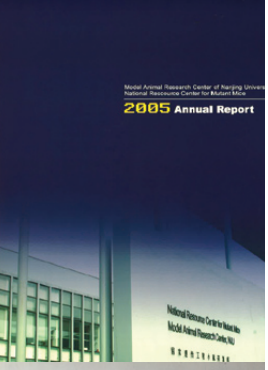

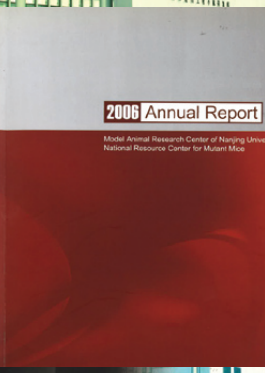

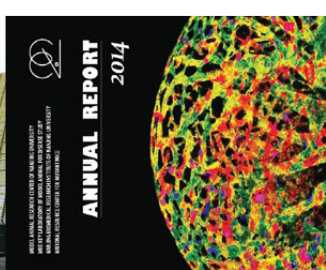

MODEL ANIMAL RESEARCH CENTER OF NANJING UNIVERSITY
MOE KEY LABORATORY OF MODEL ANIMAL FOR DISEASE STUDY
NANJING BIOMEDICAL RESEARCH INSTITUTE OF NANJING UNIVERSITY
NATIONAL RESOURCE CENTER FOR MUTANT MICE

NBRI

ANNUAL REPORT

2015

Director's Words

Great changes came to the world and to us. New local wars fighting over economic interests occurred while the old ones remained. Professor Tu You-You won China's first Nobel Prize of nature science and inspired young and old generations of Chinese scientists. The implementation of 135 program of China dramatically affected our institutional future plans. New science and technology policies to be carried out in this country are changing our funding fate. At MARC, our traditional technology platform for engineering animal which we take pride of is challenged by the new CRISPR technology. We suffered from lack of conventional instruments attributing to insufficiency of financial support. We implemented new research programs focusing on genetics, development and cell biology in hoping that they might become the mainstream of the life science at Nanjing University. Dr. Cao Y, Dr. Zhang Q, Dr. Yang ZZ, Dr. Liu G, Dr. Li CaJ, Dr. Liu JH, Dr. Zhu MS and other PIs lengthened their publication lists with impact papers published on Dev. Cell, Cell Rep, PNAS and many more. The project led by Dr. Gao Xiang is expected to be nominated for the top prize of the Ministry of Education. A new MARC core facility that will greatly improve our research condition is under construction.

Meanwhile, those that are meant to be constant remained at MARC. MARC persisted on frequent PI communication meetings, fruitful brainstorms, academic annual retreats, strict teaching systems and an effective PA (PI assistance) management. Our PIs insisted on the importance of biological significant- and hypothesis-driven researches. Our students persistently improved their abilities for academic thinking and researches conducting. Our PAs provided us with the best service on every single day.

I am proud of our MARC people, a group of people who dream and also earnestly make efforts to realize them. The changes encourage us to march ahead. The philosophies and deeds we persist upon grant us the power to do so. he programs of China and Nanjing University provided the support that we greatly needed. Let us all be thankful to the people and institutions who helped us, as well as the animals that sacrificed. Let us also keep in mind that anything has to be earned. We must look ahead.

The future is subject to even more changes and hence hard to predict, yet I have never been so confident that whatever comes to us would be the best to happen.

Min-Sheng Zhu

Director

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Research Highlight in 2015

Group Ying Cao

Kdm2a/b regulate canonical Wnt signaling via mediating the stability of nuclear beta-Catenin

Wnt/beta-catenin signaling pathway is omnipotent in that it regulates numerous essential aspects of cellular physiology, and is well recognized as a master regulator of embryonic development, the stemness of stem cells, and the homeostasis in tissue and organ differentiation and remodeling. Disregulation of the pathway is tightly correlated to the development and progress of many diseases, including cancer. Wnt signaling is regulated by complicated mechanisms, among which the regulation of beta-catenin turnover plays a critical role. In the canonical viewpoint, absence of Wnt activation will lead to the proteosomal degradation of beta-catenin in the cytosol via amino-terminal phosphorylation of beta-catenin and subsequent ubiquitylation, and hence, failure in target gene transcription. Upon Wnt activation, cytosolic beta-catenin is stabilized and imported to nucleus, where it forms a transcription activation complex with Tcf/Lef transcription factor to stimulate target gene transcription. It remained a question how the nuclear beta-catenin is regulated in response to Wnt activation, so as to maintain the signal strength at a physiological level. Our experiments have found that the protein lysine demethylaes Jhdm1a/b (Kdm2a/b) play an essential role in the regulation of Wnt/beta-catenin signaling. Mechanism analysis revealed that Kdm2a/b regulate the stability of beta-catenin in the nucleus after Wnt activation. This regulation includes the methylation/demethylation of beta-catenin, which is mediated by Kdm2a/b via direct interaction. Kdm2a/b induced beta-catenin demethylation and subsequently ubiquitylation in nucleus is necessary to restrict Wnt target gene transcription. We hence propose a model for the regulation of Wnt pathway via Kdm2a/b-modulated nuclear beta-catenin turnover (Figure1). Wnt activation leads to stabilization and translocation of beta-catenin into nucleus, where it is methylated at the lysine residues within the fourth and fifth armadillo repeats by unknown factor(s). beta-catenin forms a complex with Tcf/Lef transcription factors to activate transcription. To limit accumulation of beta-catenin in nucleus and hence target gene activation or to remove the protein at the end of signaling, Kdm2a/b compete with Tcf/Lef for beta-catenin binding and removes the methyl marks from beta-catenin, which is subsequently degraded via ubiquitylation. As a result, Wnt target gene transcription is attenuated or turned off.

Since the Wnt/beta-catenin signaling is an important pathway in regulating dorso-ventral axis patterning during embryonic development, we therefore have tested the effect of Kdm2a/b on Xenopus body axis formation. We have found that Xenopus Kdm2a/b inhibit beta-catenin-induced secondary axis formation, and knockdown of Kdm2a/b increase the level of nuclear beta-catenin and methylated beta-catenin, and enhance the expression of target genes of Wnt/beta-catenin. These data tell that Kdm2a/b regulate Xenopus body axis formation via regulating nuclear beta-catenin. The mechanism is not only important for the understanding the regulation of Wnt/beta-catenin signaling, it is also enlightening for the research in the other fields including stem cell biology and cancer biology. For detailed information, see Developmental Cell, 2015 Jun 22;33(6):660-74.

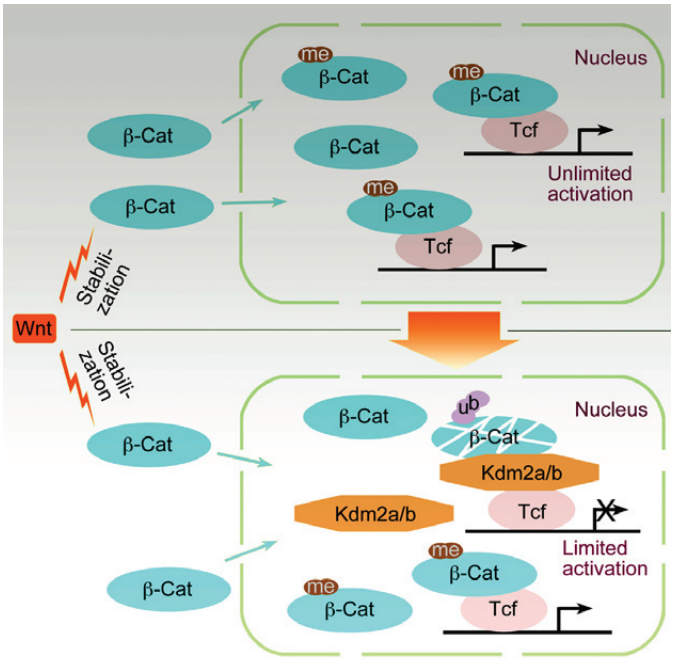

Figure 1. A proposed model for the Kdm2a/b regulated stability of nuclear beta-catenin

Group Minsheng Zhu

Regulation of DLK1 by the maternally expressed miR-379/miR-544 cluster may underlie callipyge polar overdominance inheritance

Yun-Qian Gao[a,1], Xin Chen[a,1], Pei Wang[a], Lei Lu[a], Wei Zhao[a], Chen Chen[a], Cai-Ping Chen[a], Tao Tao[a], Jie Sun[a],Yan-Yan Zheng[a], Jie Du[b], Chao-Jun Li[a], Zhen-Ji Gan[a], Xiang Gao[a], Hua-Qun Chen[c], and Min-Sheng Zhu[a,b,2]

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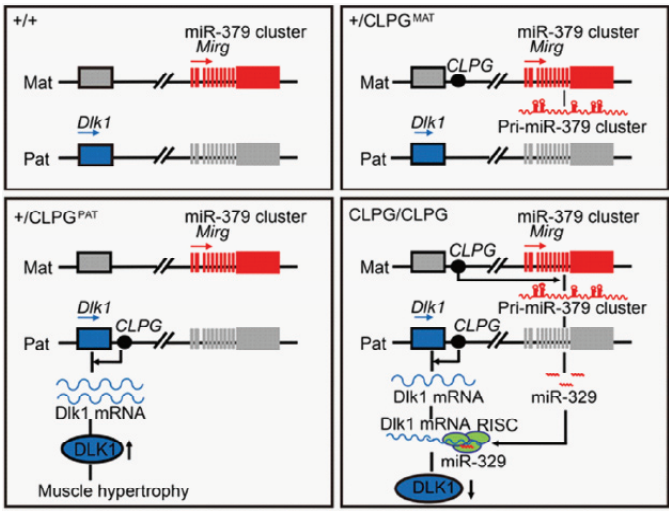
Edited by Se-Jin Lee, Johns Hopkins University, Baltimore, MD, and approved September 29, 2015 (received for review June 11, 2015)

Inheritance of the callipyge phenotype in sheep is an example of polar overdominance inheritance, an unusual mode of inheritance. To investigate the underlyingmolecular mechanism, we profiled the expression of the genes located in the Delta-like 1 homolog (Dlk1)–type III iodothyronine deiodinase (Dio3) imprinting region in mice. We found that the transcripts of the microRNA (miR) 379/miR-544 cluster were highly expressed in neonatal muscle and paralleled the expression of the Dlk1. We then determined the in vivo role of the miR-379/miR-544 cluster by establishing a mouse line in which the cluster was ablated. The maternal heterozygotes of young mutant mice displayed a hypertrophic tibialis anterior muscle, extensor digitorum longus muscle, gastrocnemius muscle, and gluteus maximus muscle and elevated expression of the DLK1 protein. Reduced expression of DLK1 was mediated by miR-329, a member of this cluster. Our results suggest that maternal expression of the imprinted miR-379/miR-544 cluster regulates paternal expression of the Dlk1 gene in mice. We therefore propose a miR-based molecular working model for polar overdominance inheritance.



