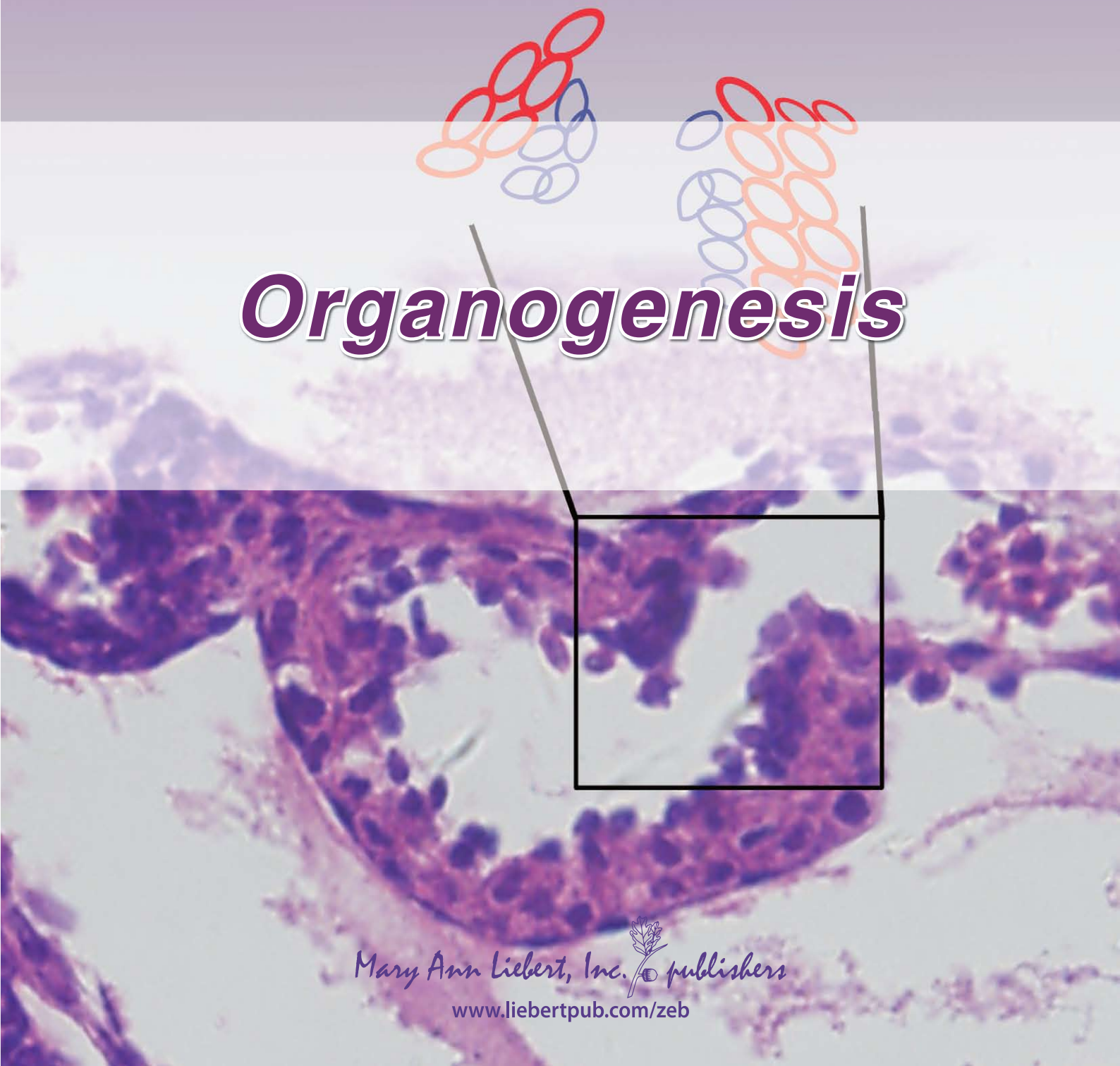
Figure 2. Mutations VIM^{Y276F} abolishes effect induced by SL-V50.

Selected publications

1. Qu, Z., Zhang, H., Huang, M., Shi, G., Liu, Z., Xie, P., Li, H., Wang, W., Xu, G., Zhang, Y., et al. (2016). Loss of ZBTB20 impairs circadian output and leads to unimodal behavioral rhythms. eLife.
2. Lei Zhang*, Zhipeng Qu* , Jianping Wu*, Qingqing Zhang, Tao Zhang, Lian Mo, Qizheng Yao, Ying Xu, Ruihuan Chen. Vimentin dysfunction mediates a pharmacologically induced methuosis. (* co-first author; submitted)
3. Qu, Z., Wang, X., Liu, D., Gao, X., and Xu, Y. (2015). Inactivation of Cipc alters the expression of Per1 but not circadian rhythms in mice. Sci China Life Sci 58, 368-372.

VOLUME 13, NUMBER 1 • JANUARY 2016
ISSN 1545-8547

Zebrafish



Organogenesis

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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. Besides developmental biology, he also focuses on the research of cancer cell differentiation.

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Mechanisms of embryonic and cancer cell differentiation

Induced differentiation of cell lines of different cancer types.

Carcinogenesis is driven by genetic variations that result in changes in gene transcription and/or protein malfunction, and consequently, leading to the reprogramming of cellular physiology. The erroneously reprogrammed cells exhibit features of immature cells due to the activation of tumor promoting genes and inactivation of tumor suppressor genes. We believe that re-differentiation of cancer cells from an incompletely differentiated state to a terminally differentiated state will possibly reduce the malignancy of cancer cells. By screening of a series of factors that are involved in the development and progression of different cancer types, we identified a few that govern the differentiation state of cancer cells. Manipulating these factors in cancer cell lines can successfully induce terminal differentiation, which leads to the loss of malignant features of cancer cells, as demonstrated by cell proliferation, growth assays, etc (Figure 1). These results provide the evidence that cancer cells can indeed undergo terminal differentiation, and there might be a unified mechanism for development and progression of different cancer types.

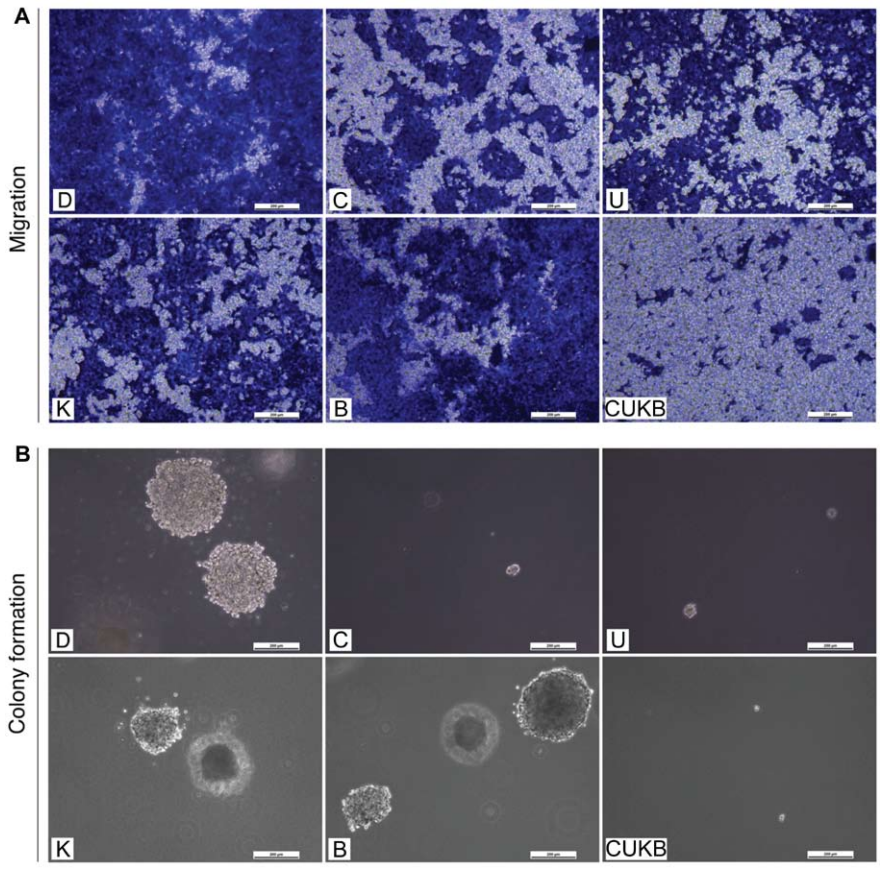

Figure 1. Inhibition of different single factors and inhibition of the factors together in HepG2 cells led to suppression of the ability of cell migration (A) and growth (B) to different extents. Note that combined inhibition almost completely eradicated these abilities in HepG2 cells.

Selected publications (*Correspondence author)

1. Gao Y, Cao Q, Lu L, Zhang X, Zhang Z, Dong X, Jia W, Cao Y*. 2015. Kruppel-like factor family genes are expressed during Xenopus embryogenesis and involved in germ layer formation and body axis patterning. Dev Dyn. 244(10):1328-46
2. Zhang X, Gao Y, Lu L, Zhang Z, Gan S, Xu L, Lei A, Cao Y*. 2015. JmjC Domain-containing Protein 6 (Jmjd6) Derepresses the Transcriptional Repressor Transcription Factor 7-like 1 (Tcf7l1) and Is Required for Body Axis Patterning during Xenopus Embryogenesis. J Biol Chem. 290(33):20273-83.
3. Lu L, Gao Y, Zhang Z, Cao Q, Zhang X, Zou J, Cao Y*. 2015. Kdm2a/b Lysine Demethylases Regulate Canonical Wnt Signaling by Modulating the Stability of Nuclear β-Catenin. Dev Cell. 33(6):660-74.
4. Cao Y*. 2015. Germ layer formation during Xenopus embryogenesis: the balance between pluripotency and differentiation. Sci China Life Sci. 58(4):336-42.
5. Cao Y*. 2013. Regulation of germ layer formation by pluripotency factors during embryogenesis. Cell Biosci. 3(1):15.
6. 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.

Group members

Principal investigator:

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Graduated students:

Cao Qing, PhD
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Zhang Xuena, PhD
Gao Yan, PhD
Yang Linan (M. Sc. Jointly supervised)

Graduate students:

Zhang Zan
Lei Anhua
Xu Liyang
Chen Lu
Yang Xiaoli
Zhang Min

Technicians:

Ma Haihua
Yan Yuelou

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 two model systems that we mainly use in the lab: the migrating border cells and the follicle epithelia in the Drosophila ovary.

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. 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 questions. 1. How extracellular factors (gradients) guide the cluster of border cells and generate asymmetry within cluster? 2. How are distinct cell polarities generated, maintained and interacting with each other during collective migration? 3. Are there novel regulatory mechanisms that link other important cellular process with collective 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 mechanism underlying apical polarity formation and maintenance using this system. Lastly, we have also collaborated with other mouse labs in MARC to study the role of actin disassembly regulators in two different morphogenetic processes in the neonatal mice, namely gonocyte migration in the testis and myofibre assembly in the heart

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

1.Mechanism of asymmetry generation through intracellular trafficking during collective migration of border cells in Drosophila ovary.

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.

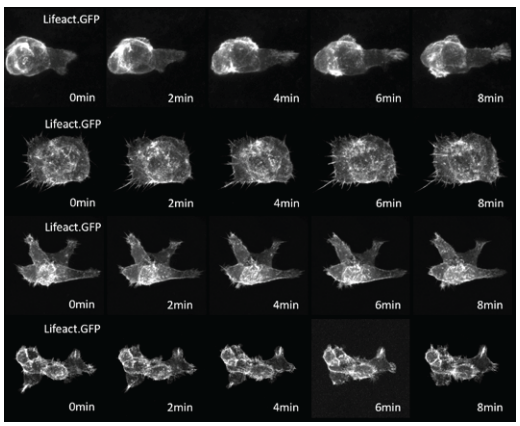

Figure 1. Time-lapsed series showing clusters of 8 border cells (WT, top row; mutants, bottom 3 rows) extending dynamic actin-rich protrusions during collective migration.

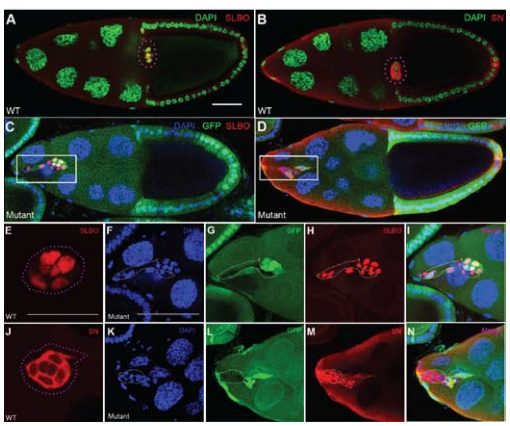

Figure 2. Wildtype border cell clusters (A,B) reached the final destination while mutant mosaic border cell clusters (C,D) displayed delayed migration within the egg chambers.

Selected Publications (*corresponding author)

1. Wu, J., Wang, H., Guo, X., & Chen, J*. (2016). Cofilin-mediated actin dynamics promotes actin bundle formation during Drosophila bristle development. Molecular Biology of the Cell, 27(16), 2554-2564.
2. Luo, J., Wang, H., Kang, D., Guo, X., Wan, P., Wang, D., & Chen, J*. (2016). Dlg5 maintains apical polarity by promoting membrane localization of Crumbs during Drosophila oogenesis. Scientific reports, 6.

Group members

Technical Staff:

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Graduate Students:

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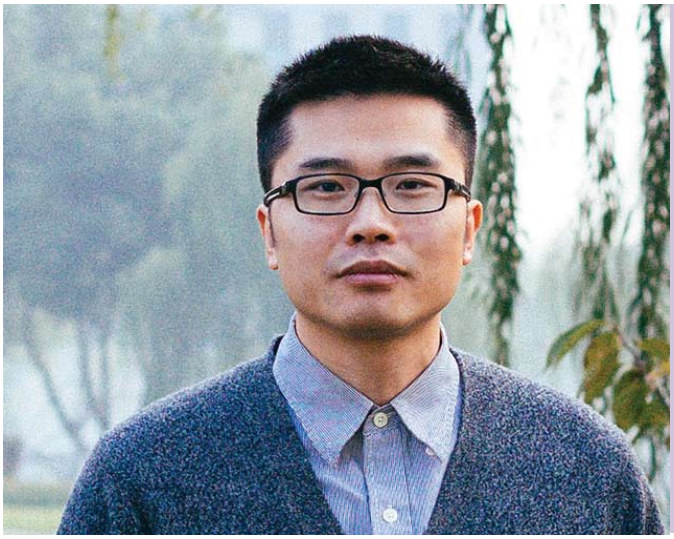
Kang Di
Wang Heng
Wang Dou
Wu Mengqi
Xu Zehao

Former Graduate Students:

Zhang Lijun (Ph.D)
Chu Dandan (Ph.D)
Wan Ping (Ph.D)
Wu Jing (Ph.D)
Luo Jun (Ph.D)
Zuo Juntao (MS)



Xin Lou Ph.D.

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

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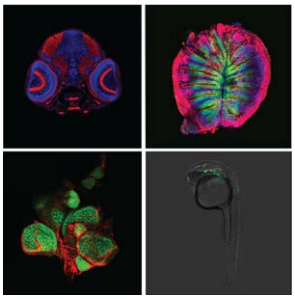
Vertebrate Organogenesis and regeneration



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:

Currently, our research focuses on the following aspects

1)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 on regenerating adult zebrafish heart, characterized the dynamic expression change of epigenetic regulators during heart regeneration. Now we are focusing on a set of chromatin modulators (including components of PRC2 complex and NuRD complex). By using a battery of strategy ranging from experimental molecular genetics to bioinformatics, we are studying the detail function and mechanism of these genes in heart regeneration.

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 working on identification of new heart development/regeneration genes and analyzing their biological function.

Selected Publications

1. Lou, X*., Burrows, J. T. A. and Scott, I. C. (2015) Med14 cooperates with brg1 in the differentiation of skeletogenic neural crest. BMC Developmental Biology 2015, 15:4
2. 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.
3. 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)

Group members

Lab Head	Lab Manager	Graduate Students
Xin Lou	Xiaoqin Wang	Lingling Zhang
		Ningning Hou
		Yuxi Yang

Zhongzhou Yang, Ph.D.

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

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Heart Development

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 second 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 β-catenin activity (Fig. 1).

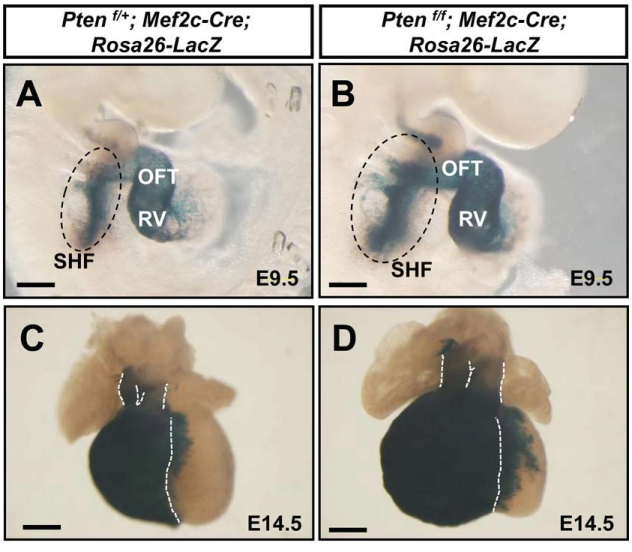

Fig. 1. Deletion of Pten in the SHF progenitors results in enlarged SHF and right ventricle.

Post-transcriptional regulation of Nkx2-5 by RHAU in heart development

RNA G-quadruplexes (G4) play important roles in RNA biology. However, the function and regulation of mRNA G-quadruplexes in embryonic development remain elusive. Previously, we identified RHAU (DHX36, G4R1) as an RNA helicase to resolve mRNA G-quadruplexes. Here, we revealed heart defects and embryonic lethality of cardiac Rhau-deletion mice. Gene expression profiling identified Nkx2-5 mRNA as a target of RHAU that associates with its 5′- and 3′-UTRs, and modulates its stability and translation. The 5′-UTR of Nkx2-5 mRNA contains G-quadruplex that requires RHAU for protein translation, whilst the 3′-UTR of Nkx2-5 mRNA possesses an AU-rich element (ARE) that facilitates RHAU-mediated mRNA decay. Thus, we uncovered the mechanisms of Nkx2-5 post-transcriptional regulation during heart development. Meanwhile, this study demonstrates the function of mRNA 5′-UTR G-quadruplex mediated-protein translation in organogenesis (Fig. 2).

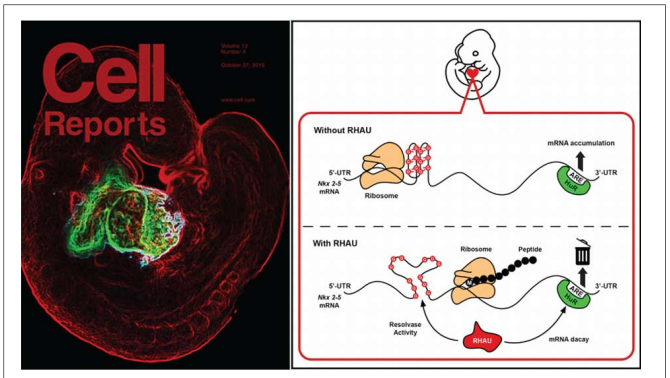

Fig.2. Post-transcriptional regulation of Nkx2.5 by RHAU in heart development.

Selected Publications

1. Junwei Nie, Mingyang Jiang, Xiaotian Zhang, Hao Tang, Hengwei Jin, Xinyi Huang, Baiyin Yuan, Chenxi Zhang, Janice Ching Lai, Yoshikuni Nagamine, Dejing Pan, Wengong Wang* and Zhongzhou Yang*. (2015) Post-transcriptional Regulation of Nkx2-5 by RHAU in Heart Development. Cell Rep. 13:723-732. (Cover featured story/*Co-corresponding author)
2. Wen Luo, Xia Zhao, Hengwei Jin, Lichan Tao, Jingai Zhu, Huijuan Wang, Brian A. Hemmings and Zhongzhou Yang*. (2015) Akt1 signaling coordinates BMP signaling and β-catenin activity to regulate second heart field progenitor development. Development 142:732-742.
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5. 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)
6. 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)
7. 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).
8. 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.
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10. 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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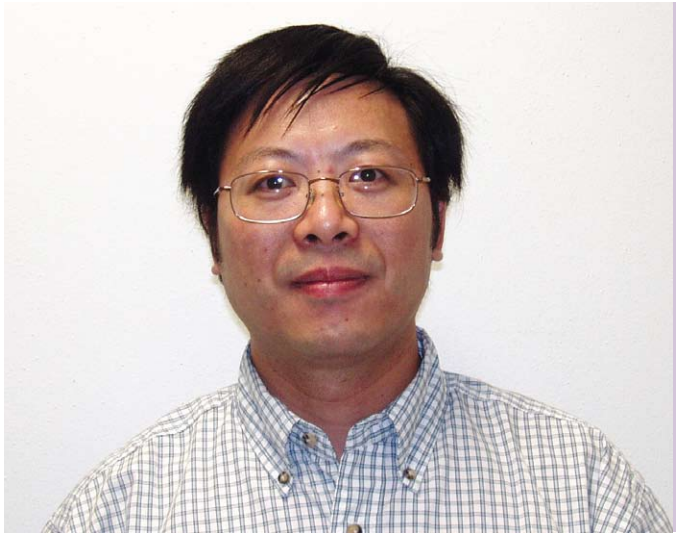

Qing Zhang, Ph.D

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.

1. Deubiquitination of Ci/Gli by Usp7/HAUSP regulates Hedgehog Signaling

Hedgehog (Hh) signaling plays essential roles in animal development and tissue homeostasis, and its misregulation causes congenital diseases and cancers. Regulation of the ubiquitin/proteasome-mediated proteolysis of Ci/Gli transcription factors is central to Hh signaling, but whether deubiquitinase is involved in this process remains unknown. Here, we show that Hh stimulates the binding of an ubiquitin-specific protease Usp7 to Ci, which positively regulates Hh signaling activity through inhibiting Ci ubiquitination and degradation mediated by both Slimb-Cul1 and Hib-Cul3 E3 ligases. Furthermore, we find that Usp7 forms a complex with GMP-synthetase (GMPS) to promote Hh pathway activity. Finally, we show that the mammalian counterpart of Usp7, HAUSP, positively regulates Hh signaling by modulating Gli ubiquitination and stability. Our findings reveal a conserved mechanism by which Ci/Gli is stabilized by a deubiquitination enzyme and identify Usp7/HUASP as a critical regulator of Hh signaling and potential therapeutic target for Hh-related cancers.

2. Stability of HIB-Cul3 E3 ligase adaptor HIB Is Regulated by Self-degradation and Availability of Its Substrates

The HIB-Cul3 complex E3 ligase regulates physiological homeostasis through regulating its substrate stability and its activity can be modulated by changing HIB abundance. However, regulation of HIB remains elusive. Here we provide evidence that HIB is degraded through the proteasome by Cul3-mediated polyubiquitination in K48 manner in Drosophila. Strikingly, HIB is targeted for degradation by itself. We further identify that three degrons (52LKSS56T, 76LDEE80S and 117MESQ121R) and K185 and K198 of HIB are essential for its auto-degradation. Finally, we demonstrate that HIB-Cul3 substrates, Ci and Puc, can effectively protect HIB from HIB-Cul3-mediated degradation. Taken together, our study indicates that there is an exquisite equilibrium between the adaptor and targets to achieve the tight control of the HIB, which is essential for maintaining suitable Hh and JNK signaling. The mechanism of adaptor self-degradation and reciprocal control of the abundance between adaptor and its substrates is also applied to BTB-Cul3 E3 ligase adaptor dKeap1, dDiablo and dKLHL18.

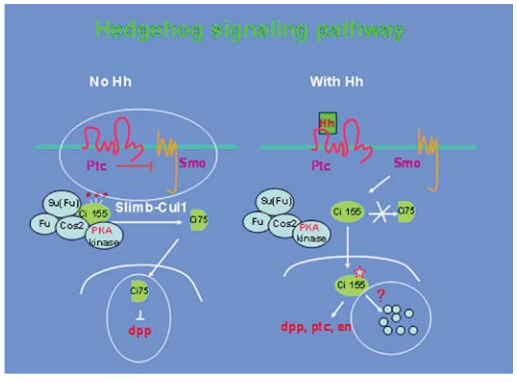

Fig1. Hedgehog signaling pathway in Drosophila.

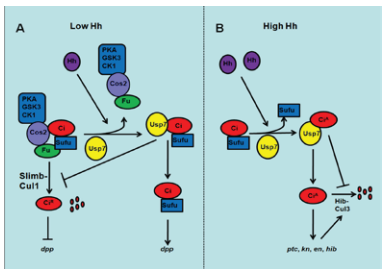

Figure 2. Regulation of the ubiquitin/proteasome-mediated proteolysis of Ci/Gli transcription factors is central to Hh signaling, but whether deubiquitinase is involved in this process remains unknown. We unveil that the deubiquitinase Usp7/HUASP positively regulates Hh signaling through stabilizing Ci/Gli, thus identifying Usp7/HUASP as a potential therapeutic target for Hh-related cancers.

3. Up negatively regulates Hh pathway through attenuating JNK signaling

Hedgehog (Hh) signaling pathway plays important roles in the pattern formation and tissue homeostasis and its misregulation causes abnormal development and kinds of cancers. Lots of external and intrinsic factors have been identified to modulate Hh signaling activities, however, whether the specific metabolic pathway involved is not explored. Through RNAi-mediated screening, we find that a nucleotide metabolic enzyme, named Up, is a novel regulator of Hh pathway. Our study demonstrates that knockdown of up promotes ci transcription and finally upregulates Hh signaling activity. Furthermore, we find that knockdown of up activates JNK signaling which is necessary but not sufficient for turning up ci transcription. The epistasis analysis shows Up functions in the upstream of combgap (CG) to modulate ci transcription. Finally, we show the regulation of Up on Hh signaling is dependent on its metabolic enzyme activity. Taken together, our finding unveils a novel link between Hh pathway and the nucleotide metabolic factor, implying that impaired nucleotide metabolism may affect Hh signaling homeostasis.

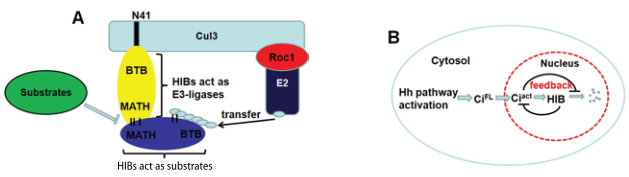

Figure 3. Above shows the model of HIB auto-regulation and the biological significance of this regulation.

(A) HIB protein associates with Cul3 through its BTB domain. HIB acting as an adaptor of HIB-Cul3 E3 ligase recruits dissociative HIB protein through the MATH domain, thus HIB-Cul3 promotes dissociative HIB ubiquitination and degradation. Substrates competitively bind the adaptor HIB, thus preventing dissociative HIB degradation. (B) The physiological significance of HIB auto-regulated degradation. In the presence of Hedgehog protein, Ci is transported to the nucleus and acts as a transcription activator. Ci promotes hib expression; conversely, HIB degrades Ci through ubiquitinating Ci. When Ci level is low, excess HIB is degraded in an auto-regulated ubiquitination. In contrast, when the HIB level is low, Ci will protect HIB from degradation. This delicate feedback loop allows appropriate Ci and HIB levels to remain in the nucleus.

Selected Publications

1. Meng H, Cao Y, Qin J, Song X, Zhang Q, Shi Y and Cao L. (2015) DNA methylation, its mediators and genome integrity. Int J Biol Sci. 11(5):604-617.
2. Zhou Z#, Yao X#, Li S#, Xiong Y, Dong X, Zhao Y, Jiang J*, Zhang Q*. (2015) Deubiquitination of Ci/Gli by Usp7/HAUSP Regulates Hedgehog Signaling. Dev Cell. 34(1):58-72.
3. Zhou Z, Xu C, Chen P, Liu C, Pang S, Yao X, Zhang Q*. (2015) Stability of HIB-Cul3 E3 ligase adaptor HIB Is Regulated by Self-degradation and Availability of Its Substrates. Sci Rep. 12;5:12709. doi: 10.1038/srep12709.
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Qingshun Zhao, Ph.D

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

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Zebrafish development

The research interests of the lab focus on investigating the molecular mechanism underlying vertebrate early development using zebrafish as a model animal.