Group Qing Zhang

Deubiquitination of Ci/Gli by Usp7/HAUSP Regulates Hedgehog Signaling

Zizhang Zhou, Xia Yao, Shuang Li, Yue Xiong, Xiaohua Dong, Yun Zhao, Jin Jiang, Qing Zhang

Background:

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.

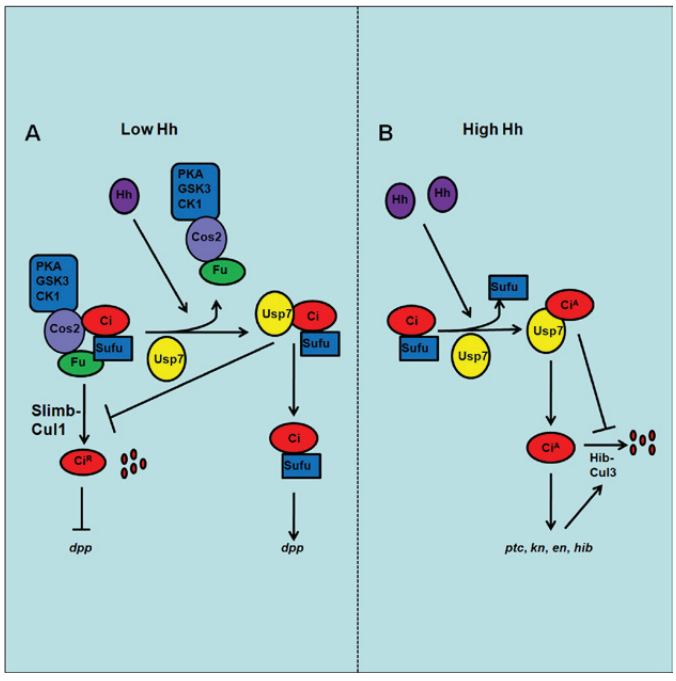
Significance:

In our study, 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.

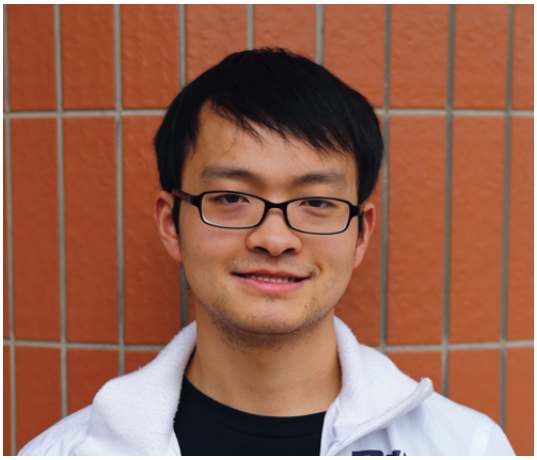
Highlights

- Ubiquitin-specific protease Usp7 positively regulates Hh signaling
- Usp7 binds and deubiquitinates Ci in a manner stimulated by Hh
- Usp7 regulates Hh pathway by forming a complex with GMPS
- Regulation of Hh signaling by Usp7 is evolutionarily conserved

Graphical Abstract



Student of the Year 2015



Zizhang Zhou

Zizhang Zhou received his Bachelor's degree of Biological Science and Technology in 2009 from School of Basic Medical Sciences, Jiangxi University of Traditional Chinese Medicine. He joined Dr. Qing Zhang's lab at the year of 2009 to study the regulatory mechanisms of Hedgehog pathway using Drosophila.

For the past several years, his study focused on the regulation of Hedgehog (Hh) pathway. Using Drosophila as a model, he and his colleagues found a deubiquitinase Usp7 positively regulates Hh signaling through binding and deubiquitinating Ci, the unique transcription factor of Hh pathway in Drosophila. The mammalian counterpart of Usp7 plays a conserved role for deubiquitinating Gli. Collectively, these data reveal a conserved mechanism by which Ci/Gli is stabilized by a deubiquitinase and identify Usp7 as a critical regulator of Hh signaling and potential therapeutic target for Hh-related cancers.

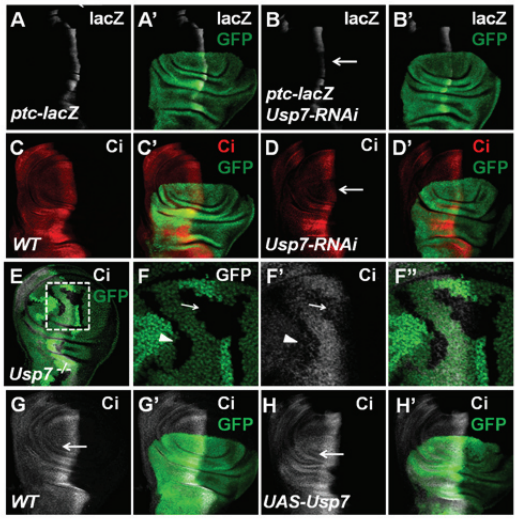


Fig1.Loss of Usp7 decreases Hh pathway activity and Ci protein level. While overexpression of Usp7 upregulates Ci.

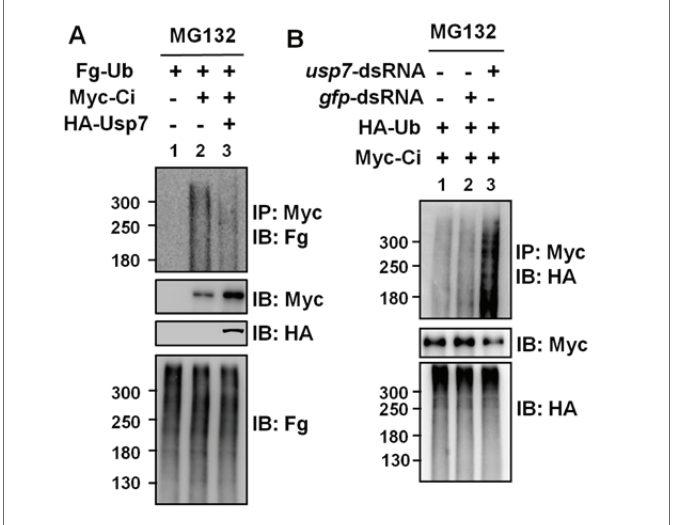


Fig2.Overexpression of Usp7 inhibits the ubiquitination of Ci. Knockdown of Usp7 promotes Ci ubiquitination.

Selected publications

1. Zhou ZZ, Yao X, Li S, Xiong Y, Dong XH, Zhao Y, Jiang J and Zhang Q. Deubiquitination of Ci/Gli by Usp7/HAUSP regulates Hedgehog Signaling. Dev.Cell, 2015; 34:58-72.
2. Zhou ZZ, Xu CY, Chen P, Liu C, Pang S, Yao X and Zhang Q. Stability of HIB-Cul3 E3 Ligase Adaptor HIB Is Regulated by Self-degradation and Availability of Its Substrates. Sci. Rep, 2015.5, 12709; doi: 10.1038/srep12709.
3. Liu C, Zhou ZZ, Yao X, Chen P, Sun M, Su MY, Chang CJ, Yan J, Jiang J and Zhang Q. Hedgehog signaling downregulates Suppressor of Fused through the HIB/SPOP-Crn axis in Drosophila. Cell Res, 2014; 5: 595-609.

Organogenesis and Birth Defect

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

a) Jmjd6 and the regulation of the transcriptional activity of Tcf7l1.

One of the major work in the last year in our lab was to investigate the functional correlation between Jmjd6 and Tcf7l1. Jmjd6 is a JmjC-domain containing protein that may exhibit histone modification activity and Tcf7l1 is a transcriptional repressor that transduces Wnt/beta-catenin signaling. We have found that Jmjd6 interacts with Tcf7l1, both by immunoprecipitation and immunofluorescence, but does not interact with beta-catenin. We observed that overexpression of Jmjd6 alleviated transcriptional repression of Tcf7l, hence enhanced the Wnt target gene transcription and vice versa, knockdown of Jmjd6 compromised the transcription of Wnt target genes. Tcf7l1 achieves its transcription repression via the recruitment of co-repressors like Groucho/TLE1. We observed that Jmjd6 interacts with Tcf7l1 to the same region where Groucho binds, suggesting that Jmjd6 relieves transcriptional repression of Tcf7l1 by competing with Groucho for Tcf7l1 binding. We thus proposed a model for the regulation of Tcf7l1 activity by Jmjd6 (Figure 1).

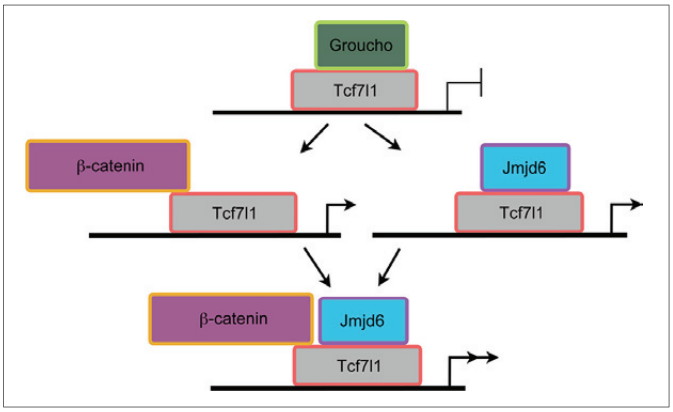


Figure 1. A proposed model for Jmjd6 modulation of the activity of transcription repressor Tcf7l1.

Tcf7l1 play a critical role in the regulation of vertebrate body axis formation. Our experiments demonstrated that loss of Jmjd6 function in Xenopus embryos led to malformation of body axis (Figure 2). Meanwhile, by a series of combinations of Tcf7l1/Jmjd6 overexpression/knockdown experiments, we observed that Jmjd6 serves to alleviate the repression effect of gene transcription by Tcf7l1. Consequently, genes that are involved in body axis formation are upregulated. These results identify a novel mechanism for body axis patterning in which Jmjd6 regulates the activity of Tcf7l1. For detailed information, see J Biol Chem. 2015 Aug 14;290(33):20273-83.

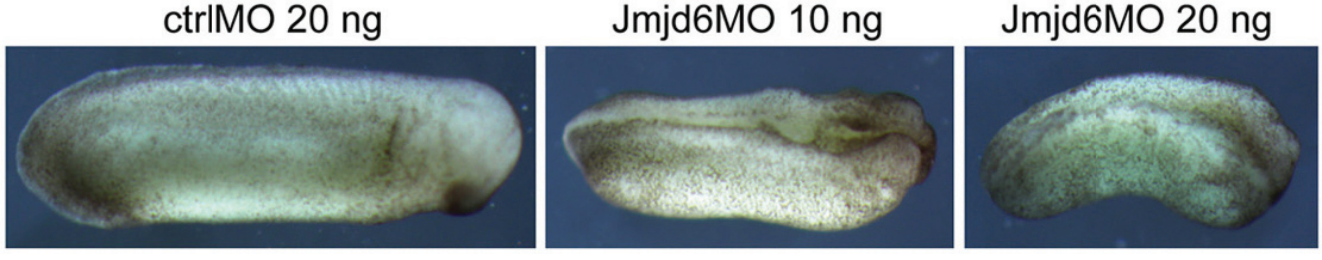


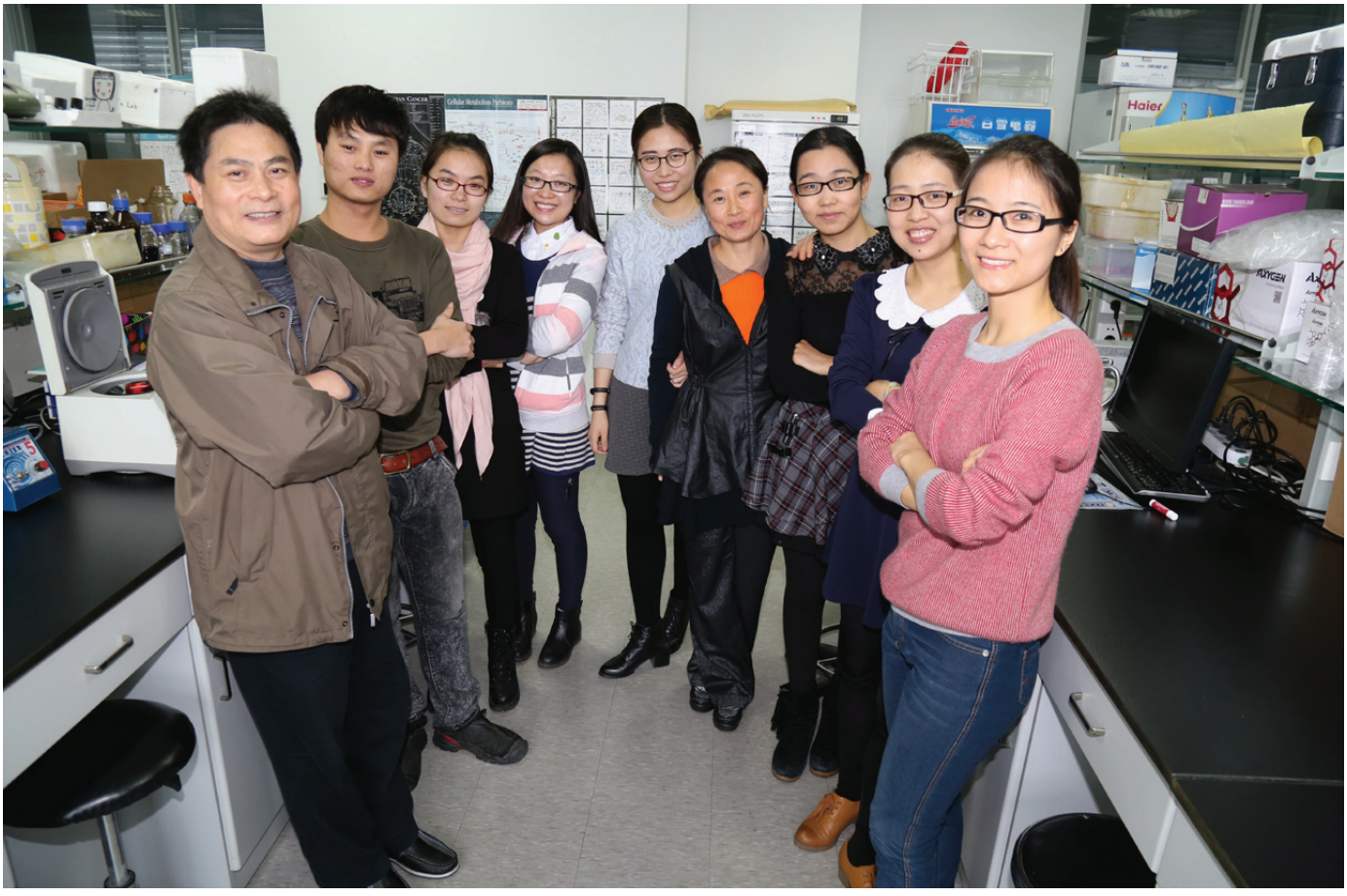
Figure 2. The dose-dependent effect of Jmjd6 knockdown on Xenopus body axis formation.

b) Kdm2a/b regulate the stability of nuclear eta-catenin, thereby mediating the axis formation during Xenopus embryogenesis

In the past 12 months, one of our major research work was to revise our manuscript according to the reviewers' comments. The work has been published in 'Development Cell' and is introduced in the Highlights in the MARC Report 2015.

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.



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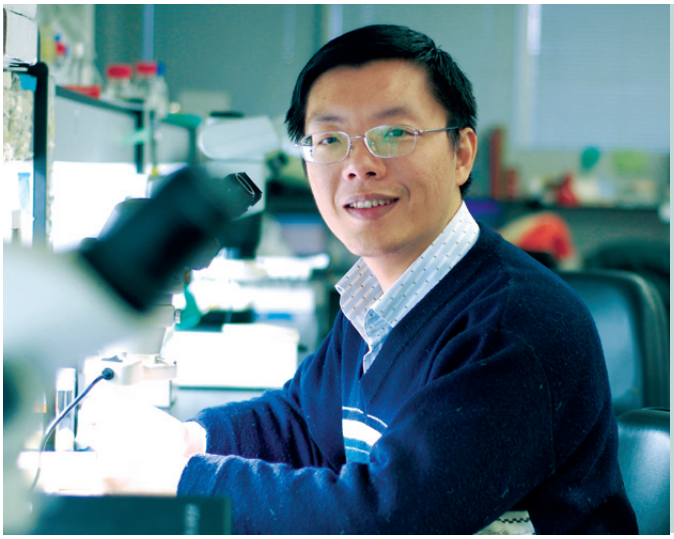

Jiong Chen Ph.D.

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

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Understanding the Driving Forces behind Morphogenesis

My lab is mainly interested in how morphogenetic processes such as cell migration and epithelial morphogenesis are regulated during development. My lab has employed a mainly genetic approach, using the model animal Drosophila melanogaster and cell biological techniques to conduct most of the experiments. And there are 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.

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

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

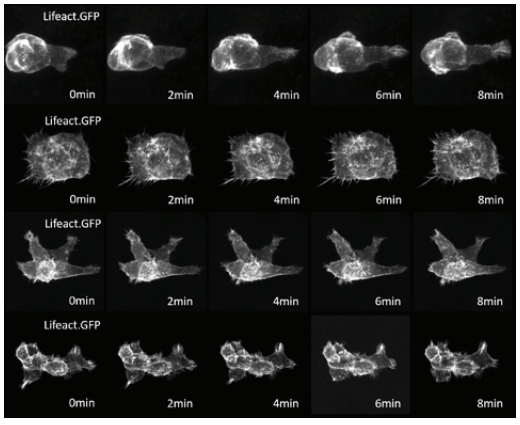

Figure 1. Time-lapsed series showing clusters of 8 border cells (WT, top row; mutants, bottom 3 rows) extending dynamic actin-rich protrusions during collective migration.

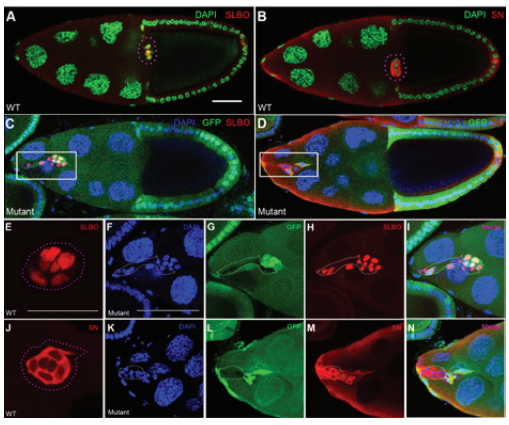

Figure 2. Wildtype border cell clusters (A,B) reached the final destination while mutant mosaic border cell clusters (C,D) displayed delayed migration within the egg chambers.