RA (retinoic acid) plays essential roles in vertebrate embryogenesis. Its homeostasis in embryos is determined by the presence of Aldh1A that produces RA and Cyp26 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, 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, 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).

RA is essential for the formation of posterior neuroectoderm in vertebrate embryos. Performing microarray analysis, we identify znfl1 (zinc finger transcription factor) is changed its expression in response to RA signaling. Functional analyses reveal that zebrafish Znfl1s mediate the roles of RA in patterning posterior neuroectoderm by acting upstream of pou5f3 and sall4 (Figure 1).

RA signaling is also 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). On the other hand, it is also essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos (Cover; Junbo Li et al., 2016). Moreover, Ncor1 and Ncor2 play essential but distinct roles in zebrafish primitive myelopoiesis (Jingyun Li et al., 2014). Other than RA signaling, the differentiation of ventral mesoderm is affected by environmental factors, excessive sodium nitrite affects zebrafish valve leaflet formation by producing too much NO signaling (Junbo Li et al., 2014).

RA signaling is genetically controlled by upstream genes. Foxc1a is a member of the forkhead transcription factors. By generating foxc1a knockout zebrafish using TALEN (transcription activator-like effector nuclease) technology, we found foxc1a null embryos exhibited defective somites at early development. Comprehensive analyses on the expressions of the key genes that control processes of somitogenesis reveal that foxc1a plays an essential role in early somitogenesis by controlling Fgf and Notch signaling through restricting the expression of aldh1a2 in paraxial mesoderm directly (Jingyun Li et al., 2015).

Engineered endonuclease (EENs) including ZFN, TALEN and CRISPR/Cas9 are powerful tools to create genome edited animals without species limitation. Using the knock out tools of ZFN and TALEN, we 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). To increase the efficiency of germline transmission of induced mutations and particularly knockin alleles created by CRISPR/Cas9, we co-microinjected yfp-nanos3 mRNA with Cas9 mRNA, sgRNA and ssDNA donor. In comparison with the common practice of selecting founders by genotyping fin clips, our new strategy of selecting founders with tentatively fluorescent-labeled PGCs significantly increases the ease and speed of generating heritable knocking and knockout animals with CRISPR/Cas9 (Dong et al., 2014). Collaborating with the groups of Professors Zhou and Zhu, we developed an alternative novel tool for DNA editing (SGN: structure-guided nuclease) without target sequence limitation (Figure 2; Xu et al., 2016).

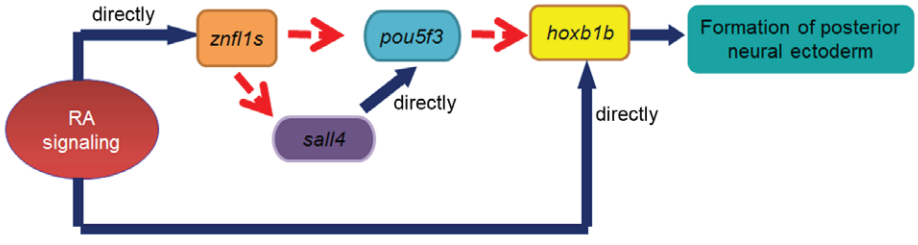

Figure 1. Proposed working model revealing that zebrafish Znfl1s mediate the roles of RA in patterning posterior neuroectoderm by acting upstream of pou5f3 and sall4. Black arrow shows direct regulation. Broken red arrow represents work upstream.

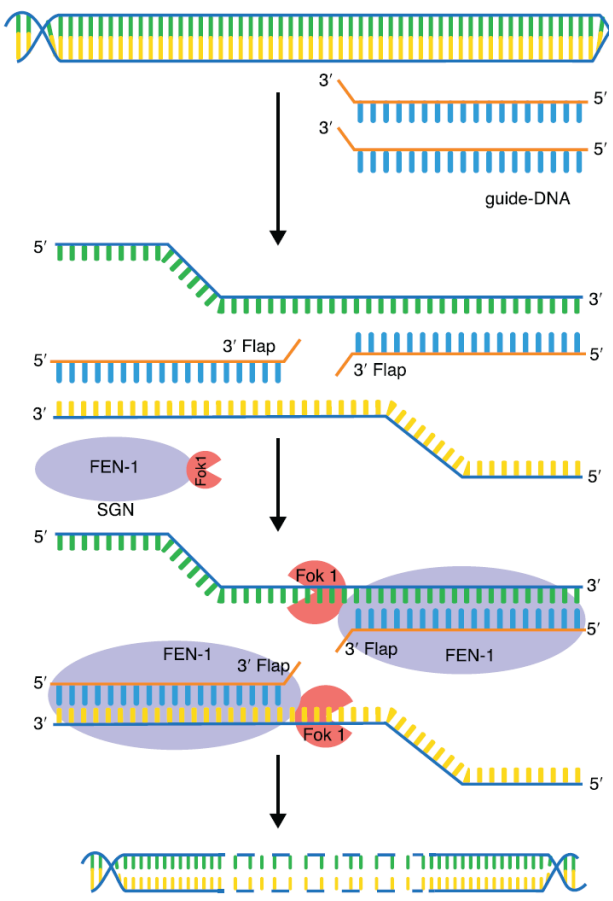

Figure 2. Genome editing using a structure-guided endonuclease (SGN).

SGN-mediated genome editing has two components: a SGN consisting of the FEN-1 enzyme fused with the Fok1 endonuclease and two 25-nucleotide target sequences with single 3′ unpaired bases. The two guides bind to the complementary sequences to form 3′ flap structure and the FEN-1 component of SGN recognizes the 3′ flap structure and guides the Fok1 dimer into position to generate a double-stranded cut, which then repairs by non-homologous end joining after what appears to be an expansion of the deleted region by a currently unknown mechanism. Taken from Varshney and Burgess [Genome Biology (2016) 17:187.DOI 10.1186/s13059-016-1055-4].

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

1. Shu Xu, Shasha Cao, Bingjie Zou, Yunyun Yue, Chun Gu, Xin Chen, Pei Wang, Xiaohua Dong, Zheng Xiang, Kai Li, Minsheng Zhu**, Qingshun Zhao**, Guohua Zhou*. 2016. An alternative novel tool for DNA editing without target sequence limitation: the structure-guided nuclease. Genome Biology. 17(1):186. doi: 10.1186/s13059-016-1038-5.
2. Junbo Li, Yunyun Yue, Qingshun Zhao*. 2016. Retinoic acid signaling is essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos. Zebrafish, 13(1):9-18. (Cover)
3. Jingyun Li, Yunyun Yue, Xiaohua Dong, Wenshuang Jia, Kui Li, Dong Liang, Zhangji Dong, Xiaoxiao Wang, Xiaoxi Nan, Qinxin Zhang, Qingshun Zhao*. 2015. Zebrafish foxc1a plays a crucial role in early somitogenesis by restricting the expression of aldh1a2 directly. The Journal of Biological Chemistry, 290(16):10216-28.
4. Zhangji Dong, Xiaohua Dong, Wenshang Jia, Shasha Cao, Qingshun Zhao*. 2014. Improving the efficiency for generation of genome-edited zebrafish by labelling primordial germ cells. The International Journal of Biochemistry & Cell Biology, 55:329-34.
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6. Dong Liang, Wenshuang Jia, Jingyun Li, Kui Li, Qingshun Zhao*. 2012. Retinoic acid signalling plays a restrictive role in zebrafish primitive myelopoiesis. PLoS ONE, 7(2): e30865.
7. Zhangji Dong, Jiachun Ge, Kui Li, Zhiqiang Xu, Dong Liang, Jingyun Li, Junbo Li, Wenshuang Jia, Yuehau Li, Xiaohua Dong, Shasha Cao, Xiaoxiao Wang, Jianlin Pan, Qingshun Zhao*. 2011. Heritable targeted inactivation of myostatin gene in yellow catfish (Pelteobagrus fulvidraco) using engineered zinc finger nucleases. PLoS ONE, 6(12):e28897.
8. Fang Xu, Kui Li, Miao Tian, Ping Hu, Wei Song, Jiong Chen, Xiang Gao, Qingshun Zhao*. 2009. N-CoR is required for patterning the anterior–posterior axis of zebrafish hindbrain by actively repressing retinoid signaling. Mechanism of Development, 126:771-780.
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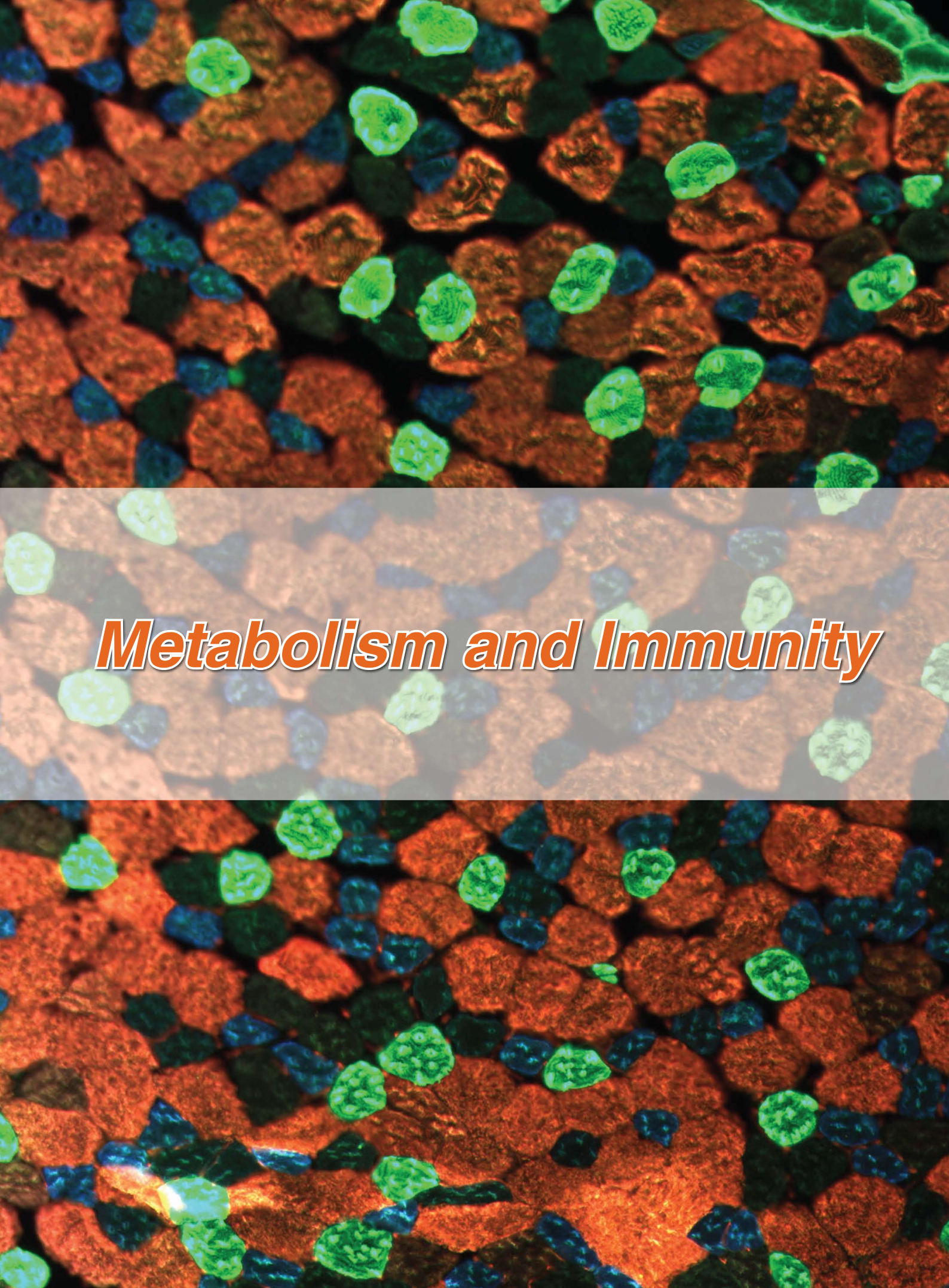
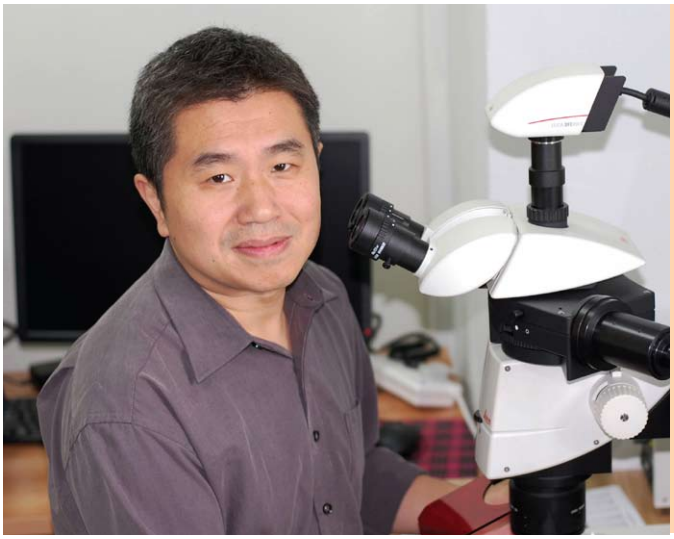


Metabolism and Immunity



Di Chen, Ph.D.

Di Chen got his Ph.D. in Genetics from the 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 developmental diapause. 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, Nanjing University as a Principle Investigator in 2013.

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Aging and Metabolism Using *C. elegans* as a Model

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. Appropriate genetic or environmental modulations not only extend lifespan but also delay age-related pathologies. Many exciting discoveries on the molecular basis 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 mechanistic Target of Rapamycin (mTOR) pathway play an important role in aging in many species. Our recently published work showed that simultaneous inhibition of DAF-2 (IGF-1 receptor) and mTOR target RSKS-1 (ribosomal S6 kinase) leads to a nearly 5-fold, synergistic lifespan extension in *C. elegans*. 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) is one of most robust environmental manipulations that slow down aging in various species. However, the molecular mechanisms of DR remain largely unknown. Previously, we demonstrated that the hypoxia inducible factor-1 (HIF-1) plays an important role in DR-induced lifespan extension by regulating the IRE-1 ER stress pathway. To gain better insights on the relationship between nutrients and aging, we performed an RNAi-based genetic screen and identified a key mediator of DR. Mutations in this gene affect DR-induced lifespan extension and lipid metabolism in a tissue-specific manner (Figure 2).

Currently, our research focuses on the following aspects:

1)Translatome analysis of the super long-lived *daf-2 rsks-1* double mutant;

2)Roles of lipid metabolism in dietary restriction-induced lifespan extension;

3)Roles of RNA metabolism in aging and age-related diseases.

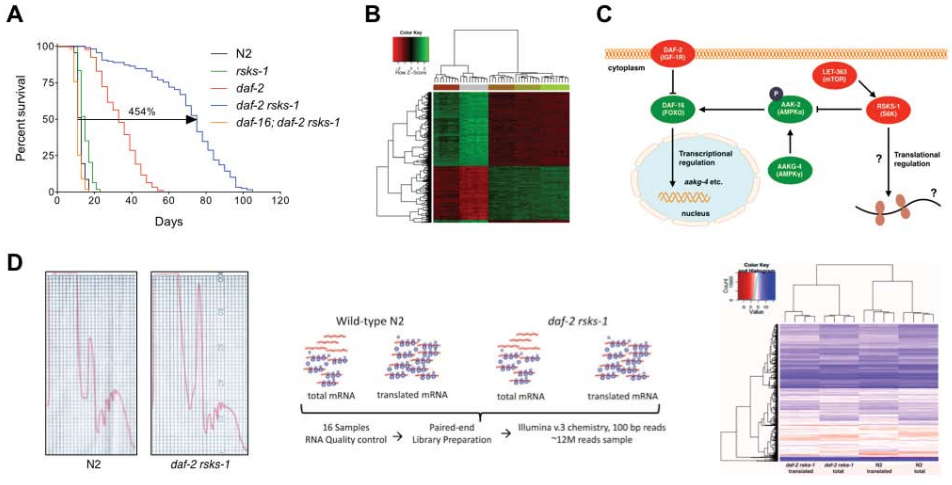

Figure 1. Functional genomics analysis of the super long-lived *daf-2 rsks-1* double mutant.

(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) Transcriptome analysis via microarrays helped to identify genes that are differentially expressed in the *daf-2 rsks-1* double mutant. (C) A model depicting the positive feedback regulation of DAF-16 via AMPK in the super long-lived *daf-2 rsks-1* double mutant. (D) Polysomal profiling and RNA-Seq were performed to identify genes that are regulated at the post-transcriptional levels in the *daf-2 rsks-1* double mutant.

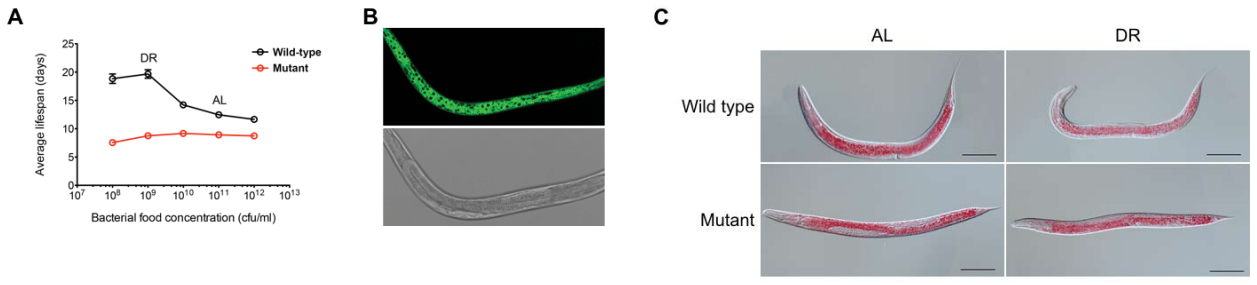

Figure 2. Characterization of lipid metabolism in dietary restriction-induced lifespan extension.

(A) Inhibition of certain lipid metabolism gene completely abolishes the lifespan extension by DR. (B) The key DR mediator gene is expressed in the epidermis. (C) Mutation in the key DR mediator gene results in excess lipid accumulation under DR.

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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.

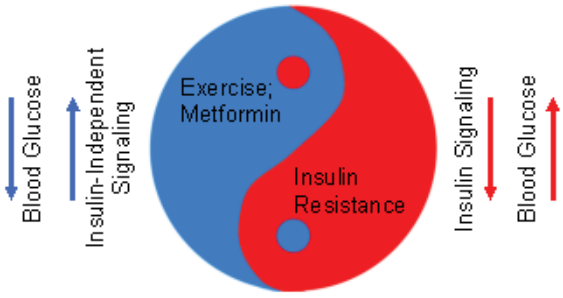

Figure 1 Insulin dependent and independent signaling in the pathogenesis of type II diabetes and its treatment.

The recent progresses of my lab is as follows:

Disruption of the AMPK–TBC1D1 nexus increases lipogenic gene expression and causes obesity in mice via promoting IGF1 secretion

Excess energy intake and physical inactivity are two major factors causing obesity, but the underlying mechanisms have not been fully understood. Both excess energy intake and physical inactivity increase cellular energy status that is monitored by the energy-sensing AMP-activated protein kinase (AMPK). We demonstrate that the AMPK–tre-2/USP6, BUB2, cdc16 domain family member 1 (TBC1D1) signaling nexus regulates insulin-like growth factor 1 (IGF1) secretion. Disruption of this AMPK–TBC1D1 signaling nexus in mice enhances lipogenic gene expression via promoting IGF1 secretion, causes obesity, and eventually leads to development of metabolic syndrome (Fig. 2). These findings reveal a novel regulatory mechanism that links energy status to development of obesity via control of IGF1 secretion (Chen L., Chen Q.L., Xie B.X.,…, Wang H.Y.*, Chen S.* 2016 PNAS).

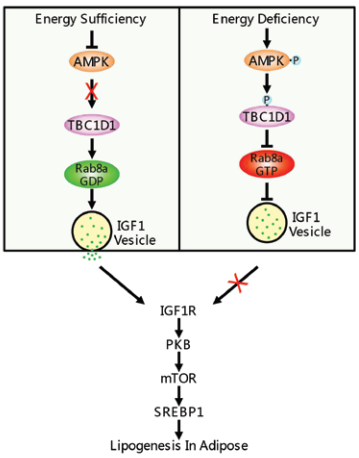

Figure 2 Disruption of the AMPK–TBC1D1 nexus increases lipogenic gene expression and causes obesity in mice via promoting IGF1 secretion

The inactivation of RabGAP function of AS160 promotes lysosomal degradation of GLUT4 and causes postprandial hyperglycemia and hyperinsulinemia

The AS160 is a Rab-GTPase activating protein (RabGAP) with several other functional domains, and its deficiency in mice or human patients lowers glucose transporter-4 (GLUT4) protein levels and causes severe insulin resistance. How its deficiency causes diminished GLUT4 proteins remains unknown. We found that deletion of AS160 decreased GLUT4 levels in a cell/tissue autonomous manner. Consequently, skeletal muscle-specific deletion of AS160 caused postprandial hyperglycemia and hyperinsulinemia. The pathogenic effects of AS160 deletion are mainly, if not exclusively, due to the loss of its RabGAP function since the RabGAP inactive AS160R917K mutant mice phenocopied the AS160 knockout mice. The inactivation of RabGAP of AS160 promotes lysosomal degradation of GLUT4 and inhibition of lysosome function could restore GLUT4 protein levels (Fig. 3). Collectively, these findings demonstrate that the RabGAP activity of AS160 maintains GLUT4 protein levels in a cell/tissue autonomous manner and its inactivation causes lysosomal degradation of GLUT4 and postprandial hyperglycemia and hyperinsulinemia (Xie B.X.,…, Wang H.Y.*, Chen S.* 2016 Diabetes).

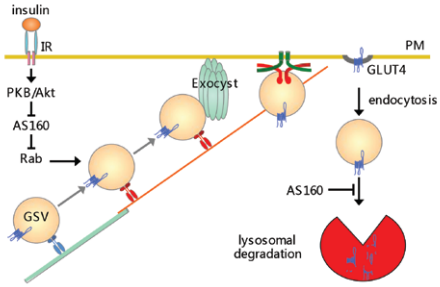

Figure 3 The inactivation of RabGAP function of AS160 promotes lysosomal degradation of GLUT4 and causes postprandial hyperglycemia and hyperinsulinemia

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1. Chen QL, Xie BX, Zhu SS, Rong P, Sheng Y, Ducommun S, Chen L, Quan C, Li M, Sakamoto K, MacKintosh C, Chen S* and Wang HY* A TBC1D1Ser231Ala knockin mutation partially impairs 5-aminoimidazole-4-carboxamide-1-ß-D-ribofuranoside- but not exercise-induced muscle glucose uptake in mice. Diabetologia (in press) (* corresponding author)
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3. Chen L., Chen Q.L., Xie B.X., Quan C., Sheng Y., Zhu S.S., Rong P., Zhou S.L., Sakamoto K., MacKintosh C., Wang H.Y. * and Chen S.* (2016) Disruption of the AMPK–TBC1D1 nexus increases lipogenic gene expression and causes obesity in mice via promoting IGF1 secretion. PNAS 113(26): 7219-24 (* corresponding author)
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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.

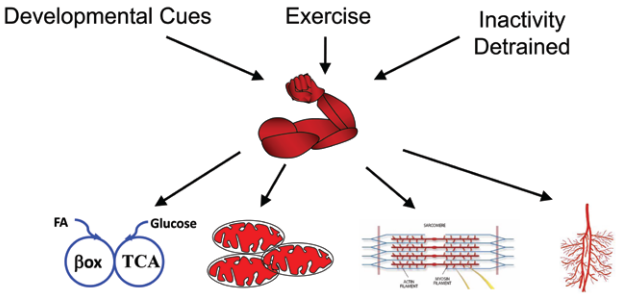

Fig 1. The muscle fitness is determined by developmental as well as physiological inputs. These inputs coordinately control the programs including fuel burning, mitochondrial ATP production, contraction, and angiogenesis.

Delineate the nuclear receptor/microRNA networks controlling muscle fitness.

Skeletal muscle contractile properties are tightly coupled to its metabolic capacity. Muscle fibers are classified into slow-twitch (Type I) and fast-twitch (Type II). Type I myofibers are characterized by high endurance and are mitochondrial-rich (red), relying largely on mitochondrial oxidative metabolism for ATP production. In contrast, Type II myofibers are low endurance and contain fewer mitochondria, and primarily rely on glycolytic metabolism for energy production. Muscle fibers exhibit remarkable plasticity, undergoing extensive metabolic and structural remodeling in response to physiological stimuli and systemic diseases.

During fiber type transition, the contractile machinery and energy production system must be precisely coordinated to maintain muscle function. However, the mechanism for precise coupling of mitochondrial function and muscle contractile machinery upon adaption to physiological stimuli remains unknown. Recently, we discovered a novel mechanism for muscle contractile property tightly coupled to its metabolic capacity during fiber type transition. Specifically, the myosin Myh7b gene encodes miR-499, which directly inhibits Fnip1, leading to activation of AMPK-PGC-1α signaling and thereby triggering a muscle mitochondrial oxidative metabolism program. We therefore propose a model for the adaptive mitochondrial function during muscle fiber type transition via the miR-499/Fnip1/AMPK circuit (Fig. 2). This mechanism likely represents a general paradigm for efficiently couple cellular energy consumption with ATP production under an array of diverse physiological and pathophysiological circumstances.

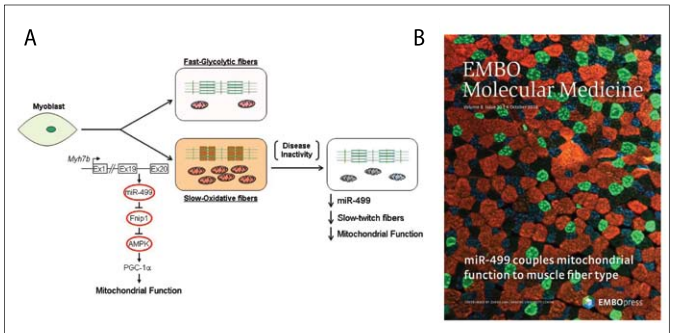

Fig 2. (A) The schematic depicts a proposed model for the miR-499/Fnip1/AMPK circuit that orchestrates mitochondrial function to match muscle contractile machinery during fiber type transition. (B) EMBO Mol Med. October 2016 Cover.

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

Exercise is known to be the best medicine for many chronic illness including obesity, diabetes, muscular diseases and aging, by promoting favorable metabolic and structural adaptations to improve muscle 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). In addition, we recently discovered a novel mechanism for exercise induced metabolic changes in skeletal muscle. Our results support the following conclusions: 1) LDHB

expression is induced by exercise in human muscle and negatively correlated with changes in intramuscular pH levels during muscle contraction; 2) exercise-induced PGC-1 α signaling directly regulates the transcription of the Ldhb gene by coactivating multiple cis-regulatory elements in the Ldhb promoter; 3) Chronic activation of Ldhb triggers a secondary mitochondrial oxidative metabolism program in skeletal muscle. We therefore identify a previously unrecognized Ldhb-driven alteration in muscle mitochondrial function and suggest a mechanism for the adaptive metabolic response induced by exercise training (Fig. 3).

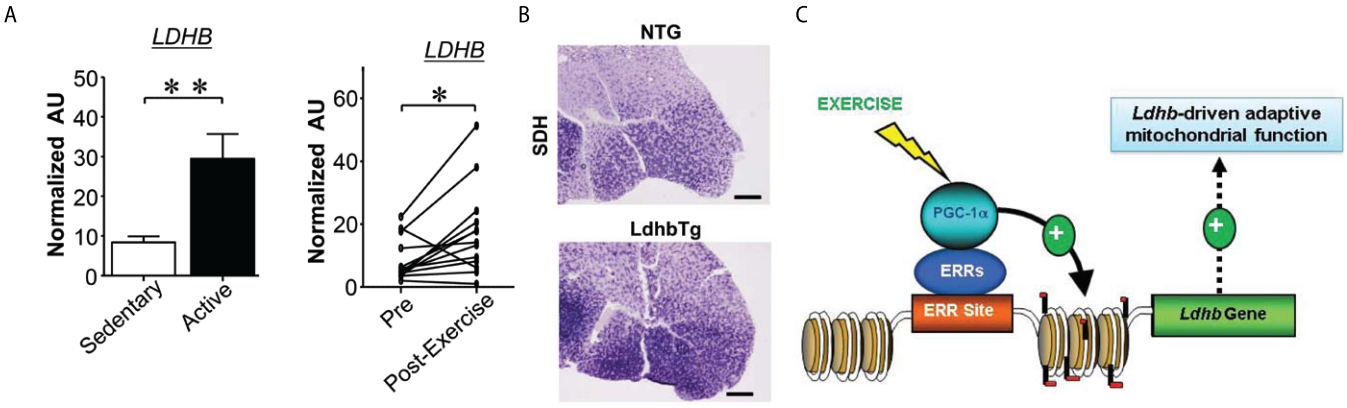

Fig 3. Exercise inducible lactate dehydrogenase B regulates mitochondrial function in skeletal muscle.