Selected Publications (*corresponding author)

1. Xu, J., Wan, P., Wang, M., Zhang, J., Gao, X., Hu, B., Han, J., Chen, L., Sun, K., Wu, J.,*, Wu. X.,*, Huang, X.,* and Chen, J.* AIP1-mediated actin disassembly is required for postnatal germ cell migration and spermatogonial stem cell niche establishment Cell Death & Disease (2015)
2. Luo, J., Zuo J., Wu J., Wan., P., Kang, D., Xiang, C., and Chen, J.* in vivo RNAi screen identifies candidate signaling genes required for collective cell migration in Drosophila ovary Science China: Life Sciences (2015)
3. Yuan, B., Wan, P., Chu, D., Nie, J., Cao, Y., Luo, W., Lu, S., Chen, J.*, Yang, Z*. A Cardiomyocyte-Specific Wdr1 Knockout Demonstrates Essential Functional Roles for Actin Disassembly during Myocardial Growth and Maintenance in Mice. American Journal of Pathology 184(7):1967-80 (2014)
4. Wan, P., Wang, D., Luo, J., Chu, D., Wang, H., Zhang, L., and Chen, J.* Guidance receptor promotes asymmetric distribution of Exocyst and recycling endosome during collective cell migration Development (2013).
5. Chu, D., Pan, H., Wan, P., Wu, J., Luo, J., Zhu, H. and Chen, J.* AIP1 Acts with Cofilin to Control Actin Dynamics during Epithelial Morphogenesis Development 139:3561-3571. (2012)
6. Zhang, Lijun, Luo, J., Wan, P., Wu, J., Laski, F., Chen, J*. Regulation of cofilin phosphorylation and asymmetry in collective cell migration during morphogenesis Development 138:455-464. (2011)
7. Chen, Jiong, Call, G., Undergraduate Research Consortium in Functional Genomics (URCFC), and Banerjee, U. Discovery-based science education: functional genomic dissection in Drosophila by undergraduates. PLoS Biology. 3(2): e59 (2005).
8. Chen, Jiong, Dorothea Godt, Kris Gunsalus, Istvan Kiss, Michael Goldberg and Frank A. Laski. Cofilin/ADF is required for cell motility during Drosophila ovary development and oogenesis. Nature Cell Biology 3(2), 204-209 (2001).

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Wang Heng
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Wu Jing
Wu Mengqi
Xu Zehao

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Chu Dandan (Ph.D)
Wan Ping (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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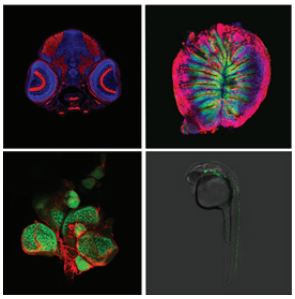
Vertebrate Organogenesis and regeneration



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:

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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)

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		Ningning Hou
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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).

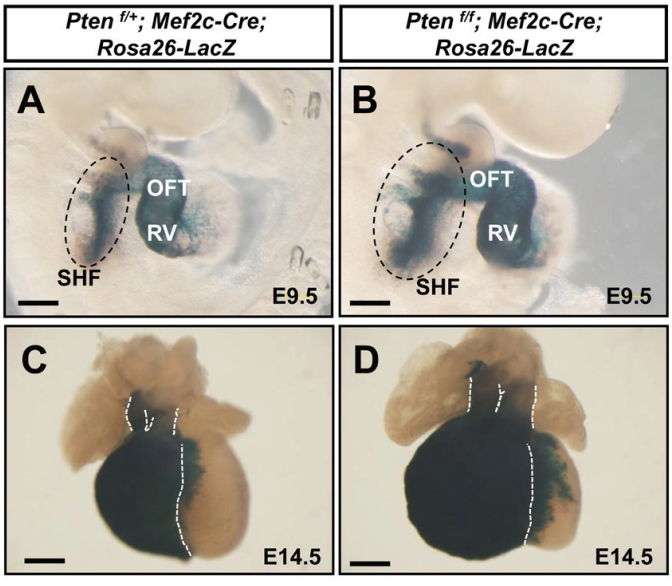

Fig. 1. Deletion of Pten in the SHF progenitors results in enlarged SHF and right ventricle.

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).

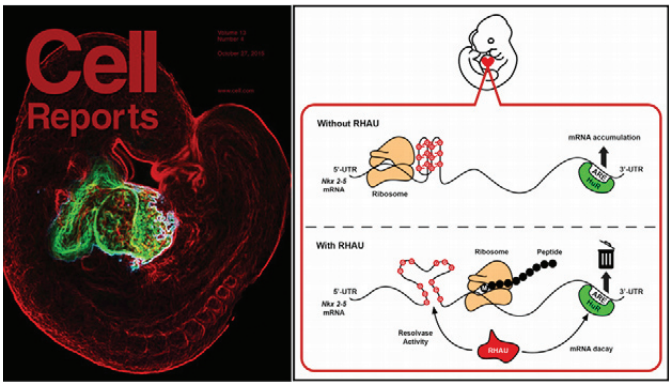

Fig.2. Post-transcriptional regulation of Nkx2.5 by RHAU in heart development.

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.
3. Meixiang Yang, Dan Li, Zai Chang, Zhongzhou Yang, Zhigang Tian and Zhongjun Dong. (2015) PDK1 orchestrates early NK cell development through induction of E4BP4 expression and maintenance of IL-15 responsiveness. J. Exp. Med. 212(2):253-65
4. Fengyuan Tang, Jason Gill, Xenia Ficht, Thomas Barthlott, Hauke Cornils, Debora Schmitz-Rohmer, Debby Hynx, Dawang Zhou, Lei Zhang, Gongda Xue, Michal Grzmil, Zhongzhou Yang, Alexander Hergovich, Georg A. Hollaender, Jens V. Stein, Brian A. Hemmings, Patrick Matthias. (2015) The kinases NDR1/2 act downstream of the Hippo homologMST1 tomediate both egress of thymocytes from the thymus and lymphocyte motility. Sci. Signal. 8(397):ra100.
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.
9. Zai Chang, Qin Zhang, Qiuting Feng, Jie Xu, Teng Teng, Qing Luan, Congjia Shan, Yali Hu, Brian A Hemmings, Xiang Gao and Zhongzhou Yang*. (2010) Deletion of Akt1 causes heart defects and abnormal cardiomyocyte proliferation. Dev. Biol.347: 384-391.
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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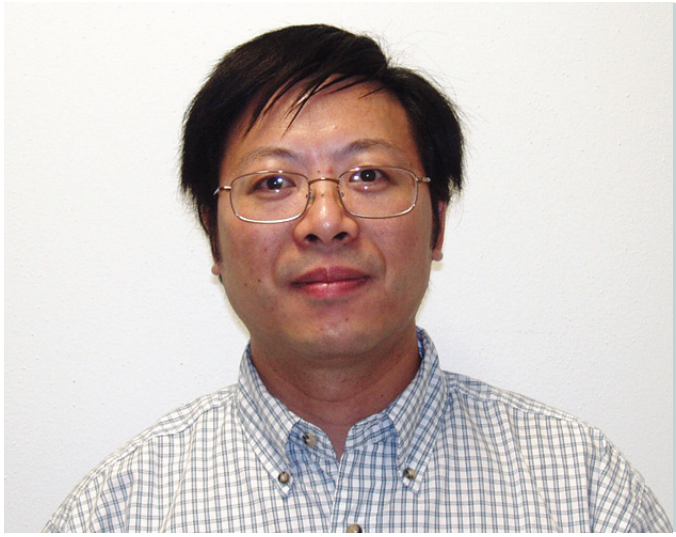

Qing Zhang, Ph.D

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.

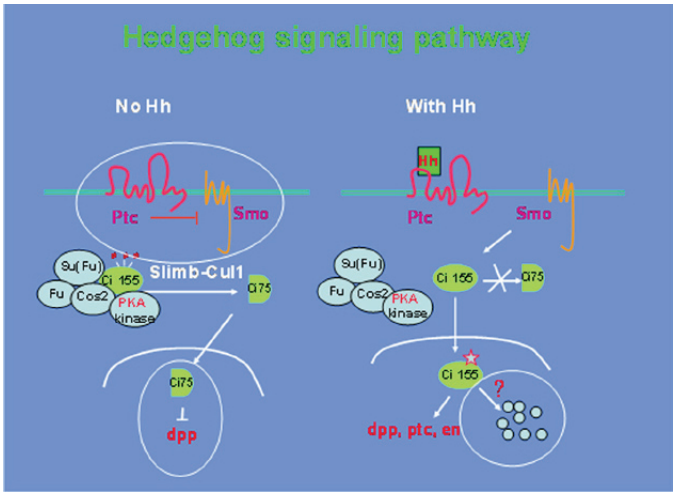


Fig1. Hedgehog signaling pathway in Drosophila.

The mechanism of mitochondrial homeostasis

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.

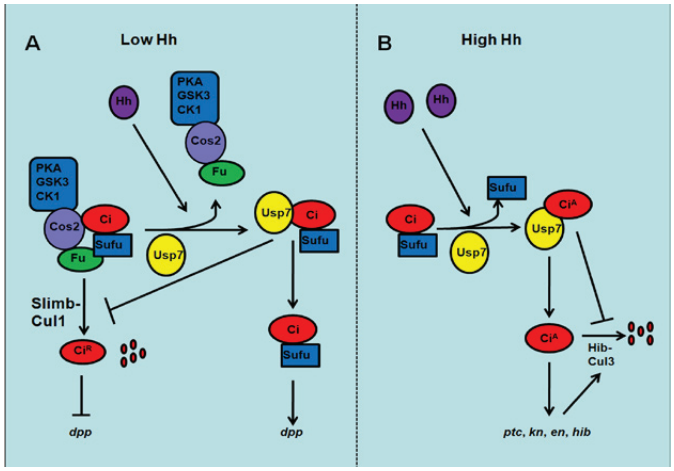


Figure 2. Regulation of the ubiquitin/proteasome-mediated proteolysis of Ci/Gli transcription factors is central to Hh signaling, but whether deubiquitinase is involved in this process remains unknown. We unveil that the deubiquitinase Usp7/HUASP positively regulates Hh signaling through stabilizing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

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.