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Metabolic homeostasis and pathogenesis

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

Recently, our research showed that duodenum-jejunum gastric bypass (DJB) surgery may be applied to cure diabetes of both genetic (mutation) and environmental (diet-induced) origin. The research was performed in theT2DM mouse modelthat mimics key symptoms including insulin resistance, high blood levels of lipids, metabolic inflammation, and obesity. These mice harbor genetic mutation in brain-derived neurotrophic factor (Bdnf) leading to Bdnf deficiency. Glucose tolerance and insulin sensitivity were greatly improved and there was less fat accumulation in liver and white adipose tissue after DJB surgery.Our data indicate that suppressed inflammation is the result, not the cause, of diabetes reversal in these genetically modified mice and DJB surgery induced gut microbiota alterations may be the key reason for diabetes remission.

Another project in our lab is to dissect the potential role of Jmjd3in control the metabolic control neurons in the brain. We found cellspecific gene targeting of Jmjd3 in RIP neuron can also lead to lateronset of obese, but only in female(Fig1). Now we know it actually control the Kisspeptin gene expression. By regulating the menstrual cycle, Kisspeptin is a key mediator for female hormone release. Therelationship between estrogen and body fat accumulation is wellestablish in previous study. Our studies added a new piece for solvingthe puzzle between the CNS complicated control among CNS function,reproductive function, and metabolic homeostasis.

Understanding the metabolic homeostasis may also be tackled atdifference stages, the establishment, maintenance, and reset of aspecific physiological status. One of the projects in my lab is try tounderstand the obesity "memory". It is commonly believed an obeseperson will obtain a tendency to get fatty again after losing weight byfood restriction or excises. We confirmed this phenomenon using mousemodels (Fig2). The mice used to be fat gain body weight fasterthan the little mate control in both normal diet as well as high fat diet,suggesting a "memory" of old physiological status. After bone marrow transplantation, we demonstrated that the "memory" existed in bone marrow and related to immune system. However, which cell types the "memory" depended on are still need more work to uncover.

The mystery of metabolic homeostasis is just beginning to beunderstood. Careful studies will not only shed the light in the regulatoryloops for the beauty of complicated life, but the potential cure fordiseases resulting from disruption of these feedback loops.

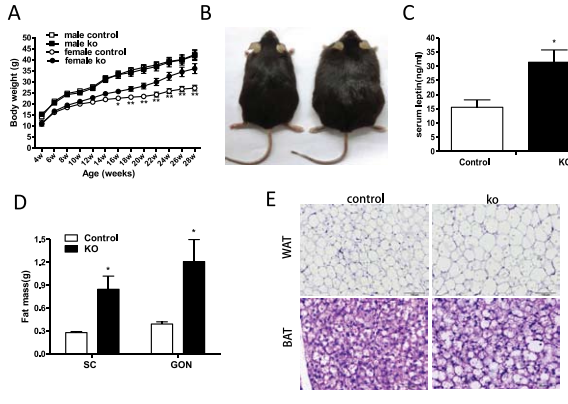

Figure1. Female specific obesity in Rip-Cre;Jmjd3lox/lox female mice.

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.

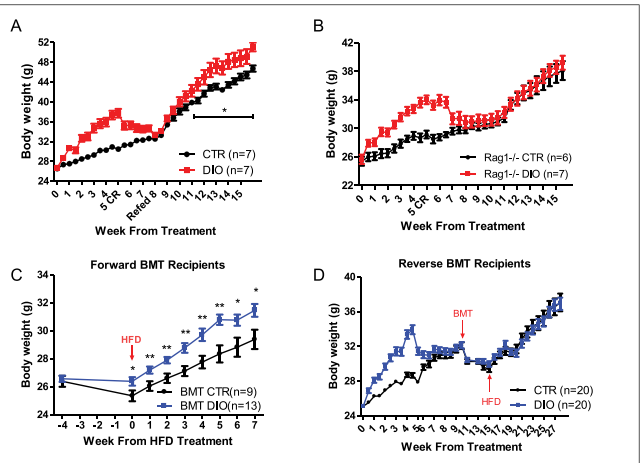

Figure2. Obesity memory relying on immune system can be revised by bone marrow transplantation.

(A) Body weight curve showing HFD-induced obesity memory in C57BL/6J mice. (B) Body weight curve of HFD-induced Rag1-/- mice. (C) Body weight curve of HFD-induced B6J mice receiving bone marrow cells from EGFP mice carrying obesity memory. (D) Body weight curve of HFD-induced B6J mice receiving bone marrow cells from normal EGFP mice.

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Chao-Jun Li, Ph.D

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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 directly target gene of Egr-1, which can positively feedback to increase Egr-1 accumulation during chronic stress stimulation through enhance Ras prenylation and membrane association (Am J Path, 2011a, 2011b; J Biol Chem 2011; EMBO J, 2011). The prenylation includes two type modifications of protein: farnesylation and geranylgeranylation. Our hypothesis is that the balance of protein farnesylation and geranylgeranylation or FPP and GGPP inside the cell is critical to cell homeostasis by affecting signal transduction and protein functions. Thus, we have constructed GGPPS Floxed mice and conditionally deleted GGPPS gene in different tissues to examine its functions on cell homeostasis and its involvements in human diseases. We found that GGPPS regulated protein prenylation balance is involved in spermatogenesis and infertility (J Exp Med, 2013; Sci Rep, 2016); hypertrophy and heart failure (J Path, 2015); insulin granule docked pool formation (J Path, 2016); lipid-induced muscle insulin resistance (J Biol Chem, 2015); pulmonary development (Am J Path, 2016). We are also exploring the function of protein dephosphorylation during liver injury and liver regeneration (J Hepal, 2016) that might mediate the interaction of hepatocyte and stellate cell.

1. PP2Acα deficiency improves acute liver injury microenvironment via NF-κB/Plg pathway (Bin Xue; Chao-Jun Li)

During early acute liver injury (ALI), damaged hepatocytes and non-parenchymal cells, such as hepatic stellate cells (HSCs), constructed the ALI microenvironment to aggravate liver injury. However, the interaction between hepatocyte necrosis and active HSC-induced fibrogenesis is still unclear. We detected high expression of protein phosphatase 2A cα (PP2Acα) in patient samples of subacute hepatitis consistent with the carbon tetrachloride (CCl4)-induced ALI animal model. Hepatocyte-specific knockdown of PP2Acα alleviated ALI by decreasing necrosis. Further study revealed that inhibiting PP2Acα relieved hepatocyte necrosis by activating nuclear factor κB (NF-κB) to inhibit ROS/TNF-α/JNK signaling. Moreover, NF-κB directly activated plasminogen (Plg) transcription, which diminished HSC activation by decreasing active TGF-β and thereby relieved hepatic fibrogenesis. Additionally, TGF-β from active autocrine HSCs stimulated the expression of PP2Acα in hepatocytes, which developed a positive feedback loop between hepatocytes and HSCs. Finally, the PP2Acα inhibitor okadaic acid also relieved hepatic fibrogenesis in in vitro experiments. Conclusions: Inhibiting PP2Acα in ALI decreased liver necrosis and fibrogenesis by improving the injury microenvironment.

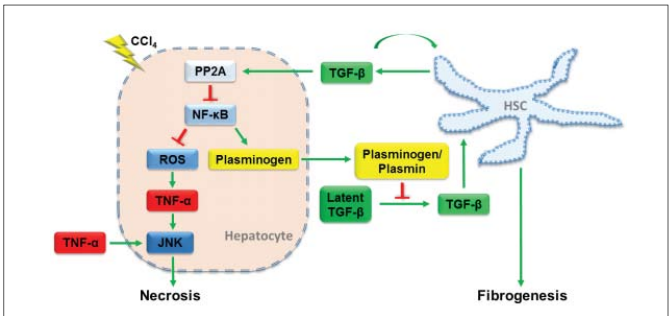

Figure 1. A positive feedback loop model between hepatocyte and HSC for the regulation of ALI by PP2Acα.

2. Hepatic exosomes remodel white adipose tissue under high fat diet via GGPPS mediated Rab27A geranylgeranylation (Yue Zhao; Chao-Jun Li)

The liver plays a central role in systemic glucose and lipid metabolism by releasing specific hepatokine. Specially, liver significantly influences adipose tissue's function under metabolic disorder, like high fat-diet induced non-alcoholic fatty liver disease (NAFLD). However, the mechanism of liver-derived secretory factors to regulate adipose tissue's function is unclear. Our results have shown that the expression of Geranylgeranyldiphosphate synthase (GGPPS) in the liver was highly increased in obesity-associated NAFLD models and GGPPS influenced hepatic lipid metabolism. Meanwhile, the hepatic metabolic disorder led to the changes of white adipose tissue's function. Further study revealed that exosome released increasingly from liver of HFD-treated mice but decreasingly from liver of L-Ggpps KO mice. Moreover, GGPPS depletion in liver decreased Rab27A prenylation, which significantly influenced exosomes secretion. Finally, miRNA microarray showed that the expression of specific miRNAs contained in exosomes obviously changed in L-Ggpps KO mice, which caused the remodeling of white adipose tissue. Our findings demonstrate the novel factor - exosome regulating the crosstalk between liver and white adipose tissue and the important role of GGPPS in the hepatic exosomes release.

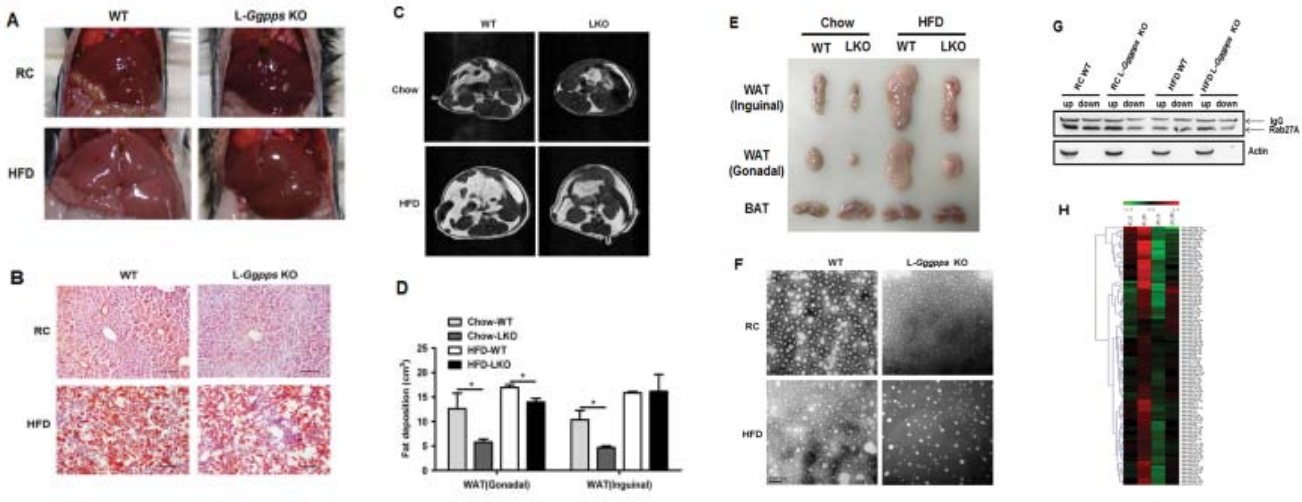

Fig.2 (A) Liver of WT mice and LGgpps KO mice. (B) H&E and oil-red staining of liver. (C) NMR of inguinal and gonadal adipose tissue. (D) Statistics of fat deposition. (E) Representative photographs of adipose tissue. (F) Exosomes of primary hepatocytes. (G) The prenylation of Rab27A in liver. (H) miRNA microarray of exosomes derived from primary hepatocytes.

3. FPP/GGPP regulates liver glucose and lipid metabolism via directly binding to its target proteins (Lei Fang; Chao-Jun Li)

With the deepening of cirrhosis and malignancy of the liver, geranylgeranyl pyrophosphate synthase (GGPPS) expression in patients with hepatocellular carcinoma (HCC) increased significantly. Phenotype analysis of GGPPS liver specific knockout mice revealed that GGPPS plays an important role in the regulation of liver metabolism. Thus, our study focuses on: (1) How FPP/GGPP regulates liver glucose and lipid metabolism via directly binding to its target proteins? Our previous study demonstrates that FPP/GGPP participate in liver glucose and lipid metabolism via directly interacting with its target proteins. However, the list of these FPP/GGPP binding proteins and the function of their interaction with FPP/GGPP are poorly defined. In our undergoing study, we attempt to establish an extensive interaction network of FPP/GGPP binding protein in mouse liver using affinity purification and mass spectrometry. Biotin GGPP was synthesized and used for the enrichment of its binding proteins. More than two hundred proteins were identified as confident GGPP binding proteins, and some of them were validated by Western blot and microscale thermophoresis. Bioinformatics analyses of GGPP binding proteins showed that a great number of key enzymes and proteins in liver glucose and lipid metabolism are enriched, indicating that GGPP does affect its target proteins independently as a small drug molecule besides its role in prenylation. To our knowledge, this study is the first report of GGPP direct binding proteins in liver, which provides alternative and additional explanations of severe phenotypes of GGPPS LKO mice. The notion that a metabolite, e.g. FPP/GGPP, functions as a modulator of signal transduction and important metabolism pathway is also novel. (2) Global identification of prenylated proteins using affinity purification and mass spectrometry. In this study, we are going to enrich prenylated proteins using liposome, subject them to mass spectrometry analysis and search against a novel in house developed prenylated proteins database. This method would be valuable for identify dynamic changes of global protein prenylation under disease and stress conditions.

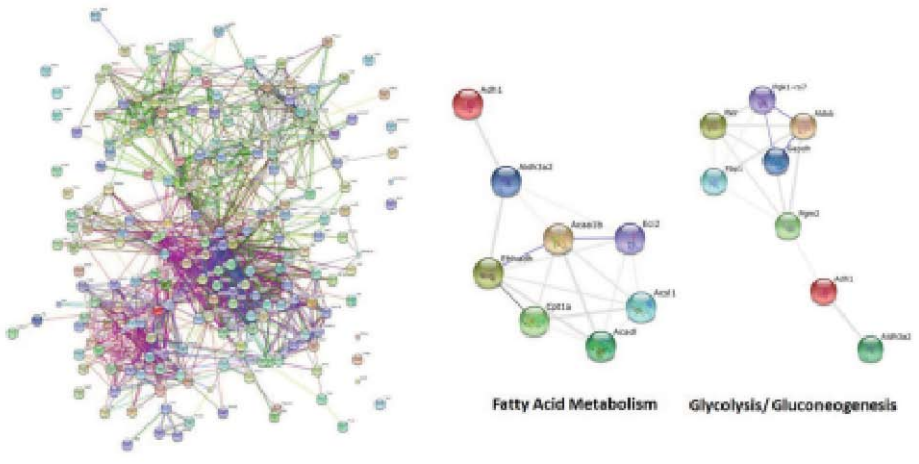

Fig.3 Protein interaction network of potential GGPP binding proteins in primary hepatocytes

Selected Publications (#:Co-first authors; *:Co-corresponding authors)

1. Fan Diao#, Chen Jiang#, et al., Bing Yao*, Chao-Jun Li*. Alteration of protein prenylation promotes spermatogonial differentiation and exhausts spermatogonial stem cells in newborn mice. Sci Reports, 2016, 6:28917 DOI: 10.1038/srep28917
2. Wen-Jun Jia#, Shan Jiang#, et al., Wen Ning*, Chao-Jun Li*. GGPPS (Geranylgeranyl diphosphate synthase) modulates fetal lung branching morphogenesis possibly through controlling K-Ras prenylation. Am J Pathol, 2016. 186(6):1454-1465
3. Shan-Shan Lai,et al., Xiang Gao*, Chao-Jun Li*, Bin Xue* PP2Acα Positively Regulates Mice Liver Regeneration Termination through AKT/GSK3β/Cyclin D1 Pathway. J Hepatology 2016, 64(2):352-360
4. Shan Jiang#, Di Shen#, et al., Bin Xue*, and Chao-Jun Li*. GGPPS mediated Rab27A geranylgeranylation regulates β-cell dysfunction during type 2 diabetes development via affecting insulin granule docked pool formation. J Pathol, 2016; 238: 109-119.(Commentary by Kowluru A. A lack of "glue" misplaces Rab27A to cause islet dysfunction in diabetes. J Pathol. 2016; 238: 375–377)
5. Weiwei Tao,et al., Jie Du* & Chao-Jun Li*. EGR1 regulates hepatic clock gene amplitude by activating Per1 transcription. Scientific Reports, 2015; 5:15212 DOI: 10.1038/srep15212
6. Weiwei Tao, et al., Bin Xue*, and Chao-Jun Li*. Lipid-induced Muscle Insulin Resistance Is Mediated by GGPPS via Modulation of the RhoA/Rho Kinase Signaling Pathway. J Biol. Chem, 2015; 290(33):20086–20097
7. Na Xu, et al., Chao-Jun Li*. The alteration of protein prenylation induces cardiomyocyte hypertrophy through Rheb/mTORC1 signaling and leads to chronic heart failure. J Pathol. 2015; 235: 672–685 (Cover Story)
8. Ning Shen#, Shan Jiang#, et al., Bin Xue*, Chao-Jun Li*. The constitutive activation of Egr-1/C/EBPa mediates the development of type 2 diabetes mellitus by enhancing hepatic gluconeogenesis. Am J Pathol. 2015, 185(2): 513-523.
9. Xiu-Xing Wang, et al., Xiang Gao*, Chao-Jun Li*. The protein prenylation alteration in Sertoli cells is associated with adult infertility resulted from childhood Mumps infection. J Exp Med. 2013, 210(8):1559-1574.
10. Ning Shen#, Yue Shao#, et al., Hua-Qun Chen*, Chao-Jun Li*. GGPPS, a new egr-1 target gene, reactivates erk1/2 signaling through increasing ras prenylation. Am J Pathol., 2011,179(6):2740-2750. (#:Co-author)
11. Xiao Yu#, Ning Shen#, et al., Bin Xue*, Chao-Jun Li*. Egr-1 enhances insulin resistance by tilting the balance of PI3K/Akt and MAPK signaling in mice. The EMBO J., 2011, 30(18):3754-3765
12. Ning Shen#, Xiao Yu#, et al., Bin Xue*, Chao-Jun Li*. An early response transcription factor, Egr-1, enhances insulin resistance in type 2 diabetes with chronic hyperinsulinism. J Biol Chem., 2011, 286(16):14508-15. (#:Co-author)

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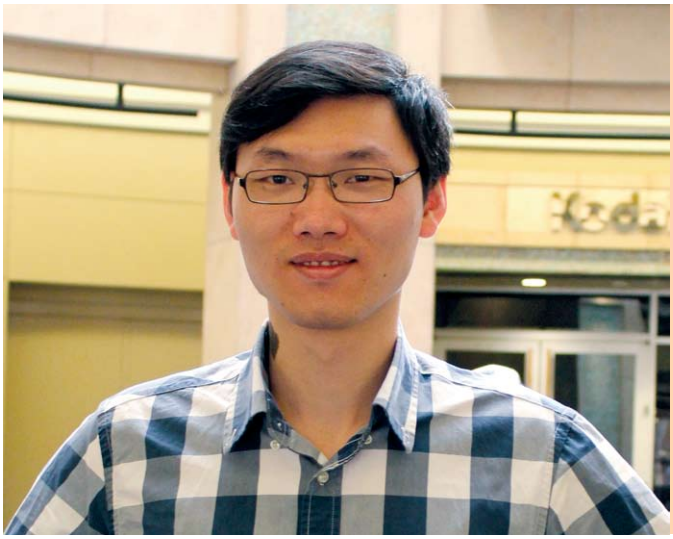

Guoqiang Wan, Ph.D.

Guoqiang Wan received both of his BSc in 2004 and PhD in 2011 from the National University of Singapore. He then had postdoctoral training with Dr Gabriel Corfas first at the Harvard Medical School/Boston Children's Hospital from 2011-2014 and then at the University of Michigan from 2014-2016. He joined MARC of Nanjing University as Principal Investigator in July 2016. His long term research goal is to regenerate cochlear cells and synapses for hearing restoration.

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Regeneration of Auditory Cells and Synapses for Hearing Restoration

In China, 27.8 million people suffer from disabling hearing loss and this number increases by 300,000 every year. Sensorineural hearing loss (SNHL) accounts for 90% of all hearing loss and in most cases it cannot be medically or surgically treated. Mechanistically, SNHL results from damages to the sensory hair cells that are essential for sound detection and/or the spiral ganglion neurons (SGNs) that are required for transmitting the acoustic signals to the brain. In addition, even with the presence of intact sensory epithelia, hearing problems can also arise from irreversible loss of the synaptic connections between hair cells and SGNs, an auditory pathology termed as cochlear synaptopathy. Therefore, restoration of auditory functions requires not only preservation or regeneration of the sensory hair cells, neurons and non-sensory supporting cells, but also re-establishment of the cochlear synaptic connections (Fig 1). Our lab aims to identify novel molecular targets and pathways for regeneration of cochlear cells and synapses and to explore therapeutic potentials of these targets for treatment of sensorineural hearing loss.

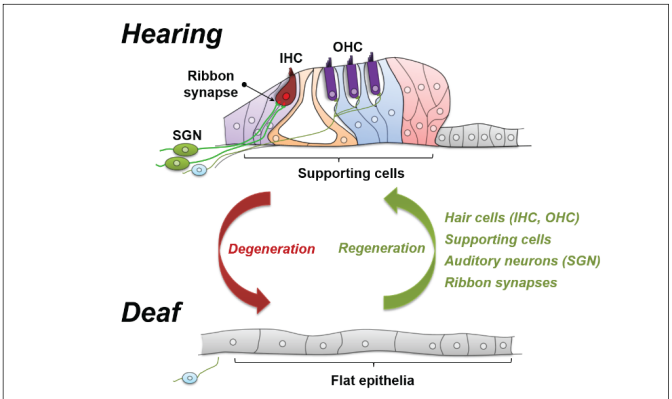


Fig 1. In mammalian cochlea, sensory hair cells (IHC and OHC), neurons (SGN), supporting cells and the auditory ribbon synapses are required for normal hearing, damage of these cells and synapses results in deafness.

1) Mechanisms and novel regulators of cochlear cell and synapse regeneration

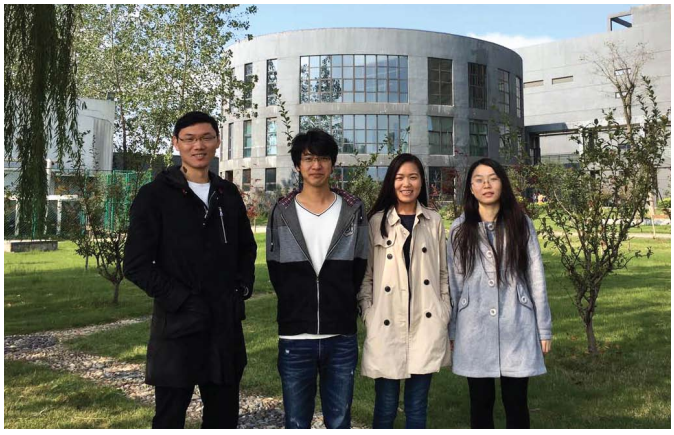
SNHL caused by loss of hair cells is associated with subsequent loss and differentiation of the supporting cells to epithelial cells, resulting in flat epithelia. Lack of supporting cells compromises the survival of hair cells. Therefore, regeneration of the sensory hair cells and non-sensory supporting cells are equally important to restore the structure and function of cochlea. Our lab will explore strategies and mechanisms to regenerate cochlear supporting cells and hair cells in the postnatal cochlea, with particular focus on Notch, Fgf and Lin28-let7 signaling pathways. Synaptic regeneration is a complex process that requires de novo transcription and translation, signaling activations, structural remodeling and functional coupling between pre- and post-synaptic sites. We will also take both targeted and unbiased approaches to identify and test novel regulatory signals for synaptic regeneration, including neurotrophic factors and cold-shock proteins.

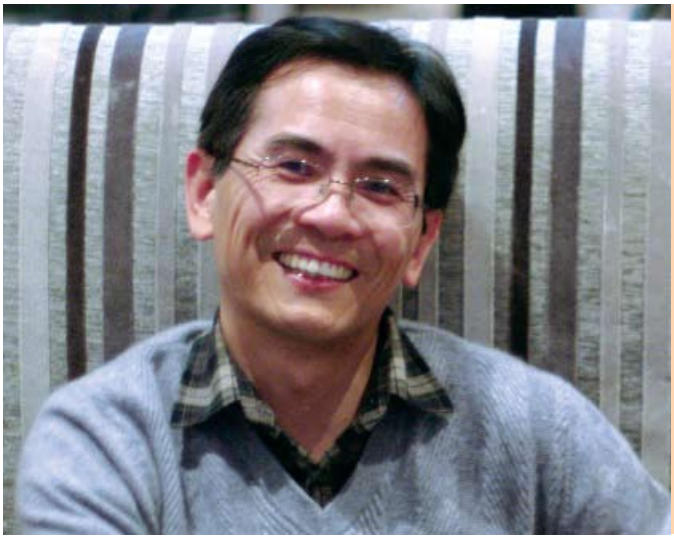
2) Identification and characterization of novel genes required for auditory function

More than 50% of prelingual deafness is genetic, most often autosomal recessive and nonsyndromic. Hearing impairment is genetically heterogeneous that may be caused by mutations in more than 100 genes. Our lab aims to establish a research platform to identify novel genes and mutations involved in auditory function and hearing loss. The candidate mutant mice will be interrogated with auditory physiology tests and inner ear histopathology analyses. These mutant mice and the research based on them will provide novel insights into the genetic caused and pathology of human deafness, and should point new ways to therapeutic interventions.

Selected Publications(*co-first authors, #co-senior authors)

1. Wan, G. & Corfas, G. (2015). No longer falling on deaf ears: mechanisms of degeneration and regeneration of cochlear ribbon synapses. Hearing Research, 329, 1-10.
2. Mellado Lagarde, M.M.*, Wan, G.*, Zhang, L., Gigliello, A.R., McInnis, J.J., Zhang, Y., Bergles, D.E., Zuo, J.# & Corfas, G.# (2014). Spontaneous regeneration of cochlear supporting cells after neonatal ablation ensures hearing in the adult mouse. Proceedings of the National Academy of Sciences of the United States of America, 111(47), 16919-24. Editor's Choice: Kiberstis, P.A. (2014). Science, 346(6214), 1197.
3. Wan, G., Gómez-Casati, M.E., Gigliello, A.R., Liberman, M.C. & Corfas, G. (2014). Neurotrophin-3 regulates ribbon synapse density in the cochlea and induces synapse regeneration after acoustic trauma. eLife, 3, e03564. Comment in: Cunningham, L.L. & Tucci, D.L. (2015). New England Journal of Medicine, 372(2), 181-182
4. Wan, G., Corfas, G. & Stone, J.S. (2013). Inner ear supporting cells: Rethinking the silent majority. Seminars in Cell & Developmental Biology, 24(5), 448-459.





Minsheng Zhu Ph.D.

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

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Smooth muscle 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, TMEM16A and Myl-9. Our observations suggest that Ca^{2+}/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; MYPT1 deletion causes phenotypic transition of phasic and tonic smooth muscles, and the myogenic alteration by MYPT1 deletion is enough for generation of hypertension; MYPT1 T694 phosphorylation is essential for sustained contraction of bladder smooth muscle, whereas MYPT T852 does not; the spontaneous contraction of sphincteric smooth muscle is mediated through a calcium spark-mediated activation of TMEM16A/VDCC/MLCK signaling (Fig.1&2). In addition to the studies on such basic principles of smooth muscle contraction, we have also been working on myogenic cause of Hirsprung disease and chronic constipation. Our mission is translate our novel knowledge of smooth muscle to the diseases.

Skeletal muscle is another important tissue of human body and its function and size may be regulated by micro RNA at multiple levels. Our previous studies suggest that the maternally expressed miR-379/miR-544 cluster might regulate skeletal muscle growth through the imprinted Delta-like 1 homolog (Dlk1) gene, thereby underlying the polar overdominance inheritance of callipyge sheep (Fig.3); miRNA23a may regulate muscular fiber property through targeting myosin gene. To understand the mechanistic pathogenesis of skeletal myopathy, we plan to assess the contribution of micro RNAs in the pathology of centered nuclear myopathy (CNM), and aim to provide with a new therapeutic strategy for this disease.

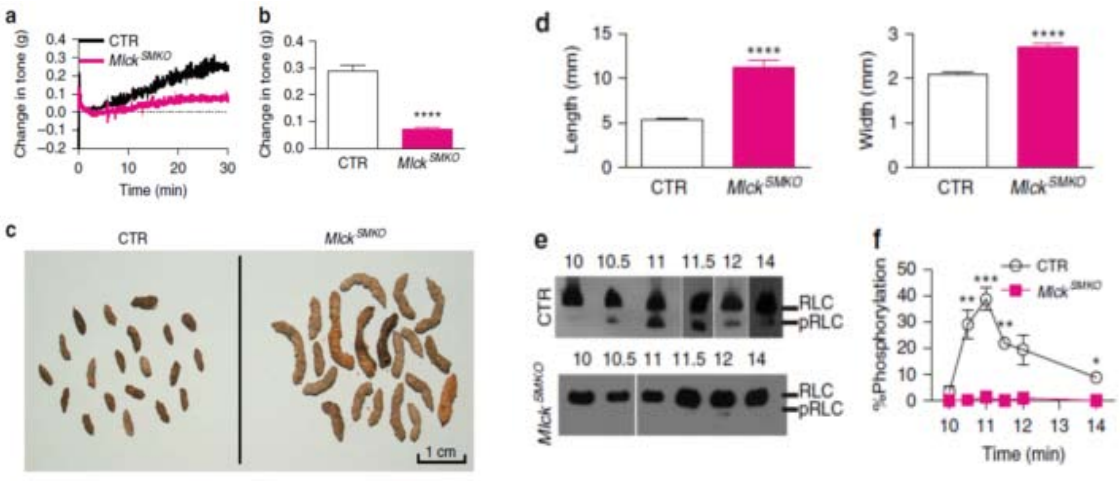

Figure 1 : MLCK and RLC phosphorylation are required for the basal tonein IAS.

(a) Time courses of changes in force after application of 0.5 g tension in CTR and $Mlck^{SMKO}$ mice. (b) Summarized data showing much smaller IAS tone in $Mlck^{SMKO}$ mice than in control (mean±s.e.m., CTRn=7, $Mlck^{SMKO}$ n=5, ****Po0.0001 by two-tailed Student's t-test). (c) Faeces from CTR mice and $Mlck^{SMKO}$ mice after 20 days of tamoxifen treatment. (d) The length (left) and width (right) of faeces were increased in $Mlck^{SMKO}$ mice compared with the controls. Bars represent mean±s.e.m., n=20, ****Po0.0001 by two-tailed Student's t-test. (e) Examples of RLC phosphorylation during the process of spontaneous tone generation in IAS from CTR and $Mlck^{SMKO}$ mice. (f) Quantification of p-RLC during basal tone generation in CTR and $Mlck^{SMKO}$ IAS. Bars represent mean±s.e.m., n=3, *P<0.05, **P<0.01, ***P<0.001 by two-tailed Student's t-test.

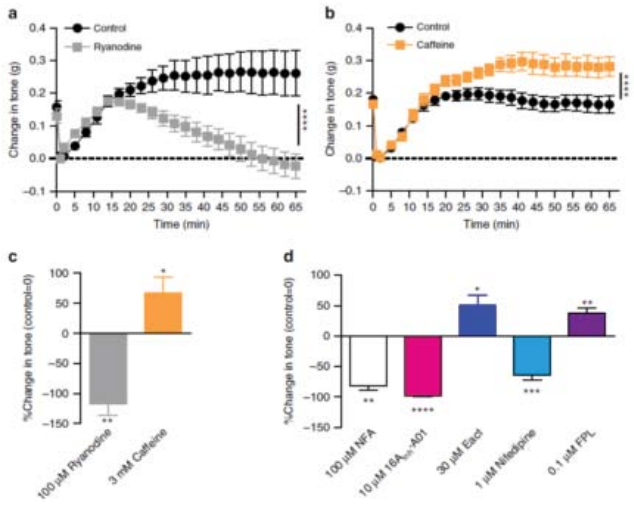

Figure 2 RyRs, ClCa channels and VDCCs are essential for the basal tone in IAS.

(a) Ryanodine (100 mM) significantly decreased the spontaneous tone in IAS. Bars represent mean±s.e.m., control n=5, ryanodine n=6, ****P<0.0001 by analysis of variance (ANOVA) comparing the sustained phases. (b) Caffeine (3mM) increased the tone in IAS. Bars represent mean±s.e.m., n=7, ****P<0.0001 by ANOVA comparing the sustained phases. (c) Summarized results on the IAS tone affected by 100mM ryanodine (n=6), 3mM caffeine (n=8). Bars depict mean±s.e.m., *P<0.05, **P<0.01 by paired two-tailed Student's t-test. (d) Summarized results on the IAS tone affected by 100 mM niflumic acid (NFA; n=8), 10 mM 16Ainh-A01 (n=4), 30 mMEact (n=5), 1 mM nifedipine (n=8) and 0.1 mM FPL64176 (n=5). Bars depict mean±s.e.m., *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 by paired two-tailed Student's t-test.

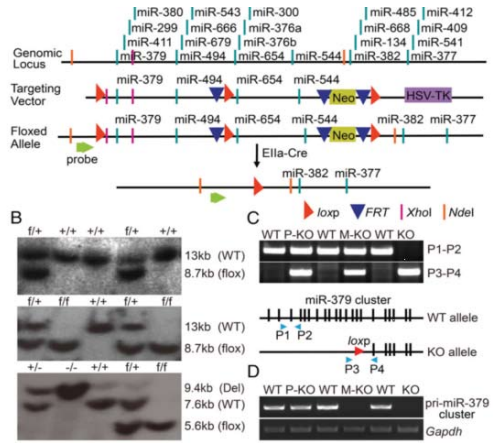

Figure 3. Generation and confirmation of miR-379/miR-544 cluster null mice.

(A) Schematic representation of the miR-379/miR-544 flox strategy. Three loxP sites were inserted into the genomic DNA. Thefirst loxP site was targeted upstream of miR-379, the second loxP site was inserted upstream of mir-376c,and the third loxP site, with a PGK-Neo cassette, was introduced downstream of miR-544. The first loxP cassette contained an XhoI site (blocks in red) designed for Southern blot analysis. The floxed allele formed after homologous recombination in ES cells. Mice containing the floxed allele were crossed with Ella-Cre mice to generate the miR-379/miR-544 cluster null allele. (B, Top) Southern blot analysis of miR-379cl floxed ES cells for blastocyst injection. Genomic DNA was digested with BamHI and hybridized with a P32-labeled probe. The 13-kb and 8.7-kb bands represent floxed and wild-type alleles, respectively (Middle) Southern blot analysis of miR-379cl floxed mice used for crosses. (Bottom) Southern blot analysis of miR-379cl floxed and miR-379cl null mice. Genomic DNA was digested with XhoI and NdeI. The 9.4-kb, 7.6-kb, and 5.6-kb bands represent the deleted, floxed, and wild-type alleles, respectively.(C) Genotype analysis of wild-type mice,heterozygotes, and homozygotes. The P1–P2 primer pair was used to detect the wild-type allele, whereas the P3–P4 primer pair was used for the miR-379cl null allele. (D) RT-PCR analysis of the expression level of the pri–miR-379/miR-544 cluster in the skeletal muscles of wild-type mice, M-KO heterozygotes, P-KO heterozygotes, and homozygotes (KO).

Selected Publications

1. Zhang CH, Wang P, Chen CP, Zhao W, Chen X, Chen C, He WQ, Qiao YN, Tao T, Sun J, Peng YJ, Craige SM, Lifshitz LM, Jr KJF, Fogarty KE, ZhuGe R, Zhu MS* The molecular basis underlying the genesis of basal tone in internal anal sphincter. Nature Communication 2016; 7:11358
2. Xu S, Cao S, Zou B, Yue Y, Gu C, Chen X, Wang P, Dong X, Xiang Z, Li K, Zhu MS*, Zhao QS*, Zhou GH*An alternative novel tool for DNA editing without target sequence limitation: the structure-guided nuclease.Genome Biol. 2016 Sep 15;17(1):186.
3. Liu J, Liang X, Zhou D, Lai L, Xiao L, Liu L, Fu T, Kong Y, Zhou Q, Vega RB, Zhu MS, Kelly DP, Gao X, Gan Z. Coupling of mitochondrial function and skeletal muscle fiber type by a miR-499/Fnip1/AMPK circuit. EMBO Mol Med. 2016 Oct 4;8(10):1212-1228

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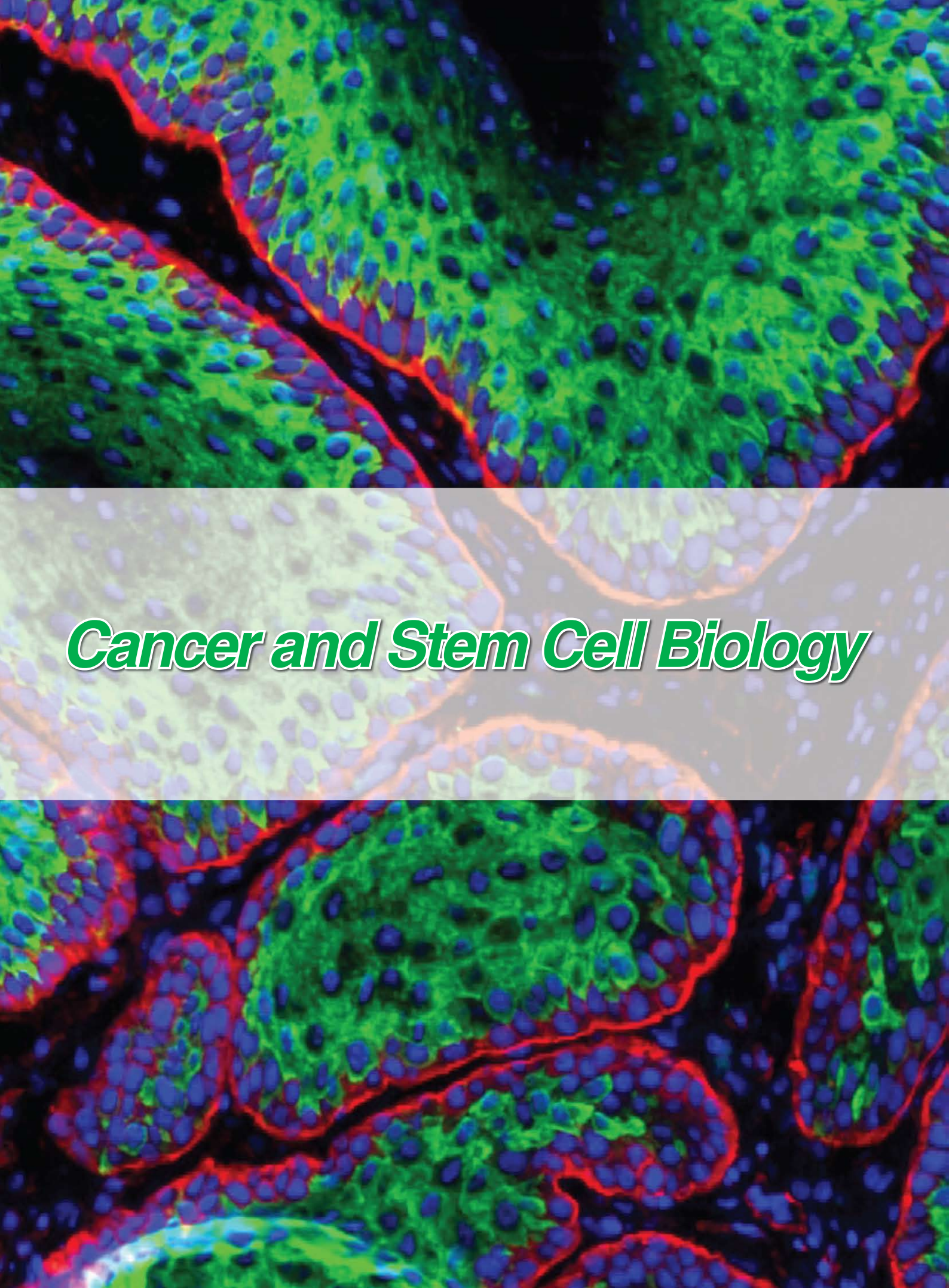
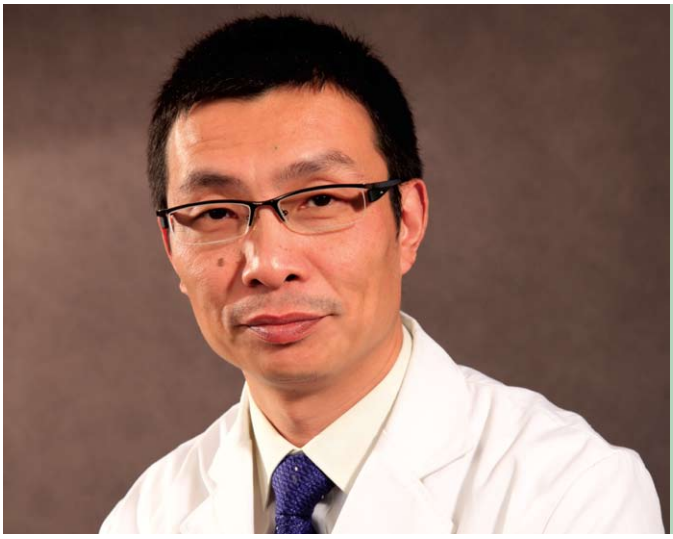
Cai-Ping Chen, Instructor of China Pharmaceutical University
Yun-Qian Gao, Postdoc research fellow of Fudan University
Yanjing Peng, Postdoc research fellow of Wiscoxin University


Cancer and Stem Cell Biology



Qing Jiang Ph.D., M.D.

Qing Jiang received his MD degree in Nanjing Medical University in 1989 and PhD degree in Beijing Medical University in 1999. In 2008, he was appointed professor in Nanjing University and moved to Model Animal Research Center (MARC) as Adjunct Professor for research on bone and joint disease. Qing Jiang's group has established human gene bank of bone and joint disease including osteoarthritis (OA), developmental dysplasia of the hip (DDH), deep venous thrombosis (DVT), ankylosing spondylitis (AS) and osteoporosis (OP). He is also the youth committee member of Chinese Orthopaedic Association (COA). Professor Jiang won the National Science Fund for Distinguished Young Scholars in 2011. He is the Master's Supervisor of Nanjing Medicine University, and the doctoral supervisor of Nanjing University.

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Skeletal System Disease

Developmental dysplasia of the hip (DDH) is the most frequent inborn deformity of the locomotors apparatus. Genetic factors play a considerable role in pathogenesis of DDH. At current stage, we have performed GWAS study of DDH. In a previous association study of DDH in North Chinese population, we had detected associations between DDH and single nucleotide polymorphisms (SNPs) in GDF5, TBX4, and ASPN by case-control studies in Chinese Han population. Our replication study indicated that the association between rs726252 and DDH in Chinese Han population was debatable. The association between PAPPA2 and DDH should be evaluated by additional studies.

Rare skeletal diseases usually are misdiagnosed, so that patient cannot get the optimal treatment. Genetic factors such as gene mutations play a considerable role in etiology and pathogenesis of these rare and developing diseases of skeletal system. Skeletal system rare hereditary disease research can help the researchers found that bone development of new genes and gene function, gene inheritance patterns. We performed genetic testing for the patient by using the next generation sequencing and direct nucleotide sequencing. Then we checked the target mutations in the proband's family members and healthy individuals by using direct nucleotide sequencing. We detected two novel mutations of WISP3 that responsible for Progressive pseudorheumatoid dysplasia, and one novel mutation in CHST3 that are responsible for Spondyloepiphyseal dysplasia with congenital joint dislocations. The DNA bank for rare skeletal diseases we had established is still enlarging.

Osteoarthritis (OA) is by far the most common type of joint disease. Our previous study also assessed the contribution of leptin gene (LEP) polymorphism(s) to knee OA among Han Chinese, and indicated that in normal weight and overweight Han Chinese, LEP polymorphisms, sex and BMI were associated with knee OA. Age was an independent risk factor for knee OA in the overweight population. Sex and BMI were risk factors for knee OA in the obese population. Our findings reveal a new paradigm for study of osteoarthritis etiology and athogenesis.

Deep vein thrombosis (DVT) remains to be major clinical problem despite decades of research effort. We evaluated the effects of NO microbubbles in an inferior vena cava (IVC) and left common iliac vein (LCIV) ligation-induced rat DVT model. We have demonstrated a clear effect of NO microbubbles on DVT resolution. Both thrombus weight and thrombus size (thrombus weight/thrombus length) significantly decreased in NO microbubbles group at day 8, suggesting that NO microbubbles had accelerated the progression of thrombolysis.

Cartilage repairation. Microfracture does not properly repair full-thickness cartilage defects. The purpose of this study was to evaluate the effect of intraarticular injection of the small-molecule compound kartogenin (KGN) on the restoration of a full-thickness cartilage defect treated with microfracture in a rabbit model. Intraarticular injection of KGN enhances the quality of full-thickness cartilage defects repair after microfracture, with better defect filling and increased hyaline-like cartilage formation (Fig 1) (Xu 2015). To confirm that KGN can induce human MSCs into chondrocytes, we compared the isolated SMSCs from the synovium tissue and the used pellets culture system with or without KGN. Our results showed that most of the cells adhered to the bottom of the culture dishes 24 h after the cell suspensions had been plated and the adherent cells exhibited a flattened and fibroblast-like morphology (Figure 2) (Shi 2016) and characteristics of MSCs. The tissue bank for cartilage and ligament has been established and enlarged.

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

1.MicroRNA as a biomarker for early diagnosis of deep vein thrombosis. (National Natural Science Foundation of China)

2.The research on whole exome sequencing of familiar Developmental dysplasia of the hip. (National Natural Science Foundation of China)

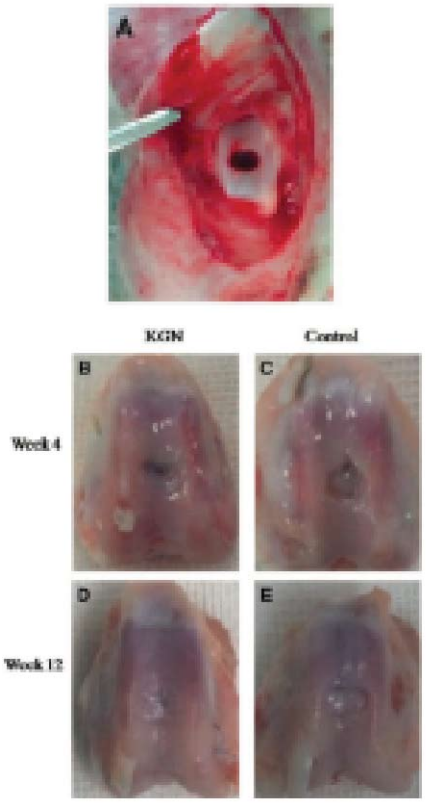

Figure 1. Microfractured cartilage defect

(A) and macroscopic appearance of the specimens in groups 1 (B, D) and 2 (C, E). Gross appearance was shown at 4 (B, C) and 12 weeks (D, E). (B) The repair tissue covered more than 50% of the cartilage defect. (C) Little repair tissue was observed in the cartilage defect. (D) Repair tissue almost completely covered the defect in the experimental group, but the defect in the control group (E) was insufficiently repaired.

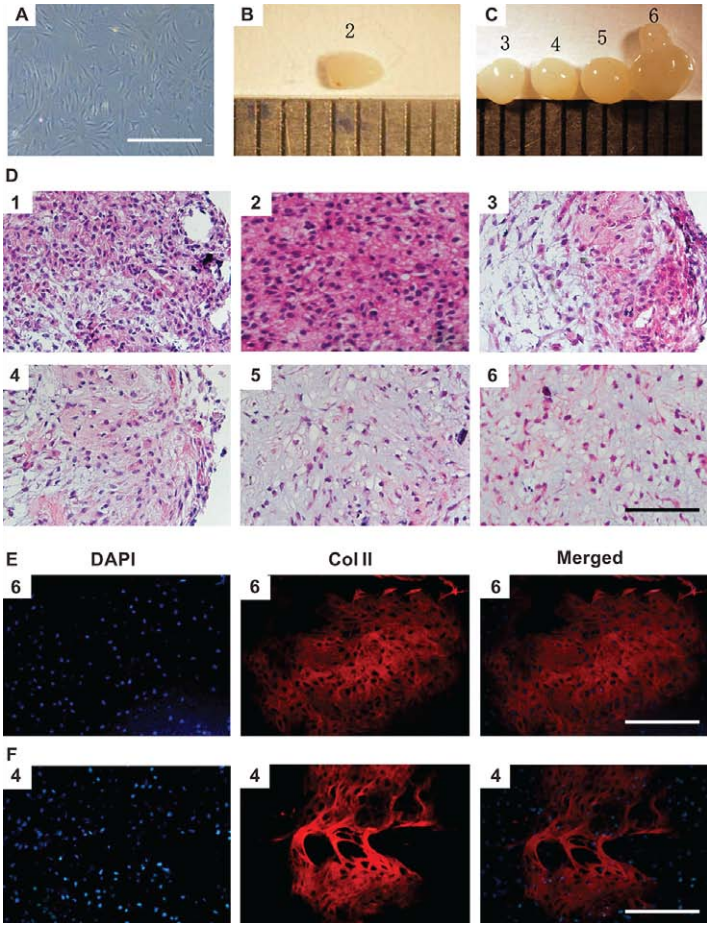

Figure 2.Chondrogenic potential of human MSCs derived from the synovium (SMSC) induced by chondrogenic medium supplemented withKGN. Passage-3 (P3) cells were pelleted and induced for 21 days in vitro.

(A) P3 SMSCs. (B) MSC pellets induced by 10 μM KGN alone,cultured for 21 days, cannot form a regular pellet. (C) Addition of KGN significantly increased the pellet size. P3 SMSCs pretreated withTGF-β3 for 1 week and induced with KGN for 2 weeks exhibited a similar pellet size to that of the TGF-β3 + KGN group and the TGF-β3 +BMP-2 group. (D1–D6) HE staining of the sections to reveal chondrocyte-like cells induced by different factors. Sections were numbered consistently with the pellets groups (1, control group; 2, KGN group; 3, TGF-β3+KGN group; 4, TGF-β3 1W, KGN 2W group; 5, TGF-β3+BMP-2 group; 6, TGF-β3+BMP-2+KGN group). (E,F) Immunofluorescence staining for collagen type II on pellets of the TGF-β3 + BMP-2 + KGN group (E) and the TGF-β3 1 week + KGN 2 weeks group (F) (n = 3). Scale bar: 100 μm in panels A, D, E, and F.

Selected publications (*corresponding author)

1. Nakajima M, Shi D, Dai J, Tsezou A, Zheng M, Norman PE, Takahashi A, Ikegawa S, Jiang Q*. Replication studies in various ethnic populations do not support the association of the HIF-2α SNP rs17039192 with knee osteoarthritis. Nat Med. 2011 Jan; 17(1):26-7.
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4. Xu Z, Mao P, Chen D, Shi D, Dai J, Yao Y, Jiang Q*. Tenosynovial giant cell tumor arising from the posterior cruciate ligament: a case report and literature. Int J ClinExpPathol 2015;8(6):6835-6840
5. Sun Y, Wang C, Hao Z, Dai J, Chen D, Xu Z, Shi D, Mao P, Teng H, Gao X, Hu Z, Shen H, Jiang Q*. A common variant of ubiquinol-cytochrome c reductase complex is associated with DDH. PLoS One. 2015 7; 10(4):e0120212.
6. Xu Z, Li L, Shi D, Chen D, Dai J, Yao Y, Teng H, Jiang Q*. The level of red cell distribution width cannot identify deep vein thrombosis in patients undergoing total joint arthroplasty. Blood Coagul Fibrinolysis. 2015, 26(3):298-301
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8. Rong Z, Xu Z, Sun Y, Yao Y, Song K, Chen D, Shi D, Dai J, Zheng M, Jiang Q *. Deep venous thrombosis in the non-operated leg after primary major lower extremity arthroplasty: a retrospective study based on diagnosis using venography. Blood Coagul Fibrinolysis. 2015, 26(7).
9. Hou H, Zheng K, Wang G, Ikegawa S, Zheng M, Gao X, Qin J, Teng H, Jiang Q*. Influence of intra-articular administration of trichostatina on autologous osteochondral transplantation in a rabbit model. Biomed Res Int 2015; 2015:470934.

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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 for cancer researchers 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. With genetic mouse models as a tool, our laboratory aims to uncover the critical functional and regulatory mechanisms of tumor suppression pathways in vivo in the hope of providing more useful strategy and targets for cancer therapy.

1. p53 regulatory mechanisms and cell fate control

P53 is extremely important for stress response and tumor suppression as exemplified by its mutations found in over 50% of human cancers. The knowledge of its functions 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 signaling pathway 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 expression pattern of endogenous p53 in the proliferating compartments during mouse development, postnatal growth, tissue homeostasis, regeneration and tumorigenesis. Importantly, p53 protein is selectively activated in the proliferating cells and tissues upon stress, highlighting p53 as a monitor of cellular proliferating state (Chen, et al., 2015).

In addition to the regulation at the expression level, it is well known that p53 protein stability and activity is tightly controlled by its negative regulators MDM2 and MDM4 in somatic cells. While we found that such regulation was also present in female germ cells and essential for mouse follicular development and fertility, our recent study further demonstrated a more tightened and rigorous regulatory mode in these cells, thus explaining the slow dynamics of p53 response in the oocytes under the treatment of chemotherapeutic drugs (Figure 1; Zhang et al., 2016).

In the presence of stress, the negative regulation is relieved and p53 is activated to exert its role in influencing the cell fate. Various degree of stresses result in different level of p53 activation. Instead of directing the classic pathways of cell cycle arrest, senescence or apoptosis, we demonstrated that low dose X-ray induced mild p53 activation affected the EMT process during valvuloseptal morphogenesis of mouse cardiac development and resulted in congenital heart defects in mice (Zhang, et al., 2012). Our more recent study found that mild p53 activation in cells renders them less competitive in a heterotypic setting when neighbored by wild type cells. These results suggest that mild p53 activation also critically influence cell behaviors and functions in distinctive manners.

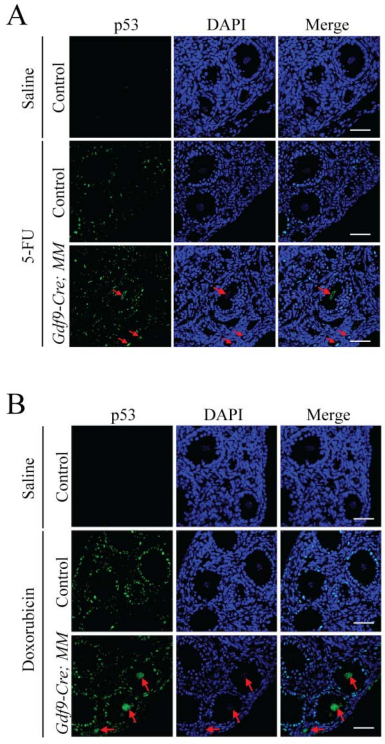

Figure 1. Treatment of 5-FU and Doxorubicin was able to induce p53 activation only in oocytes of mice with reduced dosage of both MDM2 and MDM4.

A, B: Immunofluorescent staining of p53 (green) in ovarian frozen sections of P10 Gdf9-Cre; Mdm2FM/+; Mdm4+/- (Gdf9-Cre; MM) mice and the control littermates 24 hours after a single intraperitoneal injection of 5-FU (A) or Doxorubicin (B). Arrows depicted positive stained nuclei. Scale bar=50μm.

2. Tumor microenvironment and metabolic reprogramming

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 tumor suppressors in inflammation, we found that p53 deficiency in myeloid lineage accelerated adenoma formation in Apcmin mice while p53 activation suppressed adenoma growth and colitis-associated tumor invasion. Interestingly, p53 suppressed the pro-inflammatory cytokine expression as well as M2 polarization in macrophages, both serving as targets of cancer therapy (He, et al., 2015). These and other evidence support a crucial role of tumor suppressors in modulating the tumor microenvironment, which in turn have impacts on tumorigenesis (Figure 2).