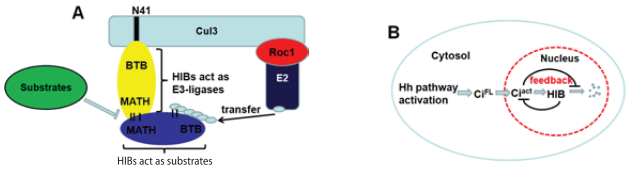
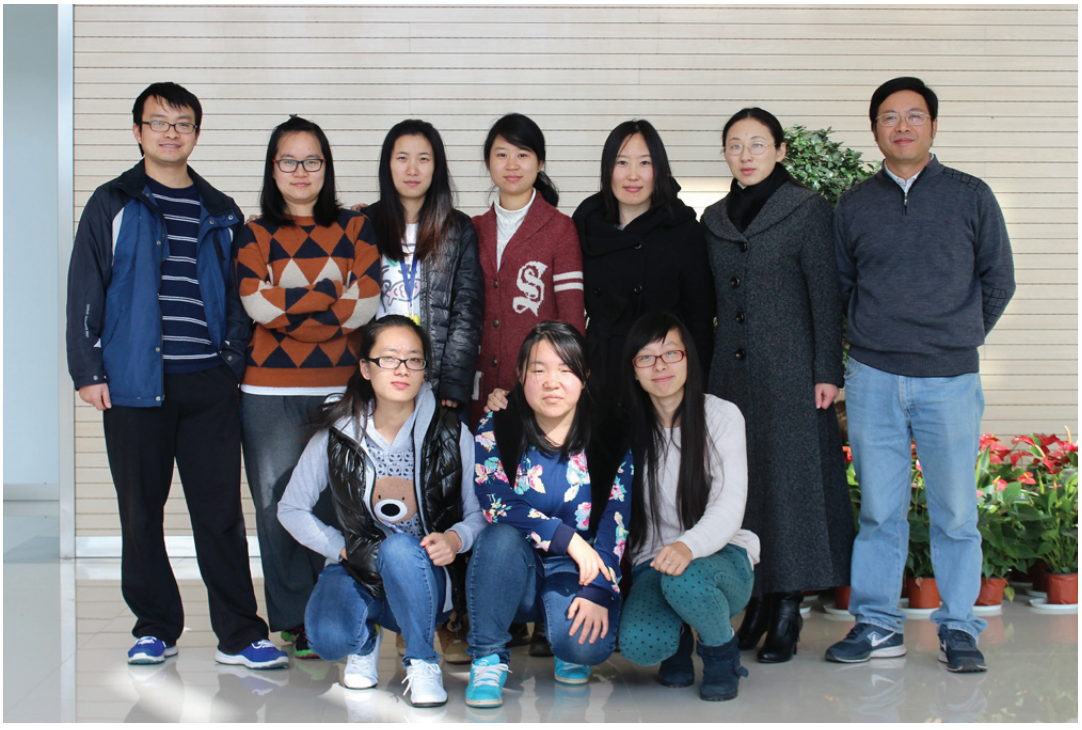


Figure 3. Above shows the model of HIB auto-regulation and the biological significance of this regulation.

(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.
4. An J, Ren S, Murphy S, Dalangood S, Chang C, Pang X, Cui Y, Wang L, Pan Y, Zhang X, Zhu Y, Wang C, Halling G, Cheng L, Sukov W, Karnes R, Vasmatzis G, Zhang Q, Zhang J, Cheville J, Yan J, Sun Y, Huang H. (2015) Truncated ERG Oncoproteins from TMPRSS2-ERG Fusions Are Resistant to SPOP-Mediated Proteasome Degradation. Mol Cell. 59:1-13.
5. Liu C, Zhou Z, Chen P, Su MY, Chang CJ, Yan J, Jiang J*, Zhang Q*. (2014) Hedgehog signaling downregulates Suppressor of Fused through the HIB/SPOP-Crn axis in Drosophila. Cell Research. 24,595-609.
6. Zhang Q, Shi Q, Chen Y, Yue T, Li S, Wang B, Jiang J. (2009) Multiple Ser/Thr-rich degrons mediate the degradation of Ci/Gli by the Cul3-HIB/SPOP E3 ubiquitin ligase. PNAS . 106(50):21191-6.
7. Zhang L, Ren F, Zhang Q, Chen Y, Wang B, Jiang J. (2008) The TEAD/TEF Family of Transcription Factor Scalloped Mediates Hippo Signaling in Organ Size Control. Dev Cell. 14, 377-387.
8. Zhang Q#, Zhang L#, Wang B, Ou CY, Chien CT, Jiang J. (2006) A Hedgehog-induced BTB protein modulates Hedgehog signaling responses by degrading Ci/Gli transcription factor. Dev Cell. 10, 719-729.
9. Jia J#, Zhang L#, Zhang Q#, Tong C, Wang B, Hou F, Amanai K, Jiang J. (2005) Phosphorylation of Cubitus interruptus by Double-time/CKIe and CKIa targets it for Slimb/b-TRCP mediated proteolytic processing. Dev Cell. 9, 819-830.



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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, such as formation and differentiation of germ layers, 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, it is strongly suggest that aldh1a2 is the major gene that is responsible for RA synthesis in zebrafish early development (Liang et al, 2008). Like mammals, zebrafish possesses a third cyp26 gene (cyp26c1) (Gu et al., 2005) in addition to cyp26a1 and cyp26b1. The cyp26c1 metabolizes RA but not retinol or retinal in a similar way to cyp26a1, the major gene that is responsible for RA metabolism in zebrafish early development (Gu et al., 2006). Like cyp26a1, proper expression of cyp26c1 at early developmental stage is essential for the development of anterior–posterior axis and left–right symmetry in zebrafish embryos (Gu et al., 2005; Gu et al., 2006). Analyzing the promoter of cyp26a1, we reveal that zebrafish cyp26a1 possesses three conserved RAREs in response to RA signal and therefore maintains RA homeostasis in a feedback way (Hu et al., 2008; Li et al., 2012). Other than Cyp26s that can limit RA signaling, we show that Ncor1 (nuclear receptor co-repressor) is essential for patterning the anterior–posterior axis of zebrafish hindbrain by actively repressing RA signaling (Xu et al., 2009).

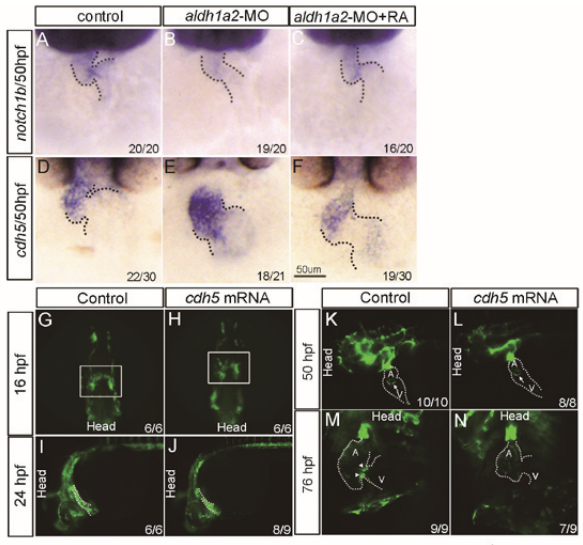

Figure 1. Administration of RA to aldh1a2 morphants well resumes the expression of notch1b and cdh5 in AVC and overexpression of cdh5 disrupts ECs formation in zebrafish embryos at 76 hpf.

Control embryos had restricted expression of notch1b in AVC (A) and that of cdh5 in heart chambers (D) of zebrafish embryos at 50 hpf. Partially knocking down aldh1a2 caused depleted expression of notch1b in AVC (B) and expanded expression of cdh5 into AVC (E). Adding 10 nM RA to aldh1a2 morphants from 1-cell stage well resumes the expression of notch1b in AVC (C) and that of cdh5 in heart chamber (F) at 50 hpf. Like control embryos at 16 hpf (G), 24 hpf (I), and 50 hpf (K), Tg(flk1:EGFP)s84 transgenic embryos overexpressed with cdh5 had normal endothelial cells (white rectangle) in the midline (H) at 16 hpf, in the endocardium of heart tube (dotted line) at 24 hpf (J), and in endothelial ring (white arrow) of AVC (L) at 50 hpf, respectively. The formation of endocardial cushions (white arrowhead) was normal in control embryos (arrowheads in M), but it was affected in the embryos overexpressed with cdh5 (N) at 76 hpf. A, atrium; V, ventricle

Aside from its global role in embryonic pattern formation, RA signaling is essential to vertebrate mesoderm differentiation. It plays a restrictive role in primitive myelopoiesis by inhibiting the specification of ventral mesoderm cells into anterior hemangioblasts through acting downstream of gata4/5/6, upstream of or parallel to cloche, and upstream to scl in a dose dependent manner (Liang et al., 2012). On the other hand, it is also essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos (Figure 1; Junbo Li et al., 2015). 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 other upstream genes. Foxc1a is a member of the forkhead transcription factors. By generating knock-out foxc1a 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 revealed 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 (Figure 2; Li et al., 2015).

Engineered endonuclease (EENs) including ZFN (zinc-finger nuclease), TALEN and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated 9) are powerful tools to create genome edited animals without species limitation. Using the knock out tools 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 single strand DNA donor, and demonstrated that founders carrying fluorescent-labeled primordial germ cells produced much higher numbers of knockin and knockout progeny. 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).