We recently developed an interest in generating novel mouse models to study cancer metabolism. Being a core hallmark of cancer, cancer metabolic reprogramming is crucial for the growth, survival and drug resistance of tumor cells. A deeper understanding of the plasticity and interplay of cancer metabolic pathways may help to unveil the "Achilles' Heel" of cancer for successful therapies. Several novel mouse models aiming to address the links of cellular metabolic pathways and redox state with cancer are currently being characterized.

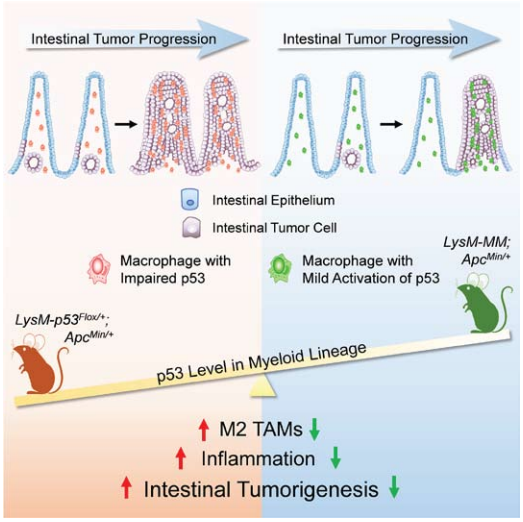

Figure 2. p53 activities critically modulate tumor microenvironment and in turn, impact on tumorigenesis.

Publications

1. Zhang CX, Zhang Q, Xie YY, He XY, Xiang C, Hou XS, Zhou Y, Chen L, Zhang GX and Liu G*. (2016) MDM2 actively suppresses p53 activity in oocytes during mouse folliculogenesis. American Journal of Pathology, doi:10.101/j.ajpath.2016.09.023.
2. He XY, Xiang C, Zhang CX , Xie YY, Chen L, Zhang GX and Liu G*.(2015) p53 in myeloid lineage modulates an inflammatory microenvironment limiting initiation and invasion of intestinal tumors. Cell Reports doi:10.1016/j.celrep.2015.09.045
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4. 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.
5. 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.
6. Gu X, Xing L, Shi G, Liu Z, Wang X, Qu Z, Wu X, Dong Z, Gao X, Liu G, Yang L and Xu Y. (2011) The circadian mutation PER2(S662G) is linked to cell cycle progression and tumorigenesis. Cell Death Differ. 19(3):397-405.
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8. Liu G, Parant JM, Lang G, Chau P, Chavez-Reyes A, El-Naggar AK, Multani A, Chang S, Lozano G. (2004) Chromosome stability, in the absence of apoptosis, is critical for suppression of tumorigenesis in Trp53 mutant mice. Nat Genet 36(1): 63-8.

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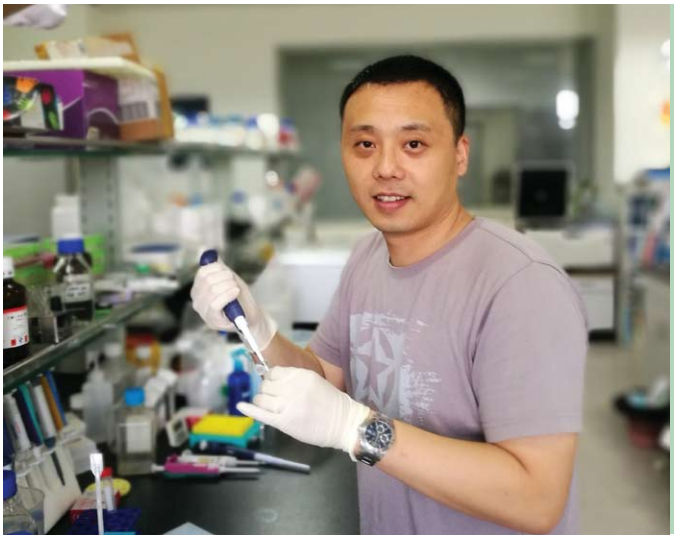

Jianghuai Liu, Ph.D.

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

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Regulation of macrophage function and molecular immunology

As a vital body department that is critical for our survival on this planet, the immune system evolved to confer strong protection against a plethora of encountering, non-self substances. Yet, such protective shield is not perfect - at times, the immune system could either fail to confer sufficient protection or become excessively activated. Focusing on macrophages, an essential innate immune cell type, we use mouse models to explore the mechanisms regulating their numbers and functions in various disease states. Ultimately, we hope that our bench discoveries can in some way impact management of human diseases.

1. The metabolic 'division' of innate immunity:

There are intricate links between metabolism and immunity which have become hot topics in today's immunology. Studies in this field shall suggest novel immuno-regulatory targets in disease states. This year, we have concluded a study to demonstrate a new immunometabolic function by virus-induced type I IFN (Jiang H. et al, 2016). The latter is mediated by IFN-triggered up-regulation of a glycolytic activator PFKFB3 selectively in macrophages, leading to a 'Warburg-like' metabolic switch that enhances efferocytosis-dependent, cell-extrinsic antiviral activity of these cells. Therefore, different from the long-standing view of glycolysis as a proviral pathway, our work has established an antiviral, immunometabolic aspect of glycolysis that may have therapeutic implications (Fig. 1A).

Currently, we are investigating whether PFKFB3 may also regulate IFN-dependent autoimmune diseases (Fig. 1B). On the other hand, we look to explore the glycolysis-independent function of PFKFB3 in the cell nucleus (Fig. 1C). To this end, the functional outputs by a mutant PFKFB3 deficient in nuclear localization are under careful examination (Fig. 1D).

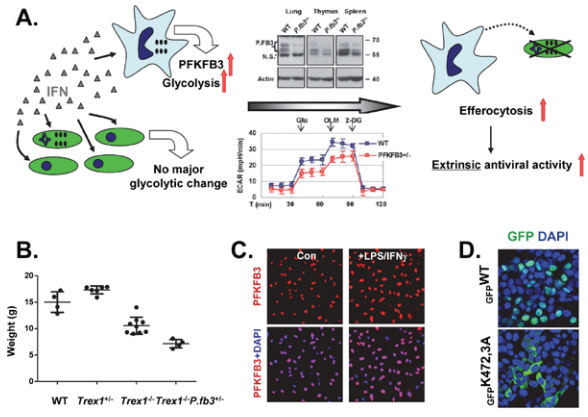

Figure 1: PFKFB3-mediated immunometabolic regulation in antiviral defense and beyond.

(A) Role of PFKFB3-driven macrophage glycolysis in the antiviral response. (B) Trex1$^{-/-}$ mice represent an IFN-dependent autoimmune disease model. Pfkfb3 heterozygosity under this genetic background appears to modulate the phenotype, whereas the Pfkfb3$^{+/-}$ genotype alone does not cause spontaneous disease phenotypes (not shown). (C) Sub-cellularly, a major pool of PFKFB3 is localized in the nucleus. (D) As reported previously, K472/3A mutant PFKFB3 is excluded from the cell nucleus. The mutant represents a tool to study the nuclear function of PFKFB3.

2. Transcriptional 're-wiring' of inflammation:

The recently emerging discipline of synthetic biology centers on construction of synthetic gene circuits that can drive new biological behaviors. As this field is developing rapidly, we envision that the synthetic biology tools will provide a new avenue for combating against the inflammatory conditions that drive many severe diseases. Taking advantage of a new CRISPR/dCas9-dependent tool for sequence-specific transcriptional regulation that we recently applied elsewhere (Du Y. et al, 2015), we are now focused on constructing a synthetic gene circuit that senses endogenous transcriptional inputs and engages synthetic transcriptional outputs in human cells (Fig. 2A, B). We found that the expression of dCas9 driven by cis-element alone was less desirable (Fig. 2C). As an important step forward, we demonstrated that further engineering efforts to connect the CRISPR activator to engage a self-amplification loop led to a more robust, signal-dependent dCas9 induction and subsequent gene activation (Fig. 2D, E, F). Our tool will be useful for various synthetic purposes, such as to rewire cells' behaviors under inflammatory conditions. In the long term, we look to further enrich the CRISPR TF toolkit and apply these tools to therapeutically relevant model systems.

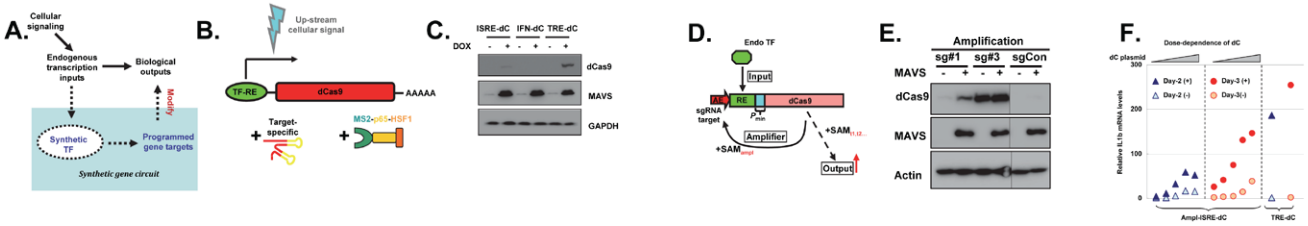

Figure 2: A CRISPR-based synthetic gene circuit that senses endogenous transcriptional inputs and leads to orthogonal downstream transcriptional outputs.

(A) The overall goal for the synthetic gene circuit. (B) The initial design of the CRISPR/dCas9-based gene circuit. (C) Cis-element-driven dCas9 expression is relatively weak. (D) The design of an amplification loop also based on CRISPR/dCas9. (E and F) The amplification loop enables robust signal-dependent induction of dCas9 (E) and downstream genes (F).

Selected publications: (*corresponding author)

1. Jiang H, Shi H, Sun M, Wang Y, Meng Q, Guo P, Cao Y, Chen J, Gao X, Li E* and Liu J*. PFKFB3-driven macrophage glycolytic metabolism is a crucial component of innate antiviral defense. J Immunol (2016), 197(7), 2080-90.
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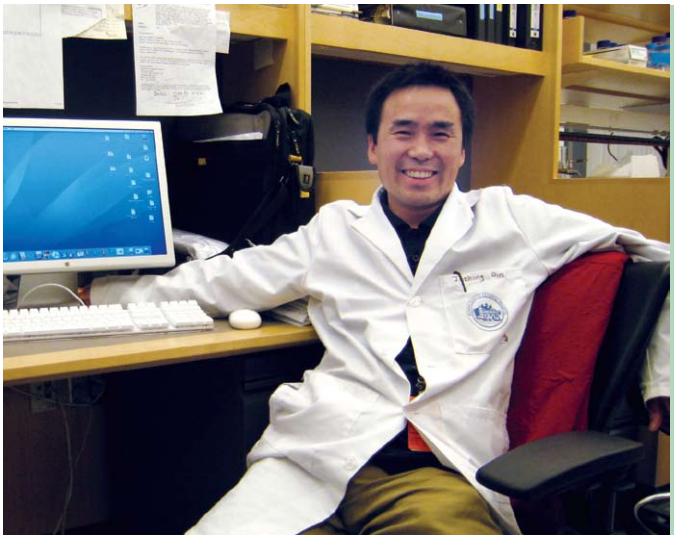

Jinzhong Qin , Ph.D.

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.

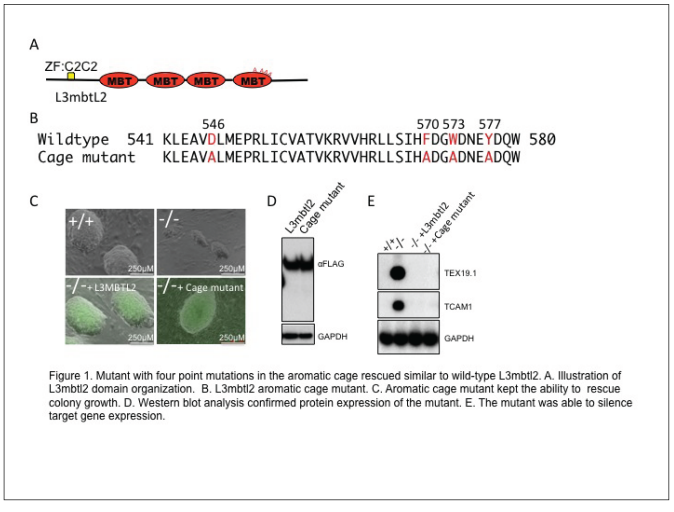

Figure 1. Mutant with four point mutations in the aromatic cage rescued similar to wild-type L3mbtl2. A. Illustration of L3mbtl2 domain organization. B. L3mbtl2 aromatic cage mutant. C. Aromatic cage mutant kept the ability to rescue colony growth. D. Western blot analysis confirmed protein expression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

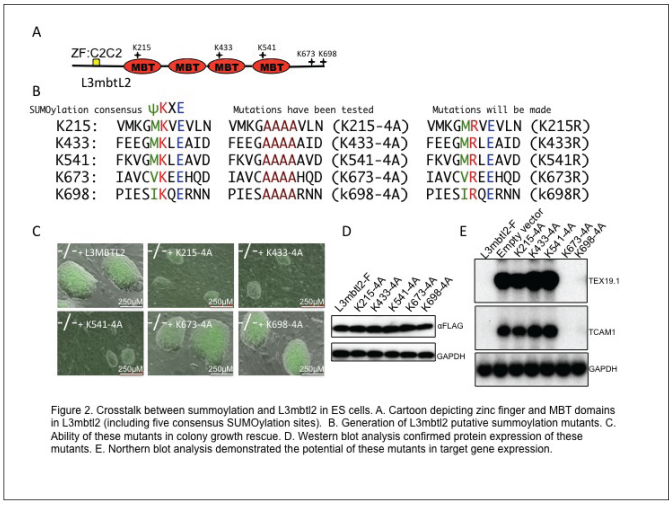

Figure 2. Crosstalk between summoylation and L3mbtl2 in ES cells. A. Cartoon depicting zinc finger and MBT domains in L3mbtl2 (including five consensus SUMOylation sites). B. Generation of L3mbtl2 putative summoylation mutants. C. Ability of these mutants in colony growth rescue. D. Western blot analysis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

Selected publications:

1. A. Foudi, D. Kramer, J. Qin, D. Ye, A. Behlich, S. Mordecai, F. Preffer, K. Hochedlinger, S.H. Orkin, and H. Hock. 2014. Distinct, strict requirements for Gfi-1b in bone marrow red cell and platelet generation. J Exp Med 211:909-27.
2. Qin, J., W. A. Whyte, E. Anderssen, E. Apostolou, H. H. Chen, S. Akbarian, R. T. Bronson, K. Hochedlinger, S. Ramaswamy, R. A. Young, and H. Hock. 2012. The polycomb group protein L3mbtl2 assembles an atypical PRC1-family complex that is essential in pluripotent stem cells and early development. Cell Stem Cell 11:319-332.
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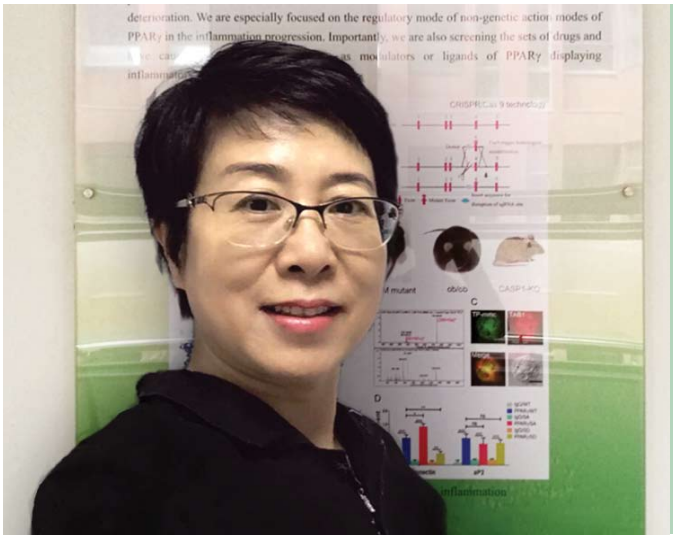

Pingping Shen , Ph.D.

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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Cancer related inflammation

Tumor-associated macrophages (TAMs) are increasingly viewed as a target of great relevance in the tumor microenvironment, because of their important role in cancer progression and metastasis. However, the endogenous regulatory mechanisms underlying TAMs differentiation remain largely unknown. With this as our goal, we are currently studying the effects of certain molecules and underlying molecular pathways such as metabolic reprogramming mediated by medium-chain acyl-CoA dehydrogenase (MCAD) (Fig.1&2). We've found that caspase-1 promotes TAMs differentiation by cleaving peroxisome proliferator-activated receptor gamma (PPARγ) and blocks fatty acid oxidation, thereby leading to the accumulation of lipid droplets and promoting TAMs differentiation. The infusion of bone marrow-derived macrophages (BMDMs) genetically engineered to overexpress murine MCAD markedly suppresses tumor growth.

In collaboration with Beijing 301 Hospital, Nanjing Gulou Hospital, we have been developing the platform to modify the surface proteins of TAMs for future clinical application, as well as building up MSC therapeutic in acute leukemia treatment.

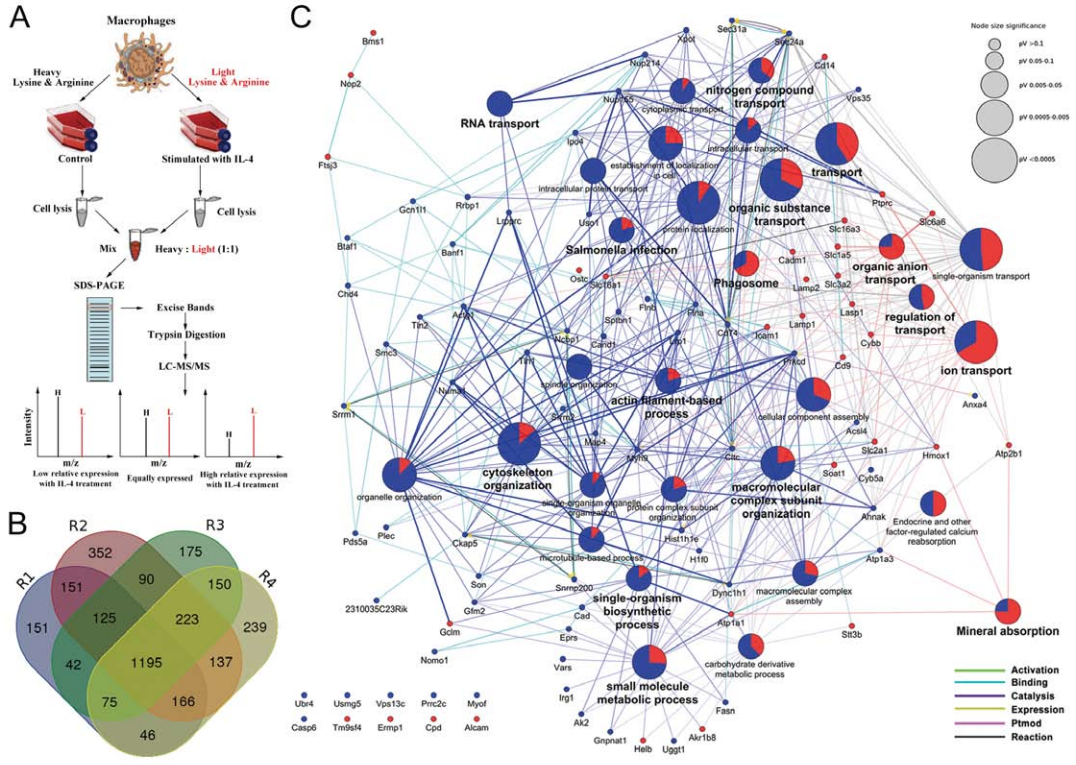

Fig 1. Quantitative proteomics analysis of the alternatively M2 activated macrophages.

(A) Schematic outline of the SILAC-based LC-MS/MS experiment. (B) In total, we identified 3317 proteins in this study by using four separate biological replicates and 2400 proteins (72%) were identified in at least two replicates. (C) Results of GOBP and KEGG enrichment analysis.

Nonresolving chronic inflammation

Nonresolving inflammation is a major driver of many diseases. Macrophages are now regarded as prominent players in metabolic disorder and associated nonresolving inflammation. We are investigating how macrophages interact with adipocytes to determine the phenotype of macrophages, and how macrophages further produce key regulators to modify extracellular microenvironment, and how these changes might play a critical role in aberrant progression of inflammation and further tumorigenesis. We are especially focused on the non-genetic actions of PPARγ which may lead to resolution of systemic inflammation. We have identified the novel phosphorylation code of PPARγ and explored related pathological role in metaflammation. We are also screening the sets of small molecules and have caught several natural products acting as modulators or ligands of PPARγ displaying inflammatory resolving activities (Fig.3).

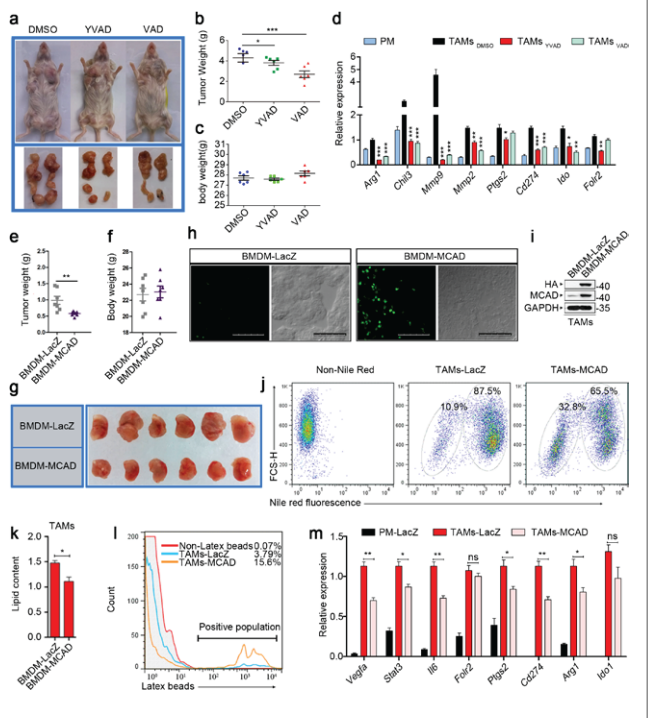

Fig 2. Targeting caspase-1/PPARg/MCAD signaling in tumor therapy

Clinical diagnostic techniques

We are collaborating with clinical colleagues to develop the novel methods for analyzing genes and molecules which are associated with human disease, particularly cancer. We have finished setting up a new, sensitive approach for nucleic acids, which demonstrate possibility of amplifying DNA in isothermal conditions without the need of a thermocycling apparatus. Working with the companies and hospitals, we are developing some specific mono-antibodies for clinical and commercial appliances, such as anti-BNP, anti-NGAL, anti-CYSL and so on. Currently, we have been trying to integrating nanomaterial, like structural modified magnetic beads, quantum dots with the test system, in order to improve the detection properties and further meet the needs of clinical diagnosis.

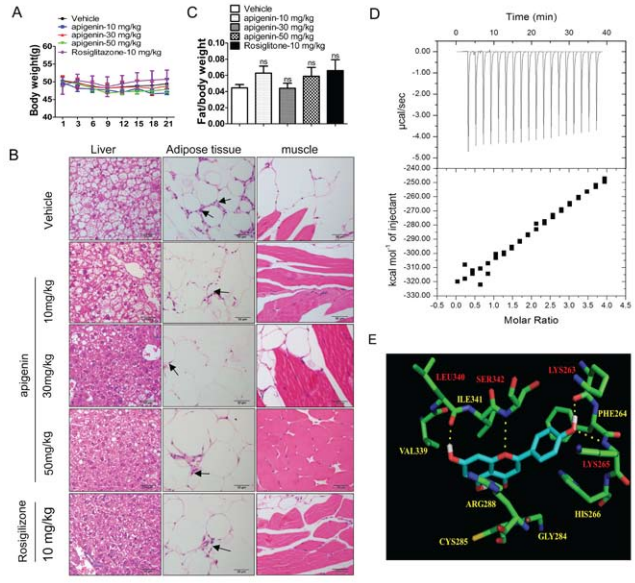

Fig. 3 Apigenin attenuates obesity-related inflammation via binding with PPARγ

A.The body weight affected by Apigenin treatment.

B.The HE staining of liver, adipose and muscle tissue

C.The fat/body weight affected by Apigenin treatment.

D.The ITC assay of the interaction between Apigenin and PPARγ

E.The molecular docking between Apigenin and PPARγ

Selected publications and awards

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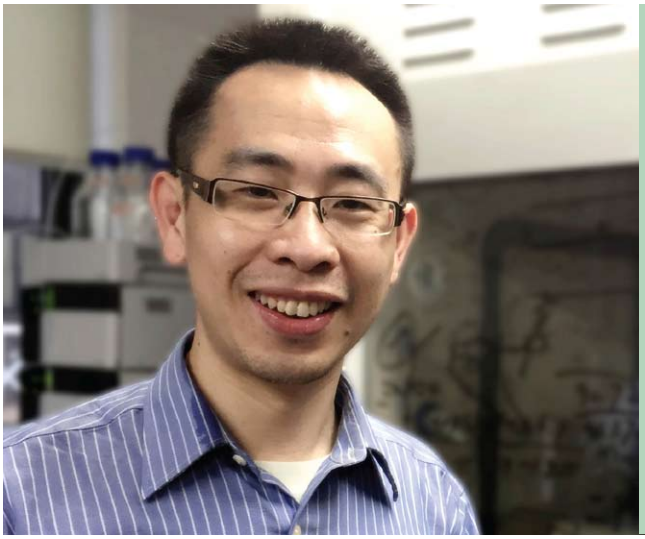

Jun Yan, Ph.D.

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 highly 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, cancers stem cells (CSCs) are resistant to conventional chemotherapeutic agents. We are focusing on identification of key regulators involved in maintenance of cancer cell stemness and potential therapeutic agents.

A) Tumor microenvironment plays an essential role in promoting tumor progression. Hypoxic condition inside tumor can enhance cancer cell stemness. Hypoxia inducible factor 1α (HIF1α) is one of the major transcription factor induced under hypoxia and essential for CSC maintenance. We and collaborators found that in prostate cancer cells hypoxia induces miR-182, which eventually reduces PHD2 and enhances HIF1α function. This functions as a vicious cycle for prostate cancer progression (Li L, et al. Sci Rep 2015). Moreover, we are the first to identify that an oncogene SRC-3 in bladder cancer directly interacts and coactivates hypoxia-inducible factor 1α (HIF1α), eventually inducing Warburg effect in bladder cancer cells. Our data indicate that blocking of glycolytic pathway can inhibit the tumorigenecity of SRC-3 overexpressing bladder cancer cells (Zhao W, et al. J Biol Chem. 2014).

B) Inflammatory microenvironment is frequently detected in tumor regions. We successfully isolated cancer-associated fibroblasts (CAFs) from human bladder specimens. Co-culture of CAFs and bladder cancer cells strikingly induces epithelial-to-mesenchymal transition (EMT) and invasion of cancer cells, but normal fibroblast cannot (Zhuang J, et al. Sci Rep 2015). With ELISA, qRT-PCR and PCR array, we identified that TGFβ1 signaling induces lncRNA-ZEB2NAT, which co-operates with ZEB2 to induce an EMT program (Fig. 1).

C) Since CSCs account for tumor recurrence and metastasis, we proposed that targeting CSCs will be an efficient approach to prevent recurrence. We screened out several small molecular inhibitors and found δ-Tricotrienol and honokiol target several signaling involved in cancer cell stemness, such as STAT3 and EZH2/miR-143 axis (Ye C, et al. PLoS One; Zhang Q, et al. Oncotarget 2015).

D) Based on recent exome sequencing data, we identified a group of epigenetic modifiers involved in carcinogenesis, especially in the maintenance of cancer cell stemness. We are striving to establish and characterize these novel mouse cancer models, which recapitulate human cancer development (Fig. 2). These mouse models will provide excellent platforms for pre-clinical study in near future.

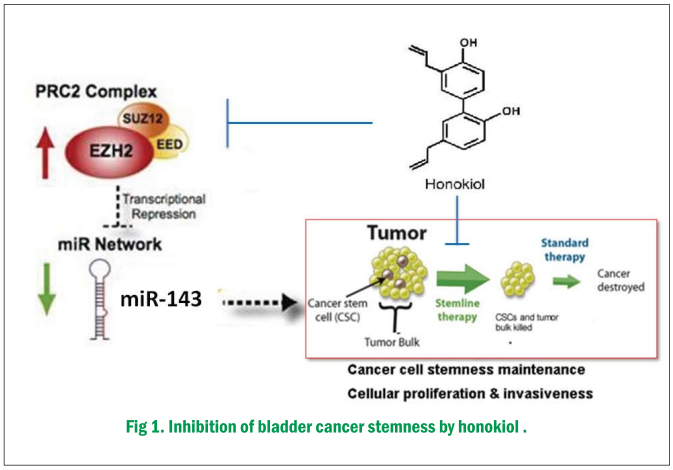

Fig 1. Inhibition of bladder cancer stemness by honokiol .

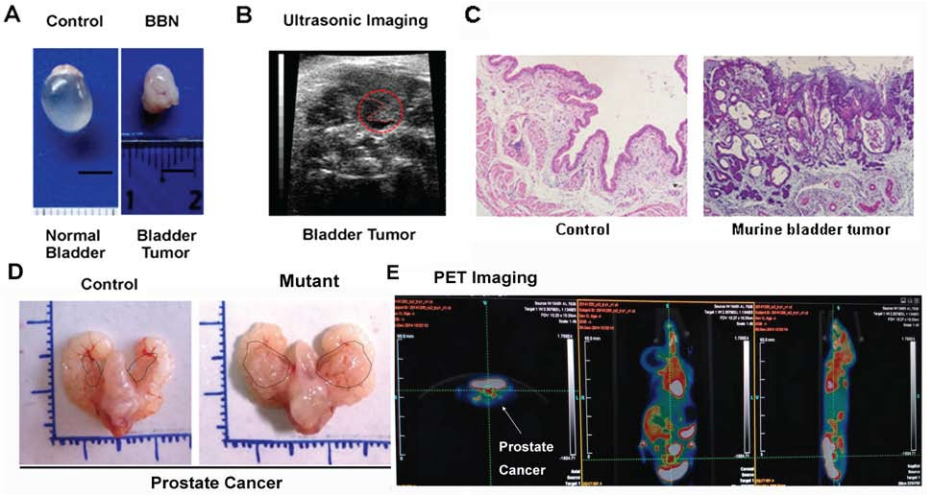

Fig 2. Bladder and prostate cancer mouse model.

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

Selected publications(* corresponding author)

1. An J‡, Ren S‡, Murphy SJ‡, Dalangood S‡, Chang C, Pang X, Cui Y, Wang L, Pan Y, Zhang X, Zhu Y, Halling GC, Cheng L, Sukov WR, Karnes RJ, Vasmatzis G, Zhang Q, Zhang J, Cheville JC, Yan J#, Sun Y*,#, Huang H*,#. Oncogenic truncated ERG proteins from TMPRSS2-ERG fusions are resistant to SPOP-mediated proteasome degradation. Mol Cell 2015;59:904-16. (# Co-senior author; Highlighted by Mol Cell and Cancer Discov.)
2. Zhang Q‡, Zhao W‡, Ye C‡, Zhuang J‡, Chang C, Li Y, Huang X, Shen L, Li Y, Cui Y, Song J, Shen B, Eliaz I, Huang R, Ying H, Guo H, Yan J*. Honokiol inhibits bladder tumor growth by suppressing EZH2/miR-143 axis. Oncotarget 2015; 6:37335-48.
3. Zhuang J‡, Lu Q‡, Shen B‡, Huang X, Shen L, Huang R, Yan J*, Guo H*. TGFβ1 secreted by cancer-associated fibroblasts induces epithelial-mesenchymal transition of bladder cancer cells through lncRNA-ZEB2NAT. Sci Rep 2015;5:11924
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7. Zhao F, Huang W, Zhang Z, Mao L, Han Y, Yan J, Lei M. Triptolide induces protective autophagy through activation of the CaMKKβ-AMPK signaling pathway in prostate cancer cells. Oncotarget 2016;7:5366-82.
8. Zhu H, Ren S,Bitler BG, Aird KM, Tu Z, Skordalakes E, Zhu Y, Yan J, SunY, Zhang R. SPOP E3 Ubiquitin ligase adaptor epigenetically promotes cellular senescence by degrading the SENP7 deSUMOylase. Cell Rep 2015;13:1183-93.

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Zongde Zhang, Ph.D.

Zongde Zhang received his Ph.D. degree in Microbiology from Huazhong Agricultural University College of Veterinary in 2012. He did his postdoctoral training in Immunology at Tsinghua University and Chicago University, where he studied the mechanisms of microbiota induced immune system maturation. Then he moved to Model Animal Research Center, Nanjing University as a Principal Investigator in 2016.

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Microbiota induced immune system maturation, Dysbiosis-related diseases

Protection of microbiological infection, preventing autoimmune disease and allergic disease, immune surveillance of cancer, both need proper functioning of immune system. Human body colonized with trillions of microorganisms also called "microbiota" are essential for postnatal immune system development, disturbing the microbiota(dysbiosis) has been shown underlie many human diseases. However, the mechanisms which microbiota induced host immune system maturation, are entirely unknown. The focus and long-term research goals in our laboratory are to interrogate the mechanisms which govern the development of the host immune system, understand the roles of microbiota in human health and disease, development of microbiota based therapy for human disease. Currently, ongoing projects in the Lab as following.

Microbiota repress food allergic challenge through modulating dendritic cells retinoic acid response

The "hygiene hypothesis" is used to explain the rising incidence of allergic disease. Recent evidences have pointed out that intestinal flora regulate the immune system's allergic reaction to food antigens and allergic airway disease. Although, it is confirmed that retinoic acid involved in allergic reactions, the intestinal flora of whether regulation of retinoic acid signaling involved in the allergic reaction is not clear. Treatment of allergic disease largely depended on immune inhibiting, side-effecting chemical drugs, which give rise to the need to develop new intervention method based on microbiota. To solve this problem, we set up a project to investigate if commensal microorganisms can modulate retinoic acid activity in immune cells. We treated the reporter mice bearing retinoic acid response element(RARE) upstream of the LacZ gene with antibacterial and anti-fungi agent, using FACS analysis of LacZ expression in immune cells. We have identified commensal bacteria which can modulate LacZ expression in dendritic cells. Dendritic cell-specific knockout of retinoic acid transcriptional factors in mice resist food allergic challenge. We will further identify if metabolites (short chain fatty lipids et.al.) from these commensal bacteria can modulate LacZ expression in dendritic cells. We will dissect the mechanisms by which retinoic acid transcriptional factors modulate food allergic response in dendritic cells. Understanding the process of microbiota regulate allergic response will lead to a new method of intervention for human food allergic disease.

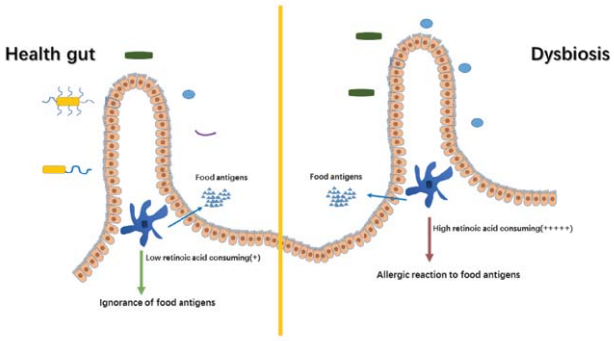

Figure 1. Microbiota repress food allergic challenge via modulating dendritic cells retinoic acid response

Microbiota upregulation of CTGF/CYR61 decorating High Endothelial Venules

High endothelial venules(high endothelial venules, HEVs), are specified small blood vessels found in the lymphoid tissues. Compared to other normal tiny veins, there are great differences in structure and function, for HEVs endothelial cells express addressin molecules(MadCam1/PNAd) and chemotactic factor CCL21, HEVs are the portal that T and B lymphocytes migrate into the lymph nodes. HEVs are also found in some solid tumors involved in tumor prognosis.

In our previous study (zhang et.al. Immunity 44-2,2016), using immunofluorescence of lymph nodes from germ-free mice (germ free, GF), we found that underdevelopment of high endothelial venules in GF mice, as reduced CCL21 expression and mixed pattern of addressin molecules (PNAd and MadCam1). This mixed mode of expression is only present in the neonatal period in SPF mice, indicating symbiotic bacteria of the gut can induce HEVs development. By germ-free mice colonization of bacteria combined high throughput transcriptome sequencing technology, we found the symbiotic bacteria colonization of germ-free mice induce RALDH+CD103+CD11b+ dendritic cells migrate to the lymph nodes, which regulated the expression of two CCN family protein gene CTGF/CYR61, previously reportedly involved in tumor blood vessels formation. Through immunofluorescence of frozen sections, we identified that CTGF/CYR61 expressed only in HEVs, not in other CD31+ microvascules. Accordingly, we will further probe the roles of CTGF/CYR61 in the intestinal flora induced HEVs Development and its functionality in lymphocytes migration into lymph nodes.

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Neurobiology

Guiquan Chen, Ph.D.

Guiquan obtained his PhD in Neuroscience at the University of Edinburgh in Scotland in 2005 and then conducted his postdoctoral research at Harvard Medical School in Boston. He joined the MARC of Nanjing University as a Principle Investigator in December of 2011. His long-term research goal is to understand molecular mechanisms by which the γ–secretase complex regulates neuronal survival and/or death. 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 and cellular mechanisms for neurodevelopmental and neurodegenerative diseases

Recent evidence has shown that loss-of-function mutations on Akt3 are associated with microcephaly, a type of neurodevelopmental disease. However, the underlying mechanisms are not known. To investigate how Akt isoform regulates brain development, we used three different lines of Akt knockout mice, Akt1-/-, Akt2-/- and Akt3-/- to conduct morphological and functional analyses.

Nissl staining revealed comparable brain structure between Akt1-/-, Akt2-/- or Akt3-/- and age-matched wildtype (WT) littermate mice (Fig.1). However, the brain size of Akt3-/- (Fig.1A) but not Akt1-/- (Fig.1B) or Akt2-/- (Fig.1C) mice was significantly reduced as compared to WT mice. These findings were consistent with previously published observations. To find out mechanism by which loss of Akt3 affects cortical development, we examined the brain's white matter. First, our IHC (immunohistochemistry) on MBP (myelin basic protein) showed significantly reduced MBP immunoreactivity in the neocortex and the hippocampus of Akt3-/- but not Akt1-/- mice (Fig.2A). Second, IHC on MAG (myelin associated glycoprotein) revealed significantly reduced MAG immunoreactivity in Akt3-/- but not Akt1-/- mice (Fig.2B). Moreover, the MAG+ fiber-like structure was largely diminished in Akt3-/- but not Akt1-/- mice (Fig.2B). These findings suggest that the white matter integrity was disrupted in Akt3-/- but not Akt1-/- mice. In contrast, IHC on MAP2 (microtubule associated protein 2) showed unchanged immuno- reactivity in the brain of Akt3-/- or Akt1-/- mice (Fig.2C), suggesting unaltered dendritic morphology.

To investigate whether Akt isoform is important for cognitive ability, we tested Akt3-/- and Akt1-/- mice using a standard Morris watermaze protocol. We found that Akt3-/- mice spent longer time in finding the submerged platform in the watermaze than wildtype animals did (Fig.3A), suggesting impaired spatial learning. In contrast, Akt1-/- mice spent equivalent time to that by WT animals, suggesting unimpaired spatial learning (Fig.3B). Interestingly, in a probe test conducted 24 hours after the training, both Akt3-/- and Akt1-/- mice exhibited preference to the target quadrant (Fig.3C-3D), suggesting that the reference memory was not impaired. We are currently using several mouse models to investigate molecular mechanisms by which Akt regulates cortical development and determines cell fate.

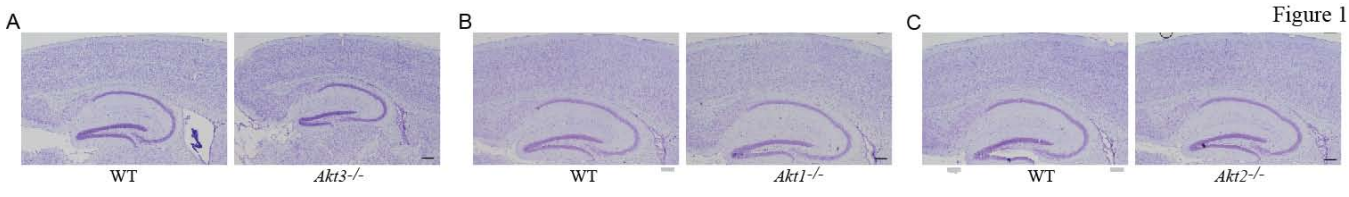

Figure 1. Morphological analysis on Akt1-/-, Akt2-/- and Akt3-/- mice. Significantly reduced size of the cortex and the hippocampus in Akt3-/- (A) but not Akt1-/- (B) or Akt2-/- (C) mice.

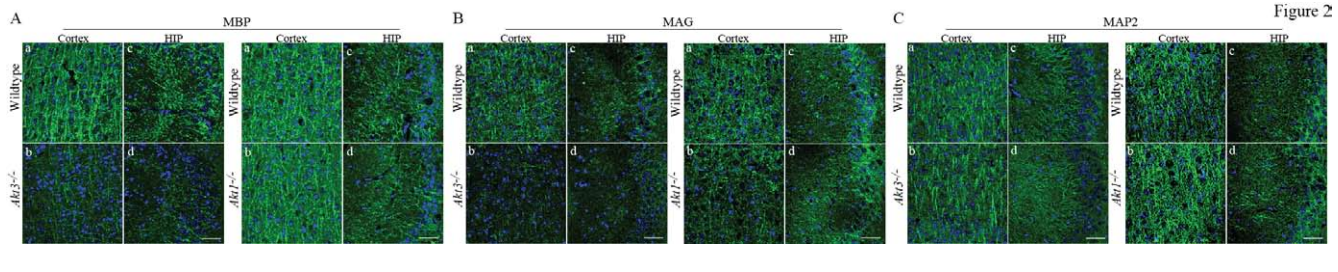

Figure 2. Disrupted white matter integrity in Akt3-/- but not Akt1-/- mice.

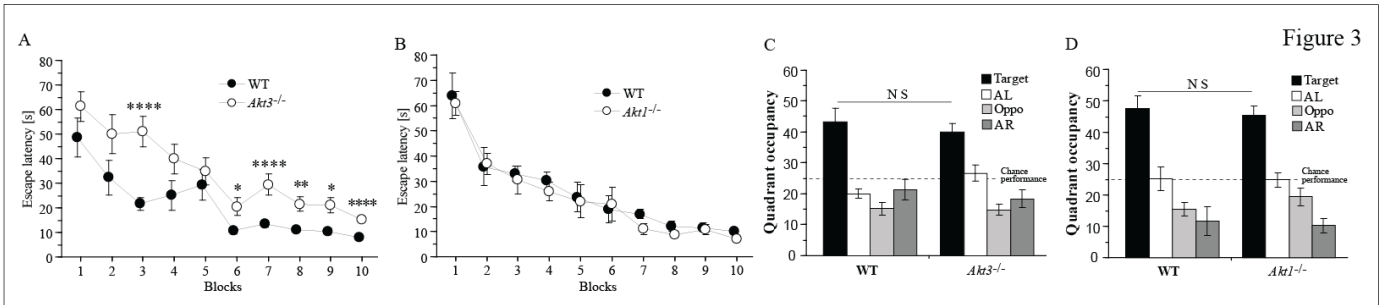

Figure 3. Impaired spatial learning in Akt3-/- but not Akt1-/- mice.

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2. He X, Li Y, Kalyanaraman C, Qiu L, Chen C, Xiao Q, Liu W, Zhang W, Yang J, Chen G, Jacobson MP, Shi Y. GluA1 signal peptide determines the spatial assembly of heteromeric AMPA receptors. PNAS, 2016; 113: E5645-E5654.
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Huiming Gao received her M.D. in 1993 and Ph.D. in 2003 from Dalian Medial University. Her Ph.D. thesis was carried out at the National Institute of Environmental Health Sciences (NIEHS)/ National Institutes of Health (NIH) in the USA. After her postdoctoral training at University of Pennsylvania School of Medicine and NIEHS/NIH, Dr. Gao joined the Faculty of Model Animal Research Center (MARC), Nanjing University in 2013. She is now a professor and a principle investigator in MARC.

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Neuroinflammation, neurodevelopment, and neurodegeneration

Neuroinflammation is a tightly regulated, self-defensive attempt by the brain to remove harmful stimuli and to initiate the healing process. 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. Chronic neuroinflammation contributes to the pathogenesis of both neurodevelopmental diseases such as Autism in early childhood and age-related neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Chronic, irreversible degeneration of brain neurons causes progressive memory loss in AD and movement impairment (e.g. tremor and rigidity) in PD. There is no cure for these devastating diseases. Importantly, what drives the decades-long progression of these diseases remains unknown. The goal of our research is to investigate a potential driving role for chronic neuroinflammation in progressive neuronal impairment in Autism and neurodegenerative diseases, to identify new therapeutic targets, and to develop novel anti-inflammatory and neuroprotective therapeutics for these diseases.

We recently identified a distinct non-cell autonomous mechanism in Endotoxin tolerance (ET) formation in brain immune cells, microglia. ET is a reduced responsiveness of innate immune cells to an endotoxin challenge following a previous encounter with the endotoxin. Although ET in the peripheral systems has been well studied, little is known about ET in the brain. We found that neurons and astroglia were indispensable for microglial ET when microglia encountered repeated lipopolysaccharide (LPS) treatments. (Figure 1). Macrophage colony-stimulating factor (M-CSF) secreted from these non-immune cells was essential for governing microglial ET. Neutralization of M-CSF deprived the neuron-glial conditioned medium (NGCM) of its ability to enable microglia to form ET. Recombinant M-CSF protein rendered enriched microglia refractory to the repeated LPS treatment (Figure 2). Activation of microglial M-CSF receptor (M-CSFR) and the downstream ERK1/2 signal was responsible for M-CSF-mediated microglial ET. Endotoxin-tolerant microglia in neuron-glial cultures displayed M2-like polarized phenotypes, as shown by upregulation of M2 marker Arg-1, elevated production of anti-inflammatory cytokine interleukin 10, and decreased secretion of pro-inflammatory mediators (tumor necrosis factor α, nitric oxide, prostaglandin E2 and interleukin 1β). Endotoxin-tolerant microglia protected neurons against LPS-elicited inflammatory insults, as shown by reduced neuronal damages in LPS pre-treatment group compared with the group without LPS pre-treatment. Thus, our studies demonstrated that neurons and astroglia govern microglial ET through M-CSF—MCSFR—Erk1/2 signals, identifying a distinct non-cell autonomous regulation of microglial ET. Loss of microglial ET could be an important pathogenetic mechanism of inflammation-associated neuronal damages.

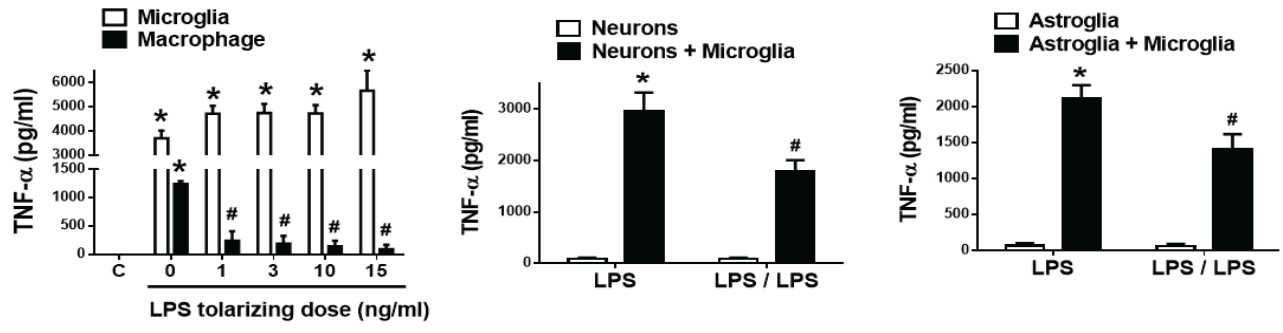

Figure 1. Neurons and astroglia are required for microglial endotoxin tolerance.

(A) Different from macrophages, enriched microglia failed to form endotoxin tolerance upon repeated LPS treatments. Pre-treated with LPS for 6 hours, brain microglia and peritoneal macrophages were washed and incubated with fresh medium for 6 hours followed by incubation with 15 ng/ml LPS. Six hours later, TNF-α in the culture supernatant was detected by ELISA assay. (B, C) Treatment of neuron-enriched (B), neuron-microglia (B), astroglia-enriched (C), and astroglia-microglia (C) cultures with 15 ng/ml LPS once or twice and measurement of TNF-α secretion were performed as described in (A). *, $p<0.05$, compared with corresponding vehicle-treated controls. #, $p<0.05$, compared with corresponding LPS-treated cultures.

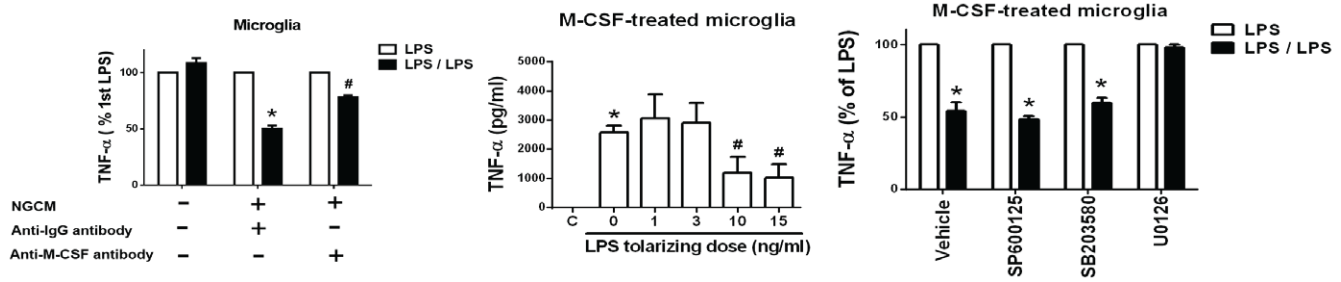

Figure 2. M-CSF secreted by neurons and astroglia governs microglial endotoxin tolerance.

(A) The neuron-glial conditioned medium (NGCM) enabled enriched microglia to form ET. After pre-incubation with anti-M-CSF neutralizing antibody for 12 hours, NGCM failed to affect microglial ET. *, p<0.05, compared with corresponding LPS-treated cultures without LPS pre-treatment. #, p<0.05, compared with LPS/LPS-treated cultures with anti-IgG antibody treatment group. (B) Recombinant M-CSF protein (500pg/ml) rendered enriched microglia refractory to the repeated LPS treatment. *, p<0.05, compared with corresponding vehicle-treated control cultures. #, p<0.05, compared with corresponding LPS-treated cultures. (C) Activation of microglial ERK1/2 signal was responsible for M-CSF-mediated microglial ET.

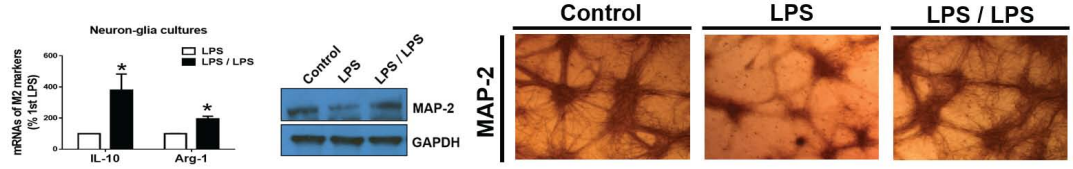

Figure 3. Endotoxin-tolerant microglia displayed M2-like polarized phenotypes and were neuroprotective.

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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 one of 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 groups 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.

The projects in our lab are: 1. The fundament of long-term potentiation. 2. Kainate receptor trafficking, synaptic targeting and function regulation. 3. Novel receptors or transporters.

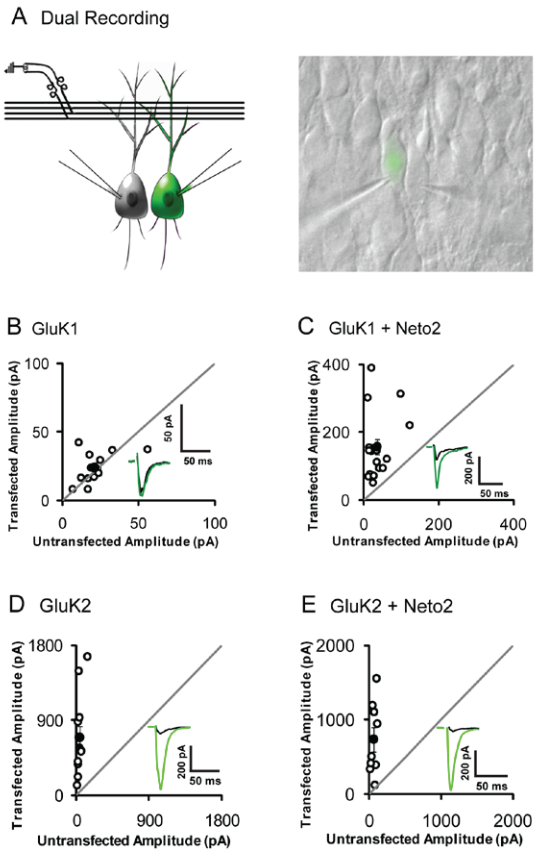

Figure 1. Synaptic response mediated by GluK1 and GluK2 expressed in CA1 neurons.

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.

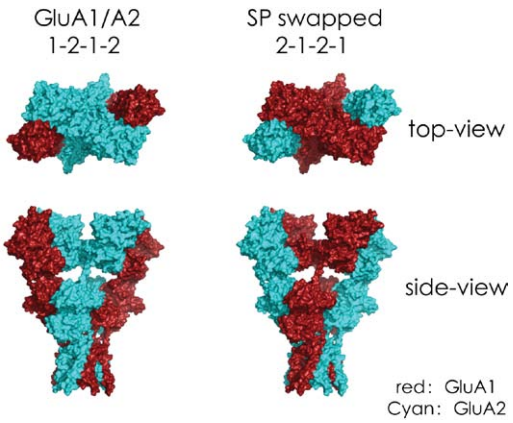

Figure 2. Signal peptides determine the spatial assembly of heteromeric AMPA receptors.

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. Left. The structural model of GluA1/A2. In the model the 2 GluA1s and 2 GluA2s form a functional assembly of AMPA receptor with 1-2-1-2 architecture. Right. Swapping signal peptides switched the spatial position of GluA1 and GluA2, so that the receptors were assembled with 2-1-2-1 pattern.

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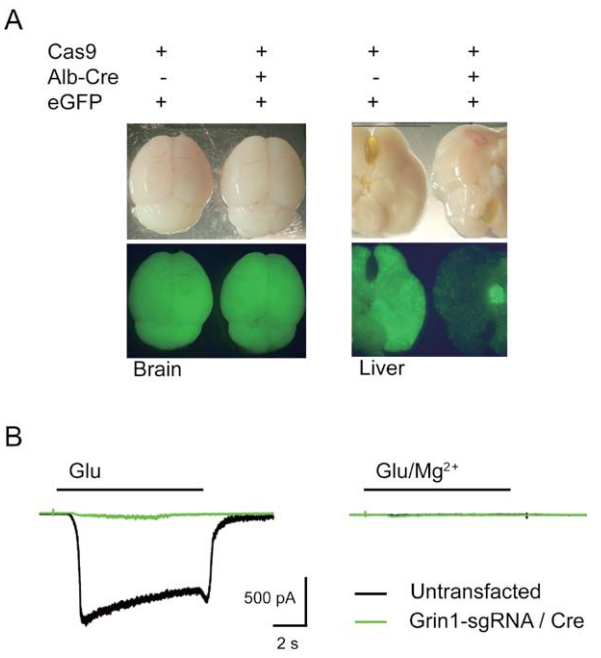

Figure 3. Characterization of a Cas9 transgenic mouse.