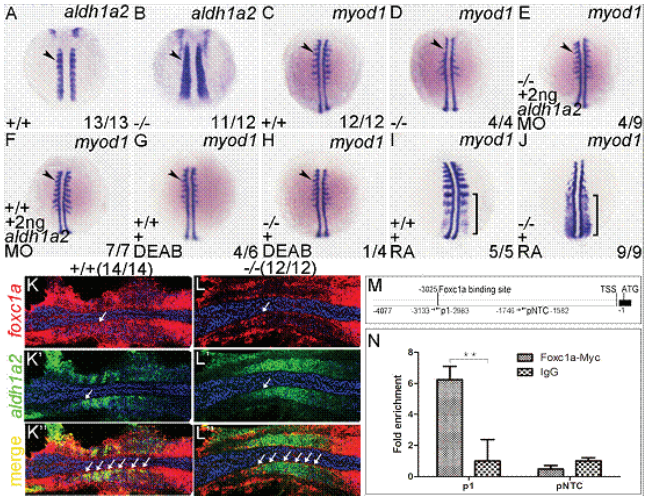

Figure 2. Foxc1a regulating myod1 expression by inhibiting aldh1a2 expression directly.

Expressions of aldh1a2 (A-B), myod1 (C-J) and foxc1a plus aldh1a2 (K, K', K'', L, L', L'') were examined in foxc1a+/+ (A, C, K, K', K'') and foxc1anju18/nju18 (B, D, L, L', L'') embryos at 9-somite stage by whole mount in situ hybridization or whole mount double fluorescent in situ hybridization (K, K', K'', L, L', L''). Expression of myod1 was examined in foxc1a+/+ (C), foxc1anju18/nju18 (D), foxc1anju18/nju18 plus aldh1a2 MO (E) and foxc1a+/+ plus aldh1a2 MO (F), foxc1a+/+ plus DEAB treatment (G), foxc1anju18/nju18 plus DEAB treatment (H), foxc1a+/+ plus RA treatment (I), foxc1anju18/nju18 plus RA treatment (J), respectively. 2 μM DEAB was treated from 0 hpf to 9-somite stage. 1 μM RA was treated from 8 hpf to 9-somite stage. Arrowhead points to the anterior somatic mesoderm region (A-H). Brackets in I-J point to the posterior somatic mesoderm region. Arrows point to individual somite (K, K', K'', L, L', L''). (M) Schematic diagram showing aldh1a2 promoter region. The predicted Foxc1a binding site was shown. Arrows around p1 and pNTC denote the position of PCR primers used for ChIP assay. (N) ChIP assay confirming that there was one functional Foxc1a binding site at aldh1a2 promoter. The relative fold enrichment of Foxc1a on aldh1a2 promoter regions of p1 and pNTC was calculated by normalizing the PCR signals obtained from ChIP with anti-Myc Tag antibody (Foxc1a-Myc) to the signals obtained from control ChIP with mouse IgG (Its average value was normalized to 1)..

Selected Publications (*corresponding author)

1. 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.
2. Junbo Li, Yunyun Yue, Qingshun Zhao*. 2015. Retinoic acid signaling is essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos. Zebrafish, (Accept).
3. 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.
4. Jingyun Li, Kui Li, Xiaohua Dong, Dong Liang, Qingshun Zhao*. 2014. Ncor1 and Ncor2 play essential but distinct roles in zebrafish primitive myelopoiesis. Developmental Dynamics, 243(12):1544-1553.
5. Junbo Li, Wenshuang Jia, Qingshun Zhao*. 2014. Nitrite affects zebrafish valvulogenesis through yielding too much NO signalling. PLoS ONE, 9(3):e92728.
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.
9. Ping Hu, Miao Tian, Jie Bao, Guangdong Xing, Xingxing Gu, Xiang Gao, Elwood Linney, Qingshun Zhao*. 2008. Retinoid regulation of the zebrafish cyp26a1 promoter. Developmental Dynamics, 237:3798-3808.
10. Xingxing Gu, Fang Xu, Wei Song, Xiaolin Wang, Ping Hu, Yumin Yang, Xiang Gao, Qingshun Zhao*. 2006. A novel cytochrome P450, zebrafish Cyp26D1, is involved in metabolism of all-trans retinoic acid. Molecular Endocrinology, 20(7):1661-1672.

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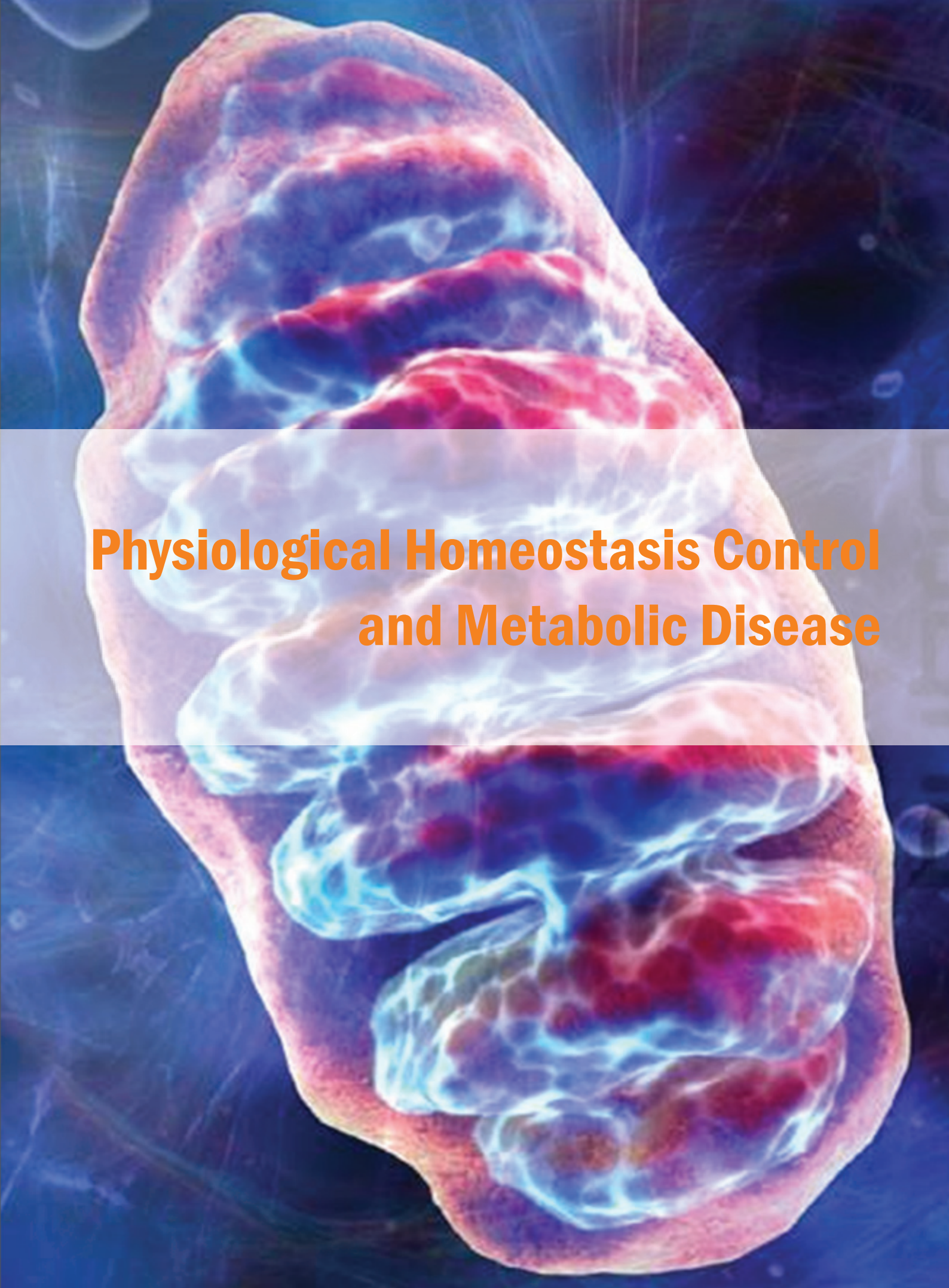

Physiological Homeostasis Control and Metabolic Disease

Di Chen, Ph.D.

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

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C. elegans Aging and Metabolism

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

The highly conserved Insulin/IGF-1 signaling (IIS) and Target of Rapamycin (TOR) pathway play an important role in aging in many species. Our recently published work showed that simultaneous inhibition of IGF-1 and TOR pathways via the *daf-2 rsks-1* double mutant leads to nearly 5-fold, synergistic lifespan extension. We further demonstrated that the underlying mechanisms involve positive feedback regulation of the DAF-16/FOXO transcription factor via the key energy homeostasis regulator AMPK, and the germ line tissue plays a key regulatory role in this process (Figure 1). Currently, we are using polysomal profiling coupled with RNA-Seq techniques to identify genes that are post-transcriptionally regulated in the *daf-2 rsks-1* double mutant and characterize their roles in aging (Figure 1).

Under harsh conditions, *C. elegans* may arrest development and enter a very long-lived, diapause stage called dauer. Previous studies have identified highly conserved pathways, including insulin/IGF-1 signaling (IIS), transforming growth factor beta (TGF-β) and target of rapamycin (TOR), as essential regulators for both aging and dauer development. Based on previous forward genetic screenings, we have isolated the *daf-31* mutant, which showed developmental arrest with partial dauer features. Using positional cloning strategies, we found that *daf-31* encodes a highly conserved acetyl transferase. Functional analysis indicated that DAF-31 is also a modulator of aging by activating DAF-16/FOXO (Figure 2).

Currently, our research focuses on the following aspects:

1.Functional genomics 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 diseases.