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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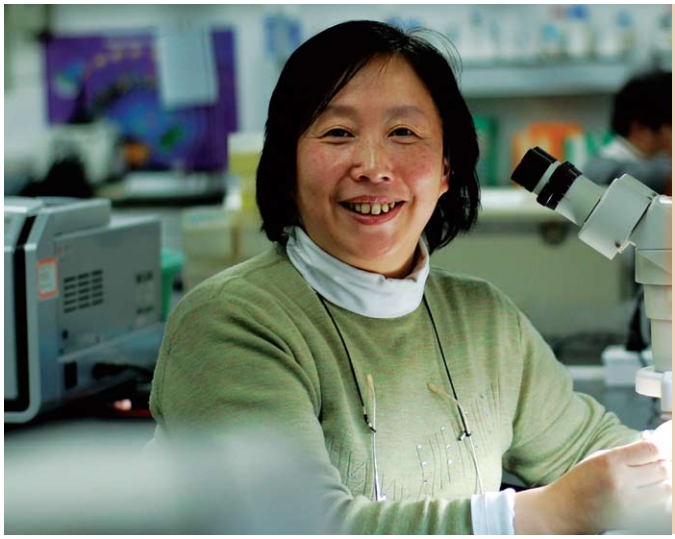

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Circadian rhythms are endogenous rhythms in physiology or behavior with a period length near 24 hours in all genetically studied organisms. In most cases, these rhythms are generated by endogenous processes referred to as circadian oscillators. These 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. Disruptions 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.

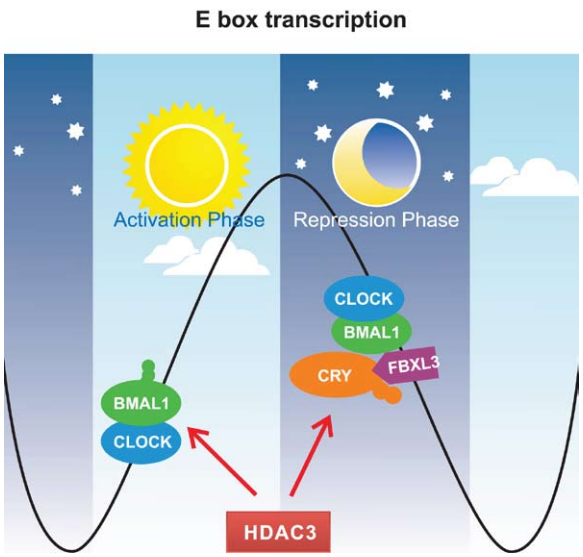

Figure 1 Model of the deacetylase-independent activity of HDAC3 with respect to the activation and repression phases of the circadian clock.

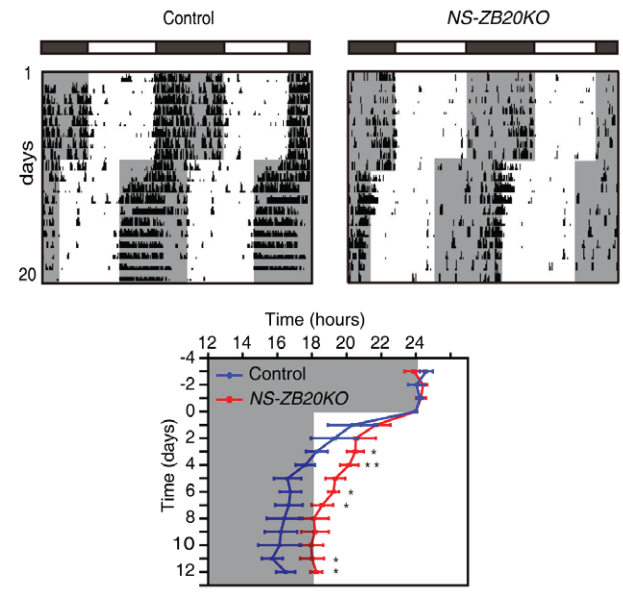

Figure 2 Loss of ZBTB20 impairs circadian output and leads to unimodal behavioral rhythms

Selected publications

1. Zhipeng Qu, Hai Zhang, Moli Huang, Guangsen Shi, Zhiwei Liu, Pancheng Xie, Hui Li, Wei Wang, Guoqiang Xu, Yang Zhang, Ling Yang, Guocun Huang, Joseph S. Takahashi, Weiping J. Zhang and Ying Xu (2016) Loss of ZBTB20 impairs circadian output and leads to unimodal behavioral rhythms. ELife. 2016; 5: e17171.
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6. ZhiPeng Qu, XiaoHan Wang, DongChuan Liu, Xiang Gao, Ying Xu (2015) Inactivation of Cipc alters the expression of Per1 but not circadian rhythms in mice. Science China. Life sciences, 58: 368–37
7. 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.
8. Zhiwei Liu, Moli Huang, Xi Wu, Guangsen Shi, Lijuan Xing, Zhen Dong, Zhipeng Qu, Jie Yan, Ling Yang, Satchidananda Panda & Ying Xu (2014) PER1 phosphorylation specifies feeding rhythm in mice. Cell Reports, 7(5) 1509-1520.
9. 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.
10. Guangsen Shi, Lijuan Xing, Zhiwei Liu, Zhipeng Qu, Xi Wu, Zhen Dong, Xiang Gao, Moli Huang, Jie Yan, Ling Yang , Yi Liu, Louis Ptacek &Ying Xu (2013) Dual roles of FBXL3 in the mammalian circadian feedback loops are important for period determination and robustness of the clock. Proc Natl Acad Sci U S A, 110 (12) 4750-4755.
11. Hsien-yang Lee, Junko Nakayama, Ying Xu, Xueliang Fan, Maha Karouani, Yiguo Shen, Emmanuel N. Pothos, Ellen J. Hess, Ying-Hui Fu, Robert H. Edwards, Louis J. Ptacek (2012) Dopamine dysregulation in a mouse model of paroxysmal non-kinesigenic dyskinesia. The Journal of Clinical Investigation, 122 (2):507–518.
12. Xiwen Gu, Lijuan Xing, Guangsen Shi, Zhiwei Liu, Xiaohan Wang, Zhipeng Qu, Xi Wu, Zhen Dong, Xiang Gao, Geng Liu, Ling Yang &Ying Xu (2012) The Circadian Mutation $PER2^{S662G}$ Is Linked to Cell Cycle Progression and Tumorigenesis. Cell Death and Differentiation, 19(3):397-405.
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15. 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	Graduate Students	Former graduate students
Ying Xu	Zhen Dong	Xiwen Gu
	Zhipeng Qu	Xiaohan Wang
	Yang An	Xi Wu
	Pancheng Xie	Zhiwei Liu
	Zhihui Zhang	Lijun Xing
	Dongchuan Liu	Guangsen Shi

Core Facilities at Model Animal Research Center is a newly founded section committing to provide cutting edge resources to our research community, enabling them to make meaningful advance in biological and biomedical research. Equipped with state-of-the-art facilities, our goal is to offer the highest quality of scientific technology in rapid turn-round time, while operating in a cost-effective manner.

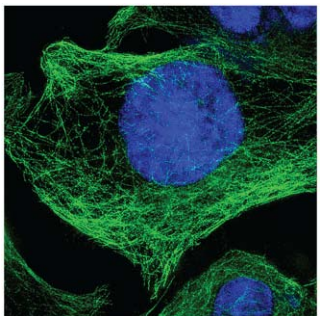
The Core Facilities of MARC provide a diverse range of services, including high resolution imaging, flow cytometry, protein and gene expression profiling, and metabolic analysis.

Microscopy and Imaging Core

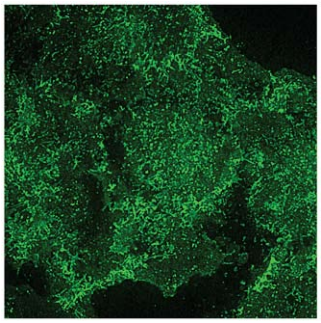
► GE DeltaVision OMX

DeltaVision OMX platform offers super resolution imaging using 3D structured illumination (3D-SIM), which allows precise visualization and measurement of features that are below the diffraction limit. 3D-SIM projects a structured light pattern onto the sample. The illumination pattern interacts with the fluorescent probes in the sample to generate interference patterns known as moiré fringes. By modulating the illumination pattern, collecting and reconstructing the subsequent images, super resolution images with double the lateral and axial resolution are obtained. 3D-SIM techniques work with traditional fluorescent proteins and dyes commonly used in much of fluorescent imaging.

Challenges arise when multiple fluorophores are too closely positioned such that images overlap and single molecules can no longer be resolved. Commercially available Localization Microscopy methods are implemented in Total Internal Reflection Fluorescence (TIRF) microscopy mode and rely on the use of either specialized dye pairs or photoswitchable fluorescent proteins.



Tubulin labeled by FITC in Hela cells was imaged using DeltaVision OMX(3D-SIM)



Membrane was visualized by WGA-FITC in Hela cells imaged using DeltaVision OMX(TIRF mode)

Feature highlights

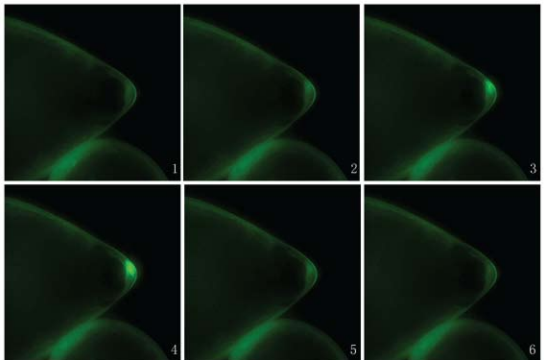
- True 3D structured illumination imaging enables resolution improvements to 120 nm in XY and 340 nm in Z, providing an overall eight-fold improvement in volume resolution
- Simultaneous photoactivation and sample imaging for fast photokinetic applications in TIRF mode(e.g. caged-calcium release or PA-GFP activation)
- Ultra-fast widefield imaging at 150 fps depending on exposure time

► GE DeltaVision Elit

The DeltaVision imaging system is a fully integrated, turnkey, deconvolution microscope system optimized for low light and live cell imaging applications.

Feature highlights

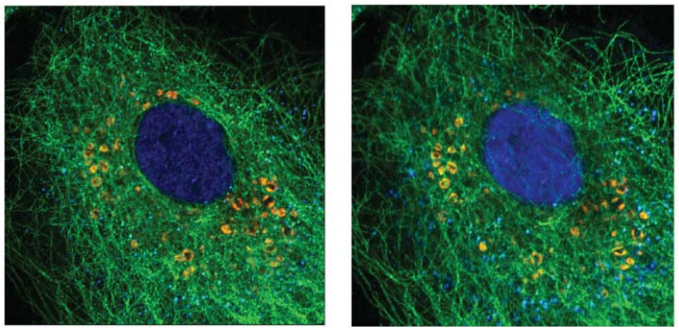
- TruLight illumination system delivers exceptional signal-to-noise performance and five times more light to the sample compared to previous illuminator assembly, enabling detection of small, dim objects such as organelles and microbial particles
- Deconvolution improves contrast and resolution compared to raw data images without sacrificing data integrity.
- UltimateFocus automatically maintains the sample z-position regardless of mechanical or thermal changes that can impact experiment.
- Cell tracking function automatically repositions the stage to accurately follow cells as they move during time-lapse experiments.



Ca^{2+} oscillates in drosophila ovary border cells showing by GCaMP6m were imaged using DeltaVision Elite in time lapse. Images were taken every 4s.

► Zeiss LSM 880

In a standard confocal microscope the out-of-focus emission light is rejected at a pinhole. The smaller the pinhole, the higher the resolution, but – equally – the bigger the loss in light. Airyscan solves this conundrum between resolution and light efficiency by using a detector array consisting of 32 single detector elements, all of which act like very small pinholes remaining open and doesn't block light – thus all photons are collected.



Tubulin labeled by Alexa fluor 488 and Mitochondrion labeled by Alexa fluor 568 in A549 cell were imaged by ZEISS (Airy Scan)

Feature highlights

- Increase the resolution with Airyscan to resolve 140 nm laterally and 400 nm axially at 488 nm, achieving 1.7× higher resolution for photon or multi photon experiments
- Working with thicker samples such as tissue sections or whole animal mounts that need a higher penetration depth, in such situation where widefield-based superresolution techniques would struggle.
- Using the Fast module to image with up to 27 frames per second at 480 × 480 pixels

Flow Cytometry Core

► BD FACS LSRFortessa

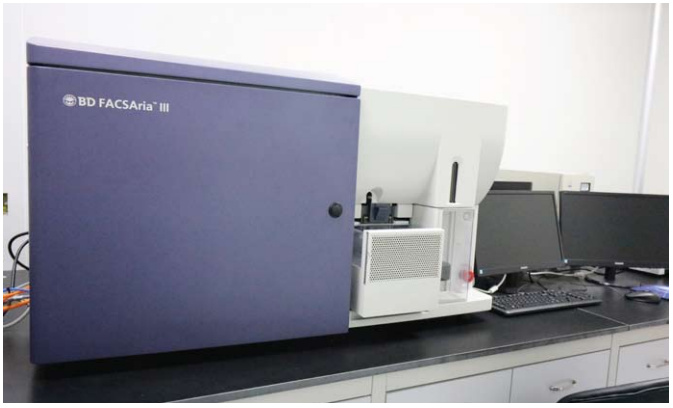
The patented collection optics are arranged in octagon- and trigon-shaped optical pathways, to maximizes signal detection and increases sensitivity and resolution, allowing to identify cells, especially dim and rare cell populations, optimizing multicolor assays and panel design for superior results.

Configured with up to 3 laser blue (488 nm), red (640nm) and violet (405nm), which enable to detection of up to 14 colors simultaneously. The 405nm laser choice expand color panel to make compensation easy.

► BD FACSAaria III

Wavelength choice includes 561nm and 405nm laser, as well as 488nm, 633nm laser. Mount up to 11 color measurement simultaneously.

At 70 psi and 90 kHz with an average threshold rate of 25,000 events per second, a four-way sort achieved a purity of >98% and a yield >80% of Poisson's expected yield. Higher threshold rates up to 70,000 events per second can be achieved without affecting purity.



Proteomics core and metabonomics core

► Agilent 6550 iFunnel Q-TOF

The Agilent 6550 Q-TOF could achieve femtogram-level sensitivity with Agilent iFunnel technology. It make high-resolution quantitation a reality with the combination of accuracy and sensitivity avoiding interference. Powerful MassHunter softwares are available for profiling, characterizing, identifying and quantifying compounds in complex mixtures via high-definition MS and MS/MS.

- METLIN Personal Metabolite Database Software

  Over 15,000 endogenous and exogenous metabolites are included in the database

- Spectrum Mill

  Faster, more accurate protein identification is possible with the advanced Spectrum Mill for MassHunter Workstation

- Mass Profiler Professional Software

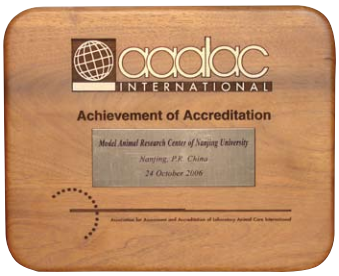
  Allows differential analysis of two or more sample sets from one or multiple MS analysis platforms in a single project.

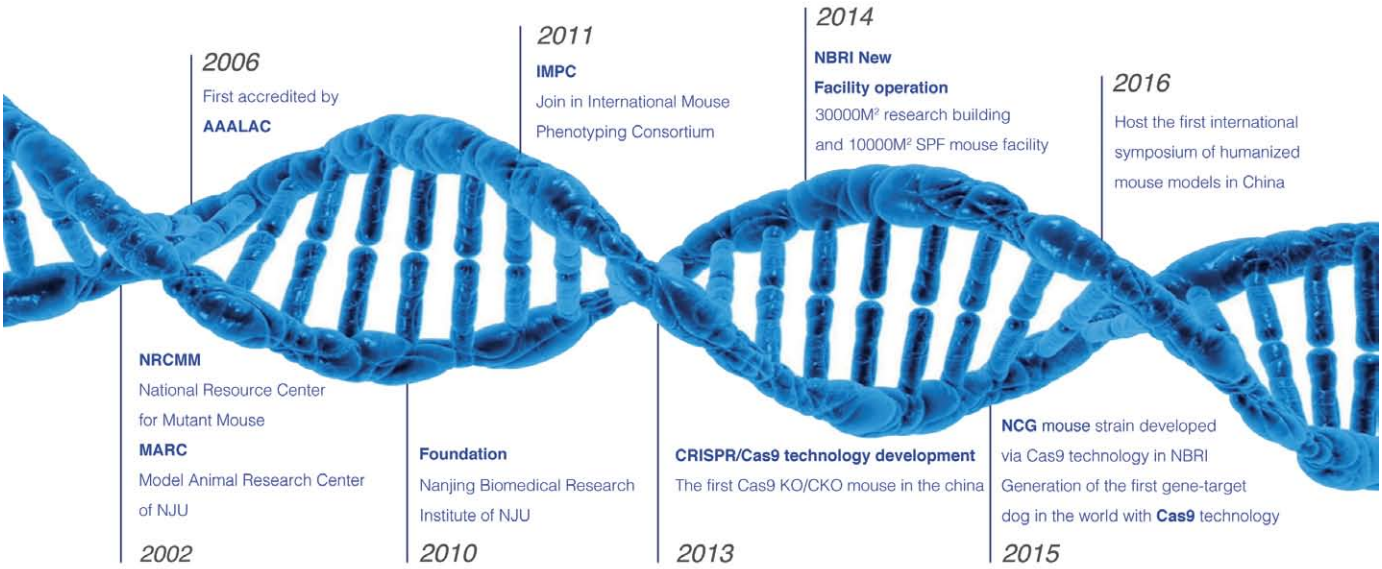
The establishment of core facilities will provide expertise support in technologies and experimental design to the investigators within or outside Model Animal Research Center. It will enhance effective usage of resources and maximize the use of expensive instruments required for these advanced technology platforms. It is expected to further promote and facilitate multi-disciplinary research studies and collaboration among the research community.

Nanjing Biomedical Research Institute of Nanjing University (NBRI)

NBRI is a leading national resource platform that provides high-quality mouse models and related services. Currently, we holds about 3,500 mouse strains, including models for cardiovascular disease, tumor, metabolic disease, autoimmune disease and neurodegeneration disease. With more than 15 years' market practices, NBRI has established the best service platform in China for generating gene-targeting and transgenic mice, cryopreservation, phenotyping, as well as mice exportation/ importation.

Now, we are striving to offer One-Stop Service for all mouse related studies in the world.





▶ Star Mice

Mouse Model	Strain	Strain Description
Diabetes Model	BKS.Cg-*Dock7*^{m}+/+*Lepr*^{db}/Nju	After 8W, blood glucose continues to rise
	B6.BKS(D)-*Lepr*^{db}/Nju	After 12W, blood glucose begins going down
	B6.Cg-*Lep*^{ob}/Nju	After 14-16W, blood glucose begins going down
Immunodeficiency Model	NOD-*Prkdc*^{em26}*Il2rg*^{em26}/Nju	The most severe immunodeficiency mouse. It lacks of T, B and NK cell, and its background is pure so the data variation is small
	STOCK-*Foxn1*^{nu}/Nju	Nude mouse (lacking of T cell)
	B6.129S7-*Rag1*^{tm1}/Nju	Lack of T cell and B cell
	NOD-*Prkdc*^{em26Cd52}/Nju	Lack of T cell,B cell and lymphocyte
Atherosclerosis Model	B6.129S7-*Ldlr*^{tm1}/Nju	Serum cholesterol level of homozygous mouse(about 200-400 mg/dl), when feeding the mouse high-fat diet(>2000 mg/dl)
	B6.129P2-*Apoe*^{tm1}/Nju	When mouse is 3 months old, fat gain can be found in eighboring arteries, they damage to the body and the injury increasing with the growth of age and reduction of lipid
Tumour Model	C57BL/6J-*Apc*^{Min}/Nju	When feeding high-fat diet, heterozygous mouse occur intestinal tumor in 100%, the kind of tumor exceed 30, and the most die before 120d
Alzheimer's disease Model	B6C3-Tg(APPswe,PSEN1dE9)/Nju	Mouse appear patches(6-7months), cognitive deficit(7 months) and synaptic loss(9 mouths) in hippocampus and cortex

▶ 2016 Highlights

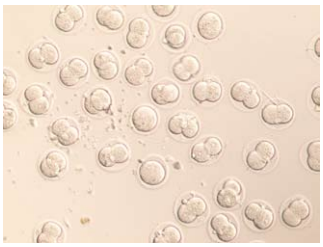
NOD-*Prkdc*^{em26}*Il2rg*^{em26}/Nju (NCG)

- NO mature lymphocytes, NO serum Ig and LOW NK cell activity
- Resistant to lymphoma development even after irradiation treatment
- Readily support engraftment of human CD34+ hematopoietic stem cells
- Suitable for patient derived tumorxerography study
- Enables transplantation of human pancreatic islets and the autoimmune lymphocytes that cause type 1 diabetes

Breeding and Cryopreservation

Cryopreservation saves the expense and space associated with maintaining live colonies and provides backups in case of colony loss due to equipment failure, genetic contaminations, diseases or natural disasters such as earthquakes and fire.

In 2016, NRCMM has finished cryopreservations of 618 strains. At the same time, NBRI provides customers with services of genotyping and maintaining live colonies. In addition, NBRI provides custom breeding services, such as strain rederivation, fast expansions via IVF and strain rescue, in order to meet specific requirments.



Animal Health Monitoring Program and Veterinary Severice

The veterinaries ensure the NBRI'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 and training.

Transgenic/Knockout Mice Services

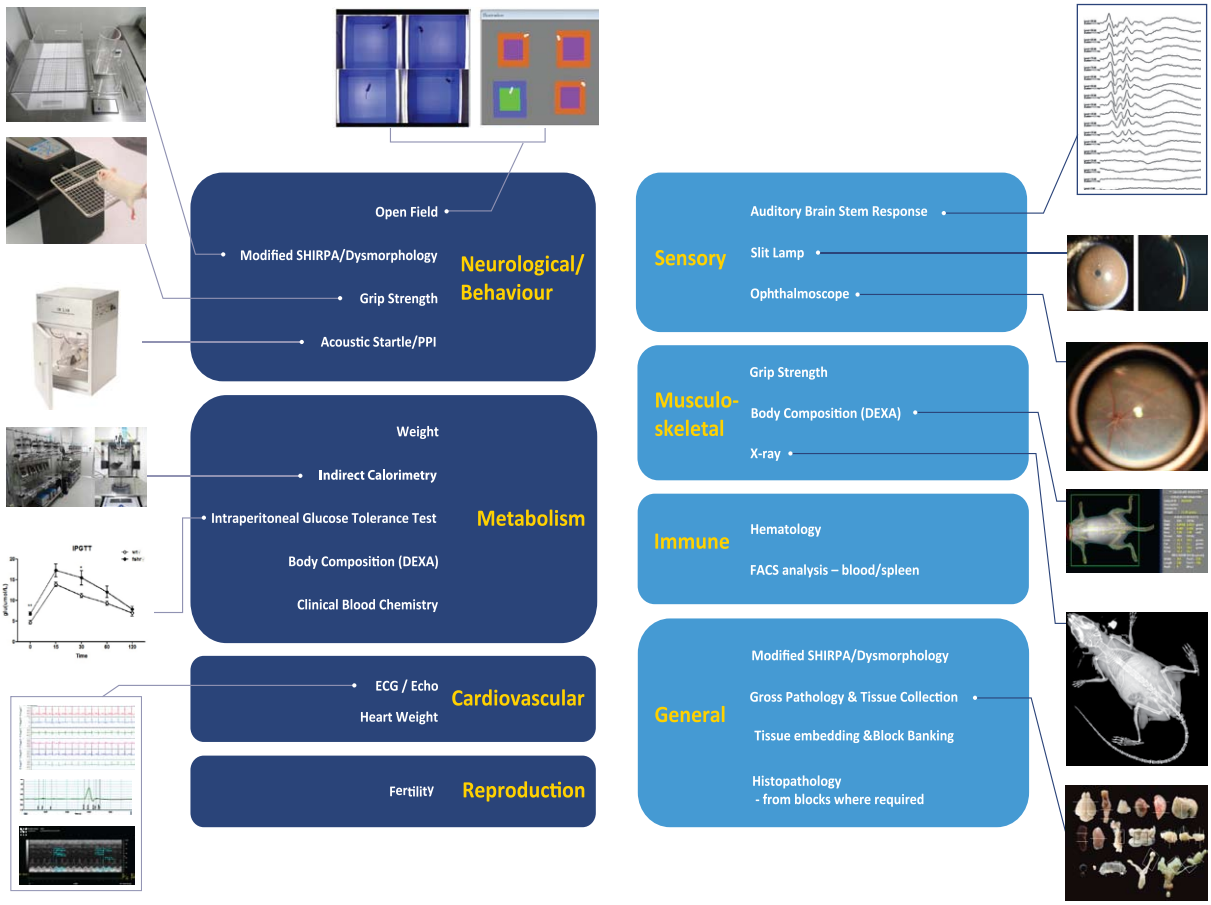
For transgenic mice, we can generate traditional transgenic mice, site-specific insertion transgenic mice (ensure the expression) and BAC transgenic mice (as large as 200kb BAC integration).

We also provide KO/CKO/KI either with traditional ES cell recombination method or CRISPR/Cas9 gene-editing technology.

In 2016, more than 500 projects of TG/KO/CKO/KI are completed in NBRI.

Phenotyping Service

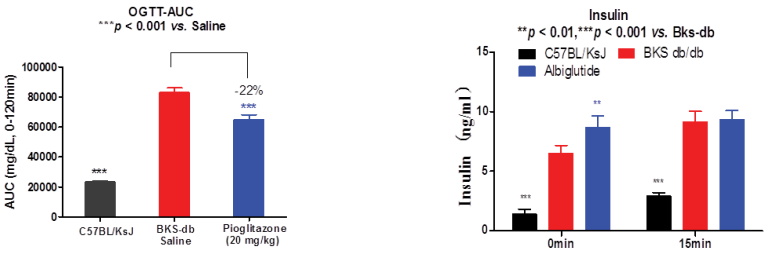
NBRI has set up a standardized phenotype analysis platform that in line with the protocols from the International Mouse Phenotyping Consortium (IMPC). As the only IMPC member in China, we have finished more than 300 KO strains' full scale phenotyping in NBRI. These phenotyping screens covered behavior tests, metabolic cage analysis, cardiovascular function analysis, sensory systems examination, musculoskeletal function analysis, blood chemistry analysis, flow cytometric analysis of blood cells, and pathology analysis.



Drug efficacy in vivo analysis for obesity, diabetes and cardiovascular disease models

Mouse models

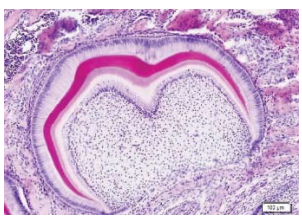
- Diet-induced obese mice
- Spontaneous mutant diabetic mice
- Drug - induced diabetic mice
- Cardiovascular disease mice
- Osteoporosis mice



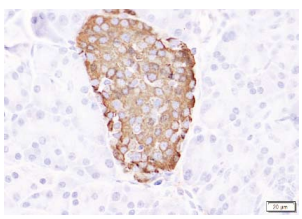
Oral Glucose Tolerance Test

Pathology examination

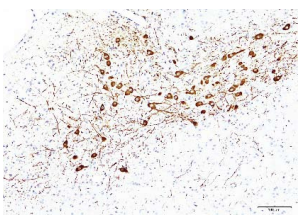
Professional molecular and histological pathology examination of individual mouse/rat tissue, including QPCR, western blot, HE staining and immuno-histochemistry.



Nfant mice odontoprisis
(H.E. staining)



Insulin
(Pancreas islet, Mouse,IHC)



Tyrosine hydrogenase
(Substantia nigra, Mouse,IHC)

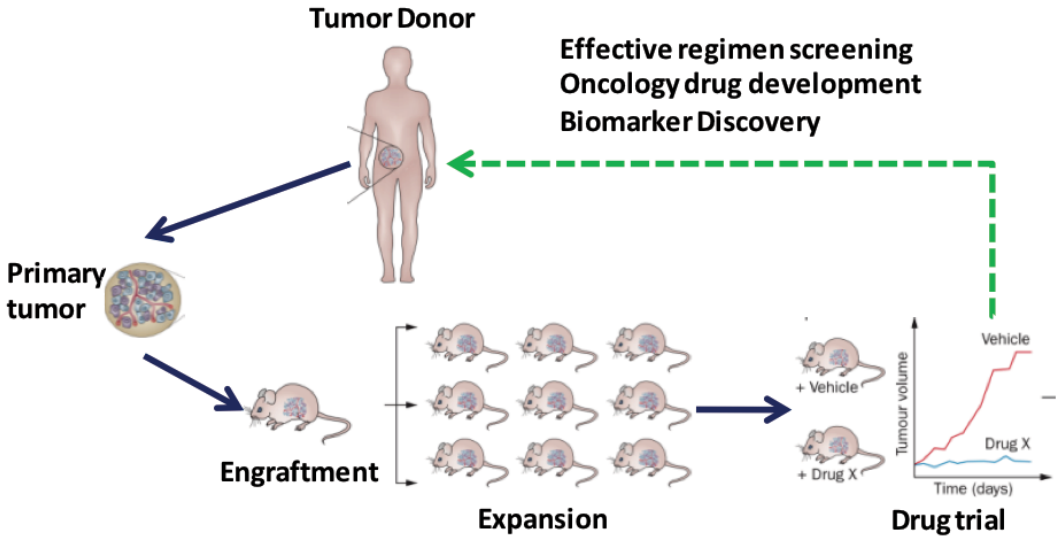
Oncology Service

CDX

Cell-line-derived xenograft tumor mouse models(CDX), are widely used for preclinical test of anti-cancer drug efficacy. Currently, 16 CDX s.c. models are available. In addition, other models can be developed for clients' projects based on request.