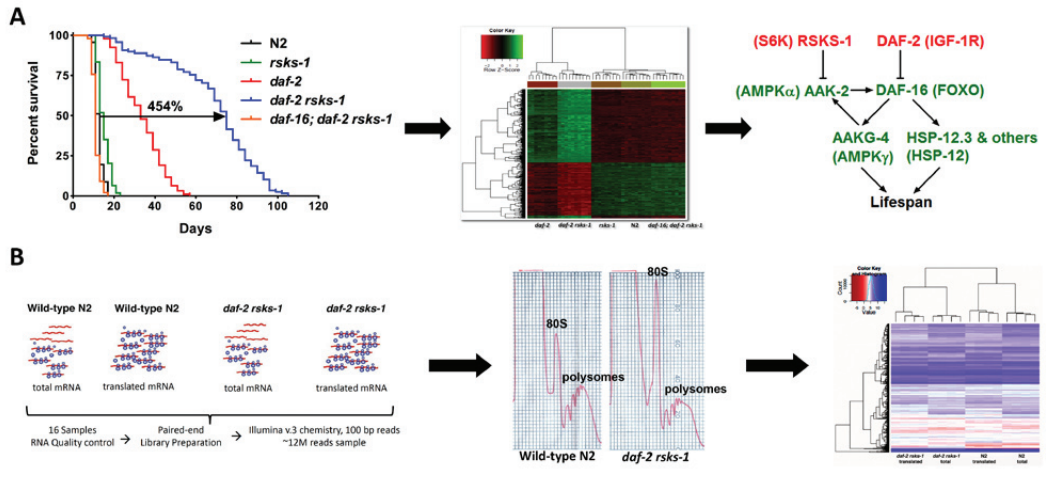

Figure 1. Functional genomics analysis of the super long-lived *daf-2 rsks-1* double mutant in *C. elegans*.

(A) Double mutations in DAF-2 (IGF-1 receptor) and RSKS-1 (ribosomal S6 kinase) leads to nearly 5-fold synergistic lifespan extension, which requires the DAF-16 (FOXO) transcription factor. Transcriptome analysis via microarrays helped to reveal AMPK-mediated positive feedback regulation of DAF-16 in *daf-2 rsks-1*. (B) Polysomal profiling coupled with RNA Seq were used to identify genes that are regulated at the post-transcriptional levels in the *daf-2 rsks-1* double mutant.

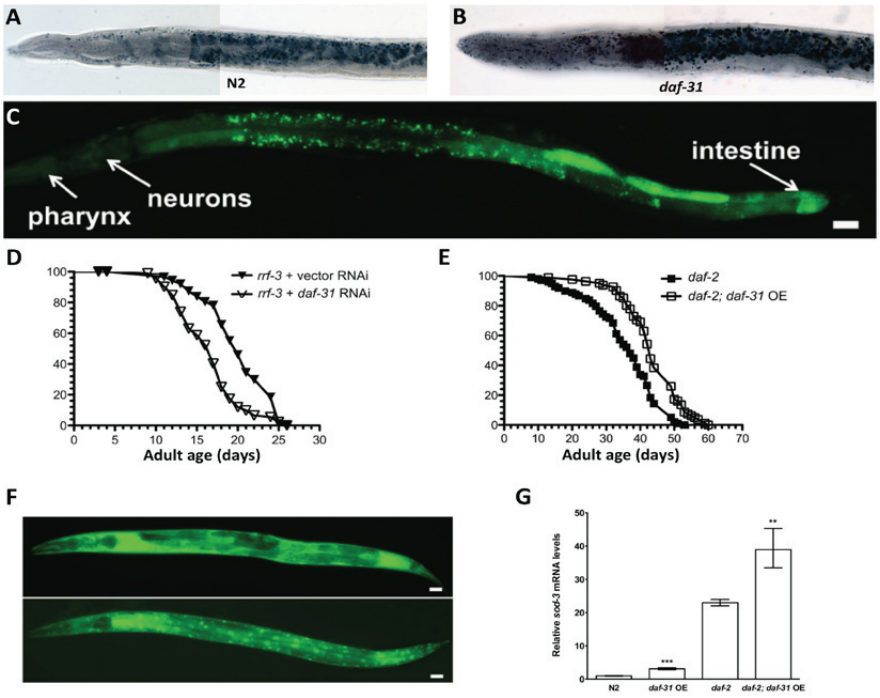

Figure 2. *daf-31* encodes the catalytic subunit of N alpha-acetyltransferase that regulates *C. elegans* development, metabolism and lifespan.

(A-B) The *daf-31* mutant showed increased fat accumulation compared to the wild-type N2. (C) *daf-31* is expressed in neurons, pharynx and intestine. (D) Inhibition of *daf-31* extended lifespan of the RNAi sensitive strain *rrf-3*. (E) *daf-31* overexpression further extended lifespan of the long-lived *daf-2* mutant. (F) *daf-31* overexpression led to increased DAF-16::GFP nuclear localization upon *daf-2* RNAi treatment. (G) *daf-31* overexpression enhanced DAF-16 activity as indicated by increased mRNA levels of *sod-3*, which is transcriptionally activated by DAF-16.

Selected Publications (*corresponding author)

1. Lan J, Zhang X and Chen D* (2015) Molecular mechanisms of dietary restriction in aging - insights from *Caenorhabditis elegans* research. Sci. China Life Sci. 58(4): 352-358.
2. Chen D*, Zhang J, Minnerly J, Kaul T, Riddle DL and Jia K* (2014) *daf-31* Encodes the Catalytic Subunit of N Alpha-Acetyltransferase that Regulates *Caenorhabditis elegans* Development, Metabolism and Adult Lifespan. PLoS Genetics 10(10): e1004699.
3. Chen D* (2014). The Nutrients-regulated Insulin/IGF-1 and TOR Pathways Play an Important Role in C. elegans Aging. Prog. Biochem. Biophys. 41(3): 305-312.
4. Chen D*, Li PW, Goldstein BA, Cai W, Thomas EL, Chen F, Hubbard AE, Melov S and Kapahi P* (2013). Germline Signaling Mediates the Synergistically Prolonged Longevity Produced by Double Mutations in *daf-2* and *rsks-1* in *C. elegans*. Cell Reports 5(6): 1600-1610.
5. Rogers AN, Chen D, McColl G, Czerwieniec G, Felkey K, Gibson BW, Hubbard A, Melov S, Lithgow GJ and Kapahi P. (2011). Life Span Extension via eIF4G Inhibition Is Mediated by Posttranscriptional Remodeling of Stress Response Gene Expression in *C. elegans*. Cell Metabolism 14(1): 55-66.
6. Kapahi P, Chen D, Rogers AN, Katewa SD, Li P, Thomas EL and Kockel L (2010). With TOR, Less Is More: A Key Role for the Conserved Nutrient-Sensing TOR Pathway in Aging. Cell Metabolism 11(6): 453-465.
7. Chen D, Thomas EL and Kapahi P (2009). HIF-1 Modulates Dietary Restriction-mediated Lifespan Extension via IRE-1 in *Caenorhabditis elegans*. PLoS Genetics 5(5): e1000486.
8. Chen D, Pan KZ, Palter JE and Kapahi P (2007). Longevity Determined by Developmental Arrest Genes in Caenorhabditis elegans. Aging Cell 6(4): 525-533.

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Shuai Chen, Ph.D.

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Cell Signaling and Type II Diabetes

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

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

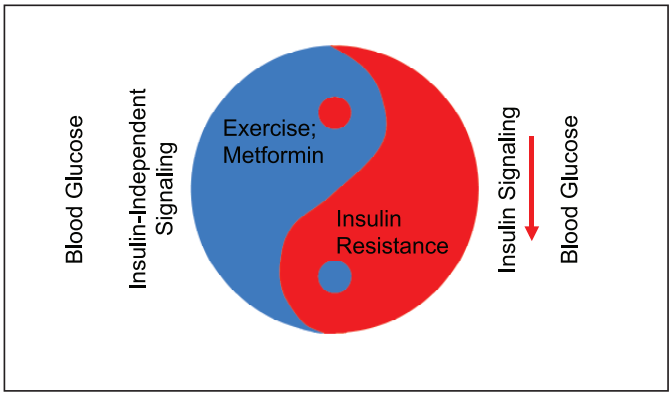

Figure 1 Insulin dependent and independent signaling in the pathogenesis of type II diabetes and its treatment.

The recent progress of my lab is as follows:

Fasting and systemic insulin signaling regulate phosphorylation of brain proteins that modulate cell morphology and link to neurological disorders

Diabetes is strongly associated with cognitive decline, but the molecular reasons are unknown. We found that fasting and peripheral insulin promote phosphorylation and dephosphorylation, respectively, of specific residues on brain proteins that included cytoskeletal regulators such as slit-robo GTPase-activating protein 3 (srGAP3) and microtubule affinity-regulating protein kinases (MARKs), whose deficiency or dysregulation are linked to neurological disorders. Fasting activates protein kinase A (PKA) but not PKB/Akt signaling in the brain, and PKA can phosphorylate the purified srGAP3. The phosphorylation of srGAP3 and MARKs were increased when PKA signaling was activated in primary neurons. Knockdown of PKA decreased phosphorylation of srGAP3. Furthermore, WAVE1, an A-kinase anchoring protein (AKAP), can form a complex with srGAP3 and PKA in the brain of fasted mice to facilitate the phosphorylation of srGAP3 by PKA. Although brain cells have insulin receptors, our findings are inconsistent with the down-regulation of phosphorylation of target proteins being mediated by insulin signaling within the brain. Rather, our findings infer that systemic insulin through a yet unknown mechanism inhibits PKA or protein kinase(s) with similar specificity and/or activates an unknown phosphatase in the brain. Ser858 of srGAP3 was identified as a key regulatory residue, whose phosphorylation by PKA enhanced the GAP activity of srGAP3 towards its substrate Rac1 in cells, thereby inhibiting the action of this GTPase in cytoskeletal regulation (Fig. 2). Our findings reveal novel mechanisms linking peripheral insulin sensitivity with cytoskeletal remodelling in neurons, which may help to explain the association of diabetes with neurological disorders such as Alzheimer's disease (AD).