PDX

PDX models are established by implanting patient-derived fresh tumor tissues into immunodeficient mice. Recognized as "Avatars" of the patients , PDX models retain the histological and genetic characteristics of their donor tumor. PDX models provide better platforms for preclinical drug evaluation, biomarker identification and oncology studies. NBRI provides services of PDX model construction and pharmacodynamic study.



- Modified from Nature Reviews/Clinical Oncology Vol.9,201

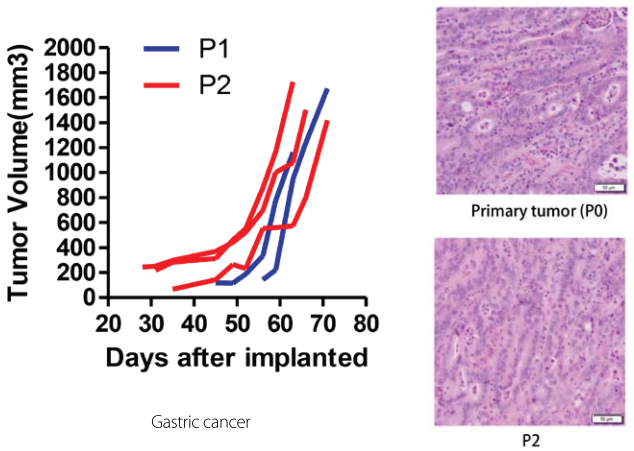
Advantages of PDX models:

- Retain the heterogeneity of donor tumor
- Clinical relevant responses to SoC
- Well characterized PDX models facilitate further biomedical study

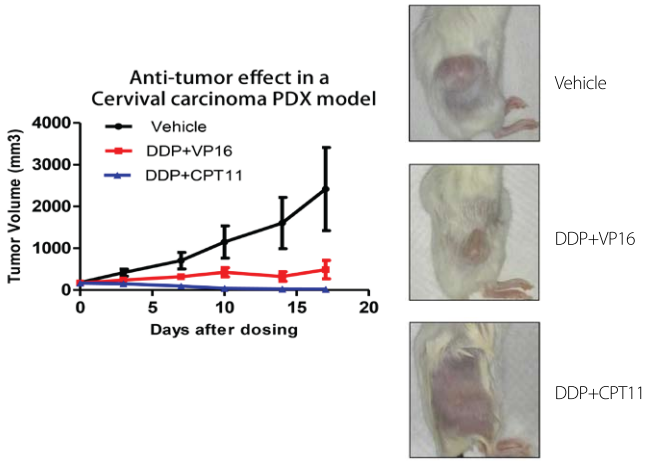
Established PDX models in NBRI

Gastric, Colorectal, Pancreatic, Cervical, Ovarian, Osteosarcoma

PDX Model Establishment



PD study on PDX



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Publications in 2016

1	Zhang YJ, Wang YF, Zhang C, Wang J, Pan DJ, Liu JH, Feng FD (2016) Targeted Gene Delivery to Macrophages by Biodegradable Star Shaped Polymers. Acs Applied Materials & Interfaces 8: 3719-3724
2	Shi DQ, Xu XQ, Ye YQ, Song K, Cheng YX, Di J, Hu QY, Li JX, Ju HX, Jiang Q, Gu Z (2016) Photo-Cross-Linked Scaffold with Kartogenin-Encapsulated Nanoparticles for Cartilage Regeneration. Acs Nano 10: 1292-1299
3	Jiang SJ, Wang QH, Huang Z, Song AY, Peng Y, Hou SY, Guo SY, Zhu WY, Yan S, Lin ZY, Gao X (2016) Gastric Bypass Surgery Reverses Diabetic Phenotypes in Bdnf-Deficient Mice. American Journal of Pathology 186: 2117-2128
4	Jia WJ, Jiang S, Tang QL, Shen D, Xue B, Ning W, Li CJ (2016) Geranylgeranyl Diphosphate Synthase Modulates Fetal Lung Branching Morphogenesis Possibly through Controlling K-Ras Prenylation. American Journal of Pathology 186: 1454-1465
5	Wang HY, Quan C, Hu CX, Xie BX, Du YA, Chen L, Yang W, Yang L, Chen QL, Shen B, Hu B, Zheng ZH, Zhu HB, Huang XX, Xu GW, Chen S (2016) A lipidomics study reveals hepatic lipid signatures associating with deficiency of the LDL receptor in a rat model. Biology Open 5: 979-986
6	Song K, Rong Z, Yang XF, Yao Y, Shen YS, Shi DQ, Xu ZH, Chen DY, Zheng MH, Jiang Q (2016) Early Pulmonary Complications following Total Knee Arthroplasty under General Anesthesia: A Prospective Cohort Study Using CT Scan. BioMed research international: 5
7	Song K, Xu ZH, Rong Z, Yang XF, Yao Y, Shen YH, Shi DQ, Chen DY, Zheng MH, Jiang Q (2016) The incidence of venous thromboembolism following total knee arthroplasty: a prospective study by using computed tomographic pulmonary angiography in combination with bilateral lower limb venography. Blood Coagulation & Fibrinolysis 27: 266-269
8	Chu CH, Wang SJ, Li CL, Chen SH, Hu CF, Chung YL, Chen SL, Wang QS, Lu RB, Gao HM, Hong JS (2016) Neurons and astroglia govern microglial endotoxin tolerance through macrophage colony-stimulating factor receptor-mediated ERK1/2 signals. Brain Behavior and Immunity 55: 260-272
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30	Zhang LL, Zhou JK, Han JX, Hu B, Hou NN, Shi Y, Huang XX, Lou X (2016) Generation of an Oocyte-Specific Cas9 Transgenic Mouse for Genome Editing. Plos One 11: 10
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35	Diao F, Jiang C, Wang XX, Zhu RL, Wang Q, Yao B, Li CJ (2016) Alteration of protein prenylation promotes spermatogonial differentiation and exhausts spermatogonial stem cells in newborn mice. Scientific Reports 6: 12
36	Hou SY, Xian L, Shi PL, Li CJ, Lin ZY, Gao X (2016) The Magea gene cluster regulates male germ cell apoptosis without affecting the fertility in mice. Scientific Reports 6: 12
37	Luo J, Wang H, Kang D, Guo X, Wan P, Wang D, Chen J (2016) Dlg5 maintains apical polarity by promoting membrane localization of Crumbs during Drosophila oogenesis. Scientific Reports 6: 14
38	Li JB, Yue YY, Zhao QS (2016) Retinoic Acid Signaling Is Essential for Valvulogenesis by Affecting Endocardial Cushions Formation in Zebrafish Embryos. Zebrafish 13: 9-18

Seminar

	Date	Speaker	Title	Unit
1	20160120	Weiyun Shi	Focus on the application of the world's first artificial bioengineered cornea	Shandong Eye Hospital
2	20160125	Juan Liang	Pancreatic Progenitors Differentiation: Lessons Learned from Development	College of dental and biomedical sciences, Queen's University Belfast
3	20160303	Zhigang Tian	NK Cells and Liver Disease	School of life sciences,University of science and Technology of china
4	20160324	Ceshi Chen	Protein ubiquitination and cancer	Kunming Institute of Zoology. CAS
5	20160401	Yongguang Yang	Humanized mice and its application in translational medicine	Institute of translational medicine, Jilin University
6	20160330	Zongde Zhang	HEVs maturation are mediated by commensal Fungi induced migration of RALDH+ DCs	Department of Pathology University of Chicago
7	20160331	Yuxin Yi	PTEN and p53: Two Guardians of The Genome	Peking University School of Basic Medical Sciences
8	20160414	Baoliang Song	Regulation of cholesterol metabolism and transport	College of Life Sciences at Wuhan University
9	20160426	Jingdong Han	Integrative Analysis of Biological Big Data	CAS-MPG Partner Institute for Computational Biology
10	20160428	Su Wang	To Study Inner Sheath Cells as the Stem Cell Differentiation Niche in Drosophila Ovary	Stowers Institute for Medical Research.USA
11	20160504	Jianliang Li	Data Mining for Cancer Biomarker Discovery	Sanford Burnham Prebys Medical Discovery Institute
12	20160509	Ziyu Zeng	Emerging interactions between cardiac progenitors and their niches	Division of Biological Sciences University of California
13	20160523	Zhonghua Hu	Mechanisms shaping synaptic circuit:microRNA and beyond	Lieber Institute for Brain Development
14	20160526	Suling Liu	The research progress on cancer stem cells	School of life sciences,University of science and Technology of china
15	20160616	Yi Zhong	Active forgetting and brian disorders	School of life sciences,Tsinghua University
16	20160614	Zhenguo Wu	A Molecular Switch that Regulates Cell Fate Choice Between Muscle Progenitor Cells and Brown Adipocytes	Division of Life Science The Hong Kong University of Science and Technology
17	20160706	Qiang Li	Fat Remodeling by PPARgamma Acetylation	Columbia University College of Physicians and Surgeons
18	20160708	Mingfu Wu	Oriented Cell Division Contributes to Cardiac Morphogenesis and Progenitor differentiation	Albany Medical College.USA
19	20160714	Zhibo Liu	Boramino Acid as A Cancer Marker for PET Imaging	College of chemistry and molecular engineering, Peking University
20	20160714	Guangmei Yan	The anti-tumor effects of a new oncolytic virus	Sun Yat-sen University
21	20160716	Dacheng Tian	Historical opportunity of plant genetic study by genome re-sequencing combined with CRISPR-Cas9 technology	Nanjing University
22	20160718	Hongbo Hu	The function of ubiquitination in immune system	Sichuan University
23	20160718	Chong Chen	The epigenetic mechanism of leukemia	Sichuan University
24	20160726	Li Xin	Prostate epithelial lineage hierarchy	Baylor College of Medicine

	Date	Speaker	Title	Unit
25	20160830	Gong Chen	In vivo reprogramming of brain repair	Pennsylvania State University
26	20160923	Lei Jiang	A novel redox pathway in anchorage-independent cell growth	The University of Texas Southwestern Medical Center
27	20161024	Jincai Luo	New regulator of endothelial exocytosis critical for maintaining vascular homeostasis	The Institute of Molecular Medicine,Peking University
28	20161024	Roger James Colbran	CaMKII: a multifunctional mediator of calcium signals in diverse tissues	Vanderbilt University
29	20161025	Quan Chen	Molecular regulation of mitochodiral autophagy	Institute of Zoology, Chinese Academy of Sciences
30	20161104	Takayuki SuzukD	Quantitative approach to understand whole organ deformation dynamics in the chick limb	Nagoya University
31	20161104	Witold Filipowicz	Mechanism and regulation of microRNA repression and metabolism in cultured cells and mouse retina	Friedrich Miescher Institute (FMI) for Biomedical Research Basel, Switzerland
32	20161110	Aibin He	Regulation network of transcriptional factors and enhancers	The Institute of Molecular Medicine,Peking University
33	20161117	Juncheng Dai	Bioinformatics vs Genomic vs Public health	Nanjing Medical University
34	20161122	Guanghou Shui	Metabolomics/Lipidomics as a principle tool to renew our understanding of metabolic disorders	Institute of Genetics and Developmental Biology, Chinese Academy of Sciences
35	20161130	Hongzhi Xue	Long noncoding RNA Braveheart harbors modular structural motifs necessary for cardiac lineage commitment	Massachusetts Institute of Technology
36	20161201	Xiaoyang Dou	Gene molecular control network	Key Laboratory of Computational Biology, Chinese Academy of Sciences
37	20161202	Chuxia Deng	Breast cancer suppressor BRCA1 in cell cycle regulation, cancer initiating cells and cancer metastasis	Faculty of Health Sciences, the University of Macau

Courses and Teachers

The MARC, as an institute of the University of Nanjing, is home to approximately 182 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 2016, the following lecture series and courses were given by MARC staff at Nanjing University (unless indicated otherwise).

Information genomics:

Zhenji Gan

Cell Biology and Molecular Biology

Shuai Chen

Guoqiang Wan

Chaojun Li

Cell signaling

Geng Liu

Jianghuai Liu

Jun Yan

Chaojun Li

Zhongzhou Yang

Genetics

Qing Zhang

Jinzhong Qin

Di Chen

Xin Lou

Doctoral qualification exam I&II

All PI in MARC

Frontier of Cell Biology

Zhigang Tian (University of Science and Technology of China)

Ceshi Chen (Kunming institute of zoology.CAS)

Yuxin Yin (Beijing University)

Baoliang Song (Wuhan University)

Suling Liu (University of Science and Technology of China)

Yi Zhong (Tsinghua University)

Mechanism of Development

Jiong Chen

Ying Cao

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

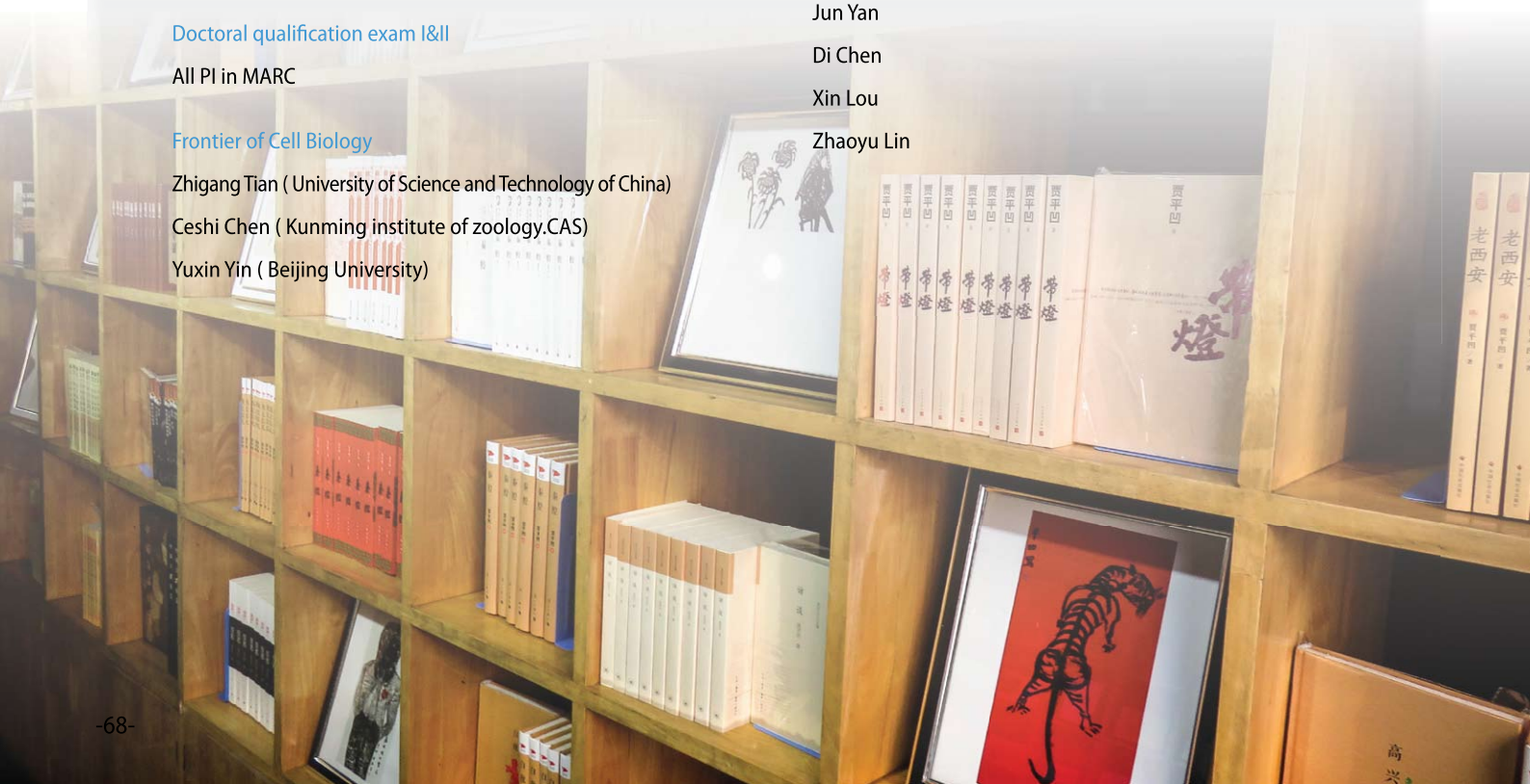
Xiang Gao

Jun Yan

Di Chen

Xin Lou

Zhaoyu Lin



PhD Theses

MARC students successfully defended the following PhD thsese in 2016

PhD Theses:

Group Xiang Gao

Peiliang Shi

Loss of Conserved Gsdma3 Self-Regulation Causes Autophagy and Cell Death

Jianghuan Zou

Relapse of Obesity is Attributed to Immune System Mediated Obesity Memory

Beibei Lai

hnRNP U controls circadian rhythm synchronization by regulating Avp and Vip

Group Qingshun Zhao

Shasha Cao

The role of sox2 in zebrafish development

Group Jianghuai Liu

Yi Lu

Construction and analysis a CRISPR device responding to endogenous signals of mammalian cells

Group Jun Yan

Cunjie Chang

Heterochromatin protein HP1γ is required for prostate cancer progression through HP1γ/miR-451a/c-Myc feedback loop

Group Xiaosong Gu

Zhanhu Zhang

Biology Function and Molecular Mechanism of Tenascin-C in Peripheral Nerve Regeneration

Group Group Ying Xu

Zhipeng Qu

Molecular analysis of the circadian clock in mice

Group Guiquan Chen

Long Wang

Akt3 is Essential for Cerebral Oligodendrogenesis and Myelination

Shanshan Cheng

Essential Role of Presenilin Enhancer2 in Cortical Development

Group Xingxu Huang

Jiankui Zhou

Dual sgRNAs facilitate CRISPR/Cas9 mediated genome engineerin g and animal model generation

Jinxiong Han

Efficient in vitro and in vivo deletion of large DNA fragments by CRISPR/Cas9

2016 Annual Conference of MARC

The 2016 MARC Annual Conference was held in Nanjing University Jinling College from November 2nd to 3rd. This conference was organized by Dr. Guiquan Chen and Dr. Huiming Gao. More than 210 scientists and students from Nanjing University, Suzhou University, and Stanford University attended the conference.

Dr. Minsheng Zhu, director of MARC, made welcome remarks at the opening ceremony, in the following sessions, PIs presented latest research progresses and scientific ideas from their laboratories, followed by lively discussions between the speakers and the audiences. About 81 posters were presented by senior students to exhibit their research results. In the Teacher-Student interaction session, interested issues and topics were discussed.

At last, Zhipeng Qu from Dr. Ying Xu's laboratory, was awarded the 2016 Student of MARC for his excellent research. Jing Liu from Dr. Zhenji Gan's laboratory and Bingxian Xie from Shuai Chen's laboratory were nominated. In addition, ten students received 2016 Outstanding Poster Prize.

Sino-Australian Joint Workshop

A joint Sino-Australian workshop between MARC and University Of Western Australia (UWA) was held in Nanjing from Septemper 14th to 16th. Prof. Robyn Owen, Vice Chancellor of UWA, along with other 12 professors from UWA, and 22 PIs from Nanjing University participated in the workshop.

There were five scientific sessions at the workshop, which were chaired by Dr. Minsheng Zhu (Nanjing University), Dr. Minghao Zheng (University Of Western Australia), Dr. Xiang Gao (Nanjing University), Dr. Ruth Ganss (University Of Western Australia), and Dr. Qing Jiang (Nanjing University), respectively. 19 speakers presented their latest research progresses and scientific ideas from their research, which inspired warm discussions. This workshop strengthened collaborative relationship between UWA and MARC, Nanjing University. The two parties also agreed to initiate a joint Ph.D program to bring the collaboration to a new level.

2016 Laboratory Open Day

To popularize scientific knowledge initiated by China Association for Science and Technology and the Chinese Society for Cell Biology, Laboratory Open Day organized by MARC, NBRI, State Key Laboratory of Pharmaceutical Biotechnology and Jiangsu Society for Cell and Developmental Biology was held on 21st May, 2016. The activity was organized by Prof. Shuai Chen and over 200 persons attended the activities to get close to laboratory work, opening the door for life mysteries exploration.

Prof. Minsheng Zhu, the director of MARC and president of Jiangsu Society for Cell and Developmental Biology, gave an introduction talk of MARC, interacted with participants and then took interview from Science and Technology News.

Prof. Shuai Chen, the vice director of MARC and secretary general of Jiangsu Society for Cell and Developmental Biology, gave another lecture of detail research work in MARC, and showed the audiences around the laboratory.

This activity exhibited four model animals, worms, flies, zebrafish and mice along with posters presenting MARC research work. Plenty of interesting lab experiments were available for people to be involved in.

Laboratory Open Day aimed to enable the general public to comprehend the lab work, gain enthusiasm in research and inspire their motivation in science. This activity was reported by several medias.

2016 Academic Conference for Young Scientists

The 4th Academic Conference for Young Scientists of Jiangsu Society for Cell and Developmental Biology organized by MARC and NBRI was held on 16th July, 2016.

Scientists from famous research institutes, e.g. Nanjing University, Southeast University, Nanjing Normal University, Zhejiang University and Nanjing General Hospital, were gathering together to share and discuss the new development and achievements in their work.

The meeting was chaired by Prof. Zhenji Gan. Prof. Xiang Gao, the founder of MARC and Dean of NBRI, gave an opening speech. The guest speaker, Prof. Dacheng Tian, Prof. Chaojun Li and Prof. Zhongzhou Yang made interesting and inspiring speeches followed by talks giving by young scientists. The PhD candidates at MARC, Pei Wang, Liang Chen and Jing Liu, who did excellent research performances this year, reported their work, and Jing Liu was voted as the best presentation among the three.

This conference provided the chance for young scientists to develop their knowledge and be inspired by a wide range of inspiring speakers, enabling them to share their work with the audience as well as networking with colleagues, peers and like-minded individuals working in the field of cell and developmental biology.

2016 Summer Camp

As the primary task for scientific research and education of MARC, we treat the graduate students to treasure. In order to attract more outstanding students to MARC, we held the 7th Summer Camp from July 11–15 this summer. 45 excellent undergraduates were selected from a pool of 265 applicants from 23 universities nationwide.

Wonderful programs have been organized in order to increase the interaction between undergraduate students and our faculty members/graduate students. 7 faculty members gave lectures on the current progress in biomedical researches, ranging from circadian rhythms, cell migration to heart regeneration and neurodegeneration.

To enhance the students' interest in the experiment, the summer camp also has carried on the experimental demonstration. Students have observed and participated in the experimental Mice, Drosophila melanogaster and Zebrafish.

We respectively held academic salons, dedicates PI with summer camp students communicate with each other at three nights .Moreover, 2 of our outstanding graduate students, Liang Chen and Pei Wang, communicated with the Summer Camp students on their own research lives at MARC.

The purpose of the Summer Camp is to train and attract students for future biomedical researches involving model animals both at MARC and at other institutes in China.

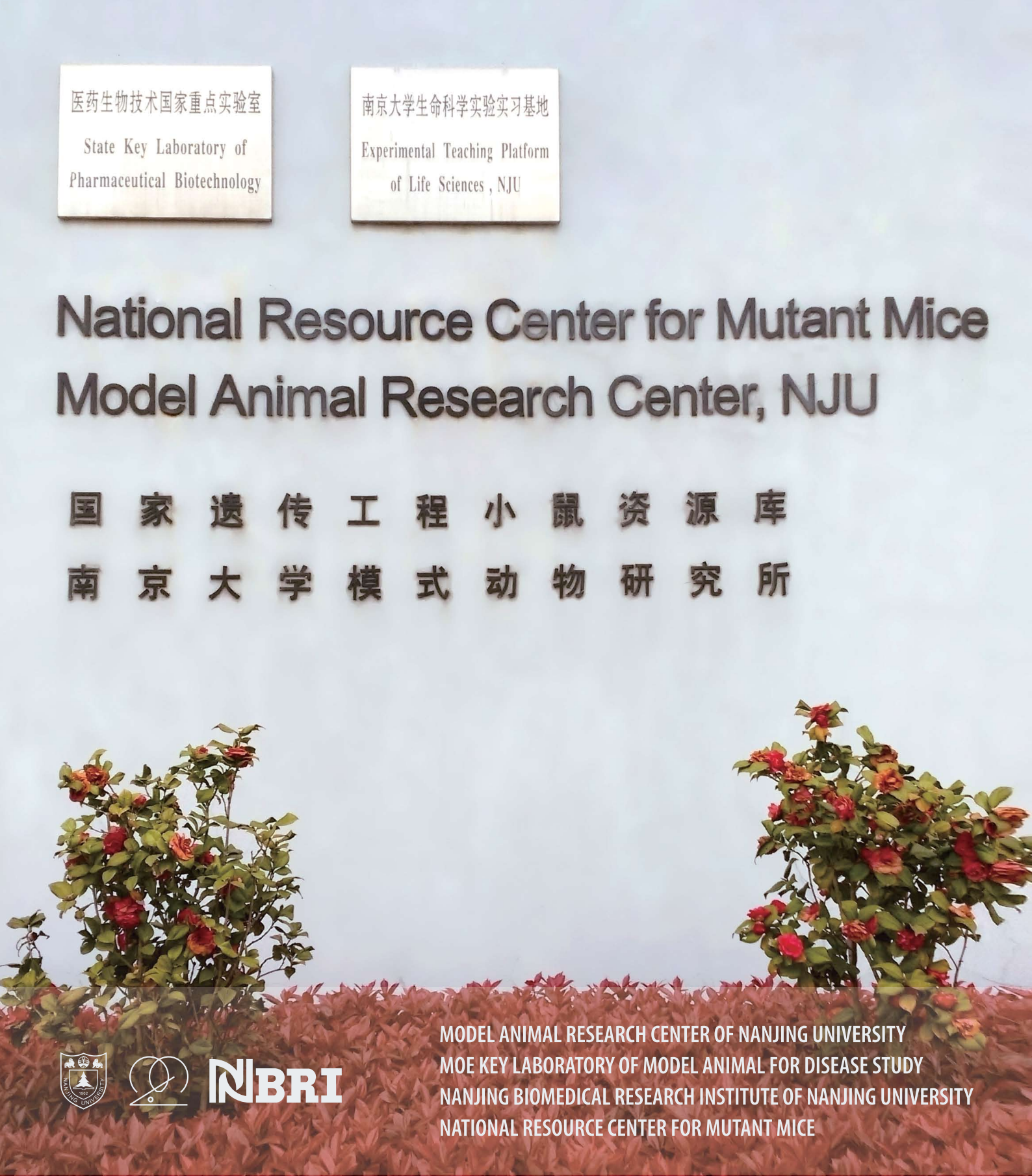
2016 Students Union

In MARC, we have rich and wonderful activities between the professors and students.

The about two hundred "MARC's person" also found themselves cultivated by a culture promoting humanity, and critical thinking. Thanks to the generous financial support guaranteed by both MARC and government, we have adequate resources to enrich our lives here.

In the year of 2016, the annual badminton and table tennis game came at the appointed time in May and Dec. this year, respectively, and being attractive as usually. Moreover, many students also take part in marathon from Nanjing Jiang Su province, and get good grade between the exercises. Sports will not only spice up our life, but also more importantly inspire passion and enthusiasm for physical exercises, which is more than necessary for scientific students like us.

The first stage of weekly held student seminar ended successfully with all the PhD candidates over the third year demonstrated their academic results. A platform to show ourselves and learn from each other is always among our pursuits, and the student seminar was held in this perspective. Also, our lectures with invited speakers were quite abundant this year. Plenty of renowned scientists, have given lectures here. And more speakers form non-science background, such as experts of literature, arts and social science were invited as well.



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