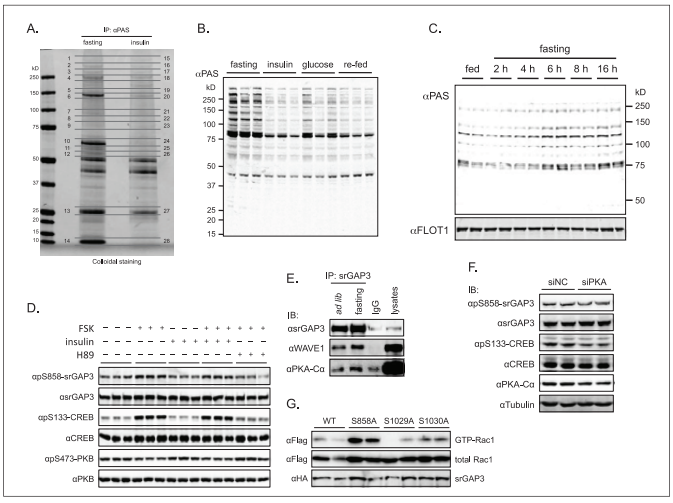

Figure 2 Fasting and systemic insulin signaling regulate phosphorylation of brain proteins that modulate cell morphology and link to neurological disorders

A. PAS antibody-reactive phosphoproteins were immunoprecipitated, using immobilised PAS antibody beads, from brain lysates of mice subjected to overnight fasting (16 h) or intraperitoneal insulin injection (20 min) after an overnight fast.

B. Overnight fasted mice were intraperitoneally injected with insulin (20 min) or glucose (20 min), or allowed to re-feed ad libitum for 90 min (3 mice for each condition) before being culled for brain sampling. The PAS antibody-reactive phosphorylation was detected in brain lysates.

C. Mice were subjected to ad libitum feeding or deprived of food for the indicated time before termination for brain sampling. The PAS antibody-reactive phosphorylation was detected in brain lysates with FLOT-1 as a loading control.

D. Primary cerebellar granule cells were isolated from neonatal mice and subjected to stimulation with forskolin (FSK) or insulin or both. The total and phosphorylated PKB, srGAP3 and CREB were detected in cell lysates.

E. Endogenous srGAP3 was immunoprecipitated from homogenates of brains of mice that were subjected to either ad libitum (ad lib) or overnight fasting (16 h). Endogenous WAVE1 and PKA catalytic subunit were detected in the immunoprecipitates.

F. Ser858 phosphorylation of srGAP3 upon knockdown of PKA. PKA catalytic subunit was knocked-down via siRNA in HEK293 cells expressing HA-srGAP3. Phosphorylation and expression of HA-srGAP3, CREB and PKA were determined via western blot using tubulin as a loading control.

G. The HA-tagged wild-type and mutant srGAP3 proteins were co-expressed with the Flag-tagged Rac1 in HEK293 cells. The GTP-Rac1 was determined via immunoblotting after being isolated from cell lysates using recombinant Pak1-RBD.

Selected Publications(* corresponding author)

1. Li M., Quan C., Toth R., Campbell D.G., MacKintosh C.*, Wang H.Y.* and Chen S.* (2015) Fasting and systemic insulin signaling regulate phosphorylation of brain proteins that modulate cell morphology and link to neurological disorders. J Biol. Chem. doi:10.1074/jbc.M115.668103 (* corresponding author)
2. Quan C., Xie B.X., Wang H.Y.* and Chen S.*. (2015) PKB-mediated Thr649 phosphorylation of AS160/TBC1D4 regulates the R-wave amplitude in the heart. Plos One 10(4):e0124491. Doi:10.1371/journal.pone.0124491 (* corresponding author)
3. Qiaoli Chen, Chao Quan,Bingxian Xie, Liang Chen, Shuilian Zhou, Rachel Toth, David G. Campbell, Shuangshuang Lu, Ryutaro Shirakawa, Hisanori Horiuchi, Chaojun Li, Zhongzhou Yang, Carol MacKintosh, Hongyu Wang*, Shuai Chen*. (2014) GARNL1, a major RalGAP a subunit in skeletal muscle, regulates insulin-stimulated RalA activation and GLUT4 trafficking via interaction with 14-3-3 proteins. Cell Signal 26(8): 1636-1648 (* corresponding author)
4. Wang H.Y., Ducommun S., Quan C., Xie B.X., Li M., Wasserman D.H., Sakamoto K., MacKintosh C.* and Chen S.* (2013) AS160 deficiency causes whole-body insulin resistance via composite effects in multiple tissues. Biochem J. 449 (2): 479-489 (* corresponding author)
5. Ducommun S., Wang H.Y., Sakamoto K., MacKintosh C. and Chen S.* (2012) Thr649Ala-AS160 knockin mutation does not impair contraction/AICAR-induced glucose transport in mouse muscle. Am J Physiol Endocrinol Metab 302: E1036-E1043 (* corresponding author)
6. Chen S., Synowsky S., Tinti M. and MacKintosh C. (2011) Insulin triggers 14-3-3 capture of many phosphoproteins. Trends Endocrinol Metab 22(11): 429-36 (review; Cover story)
7. Chen S.*, Wasserman D., MacKintosh C. and Sakamoto K. (2011) Mice with AS160/TBC1D4 Thr649Ala knockin mutation are glucose intolerant with reduced insulin sensitivity and altered GLUT4 trafficking. Cell Metabolism 13(1): 68-79 (* corresponding author)
8. Chen S.* and MacKintosh C. (2009) Differential regulation of NHE1 phosphorylation and glucose uptake by inhibitors of the ERK pathway and p90RSK in 3T3-L1 adipocytes. Cell Signal, 21(12): 1984-93 (* corresponding author)
9. Chen S., Murphy J., Toth R., Campbell D.G., Morrice N.A. and MacKintosh C. (2008) Complementary regulation of TBC1D1 and AS160 by growth factors, insulin and AMPK activators. Biochem J. 409(2): 449-459
10. Geraghty K.*, Chen S.*, Harthill J.E., Ibrahim A.F., Toth R., Morrice N.A., Vandermoere F., Moorhead G.B., Hardie D.G. and MacKintosh C. (2007) Regulation of multisite phosphorylation and 14-3-3 binding of AS160 in response to insulin-like growth factor 1, EGF, PMA and AICAR. Biochem J. 407(2): 231-241. (*co-first 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.

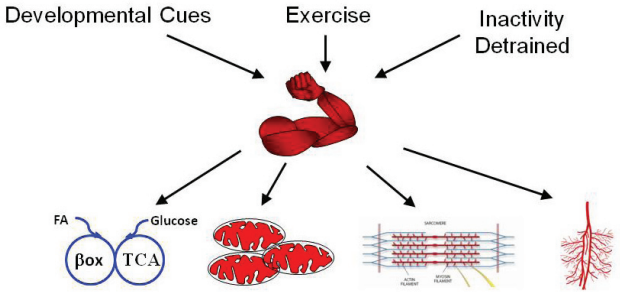


Fig 1. The muscle fitness is determined by developmental as well as physiological inputs. These inputs coordinately control the programs including fuel burning, mitochondrial ATP production, contraction, and angiogenesis.

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.

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

Recently, we found that in addition to the established role in the control of genes encoding muscle contractile proteins, miR-499 also activates a broad program of mitochondrial oxidative metabolism genes in muscle. Our findings uncover a miR-499 mechanism for tight coordinate control of mitochondrial metabolism and muscle fiber type, and suggest therapeutic potential for the miR-499 circuit in muscular dystrophy.

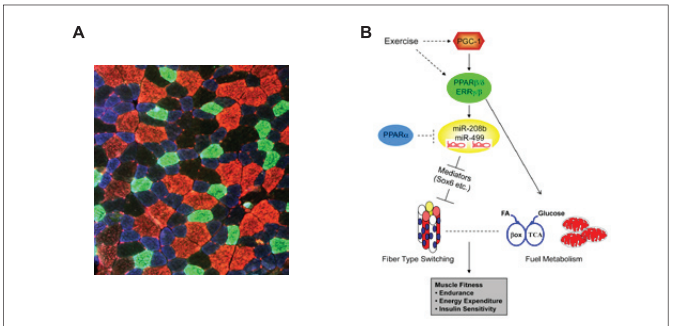
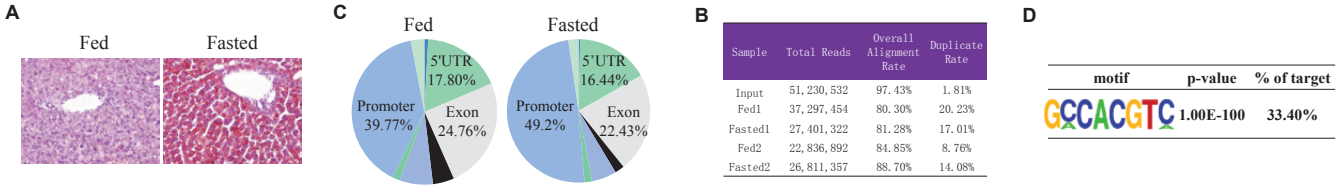


Fig 2. (A) Muscle fiber typing by immunofluorescence staining of gastroc muscle from 3 month old C57BL/6J mice. MHC1 (green), MHC2a (blue), MHC2b (red), and MHC2x (unstained). (B) Schematic depicts proposed model for nuclear receptor signaling and miRNA network in the control of muscle fiber type switching.

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

We are trying to conduct genome-wide chromatin state mapping to identify regions with cis-regulatory potential in the genome of muscle cells undergoing beneficial reprogramming. We hypothesize that epigenetic genome-wide chromatin state mapping to find cis-elements exhibiting dynamic changes under beneficial muscle reprogramming (such as endurance exercise training), computational motif finding with these regions will allows us to discovery of novel transcriptional pathway of importance. Then proof-of-concept studies will be conducted using cell-based and mouse genetic approaches by manipulation of the new candidates. Fig. 3 show a genome-wide approach to delineate novel transcriptional components involved in liver metabolism.

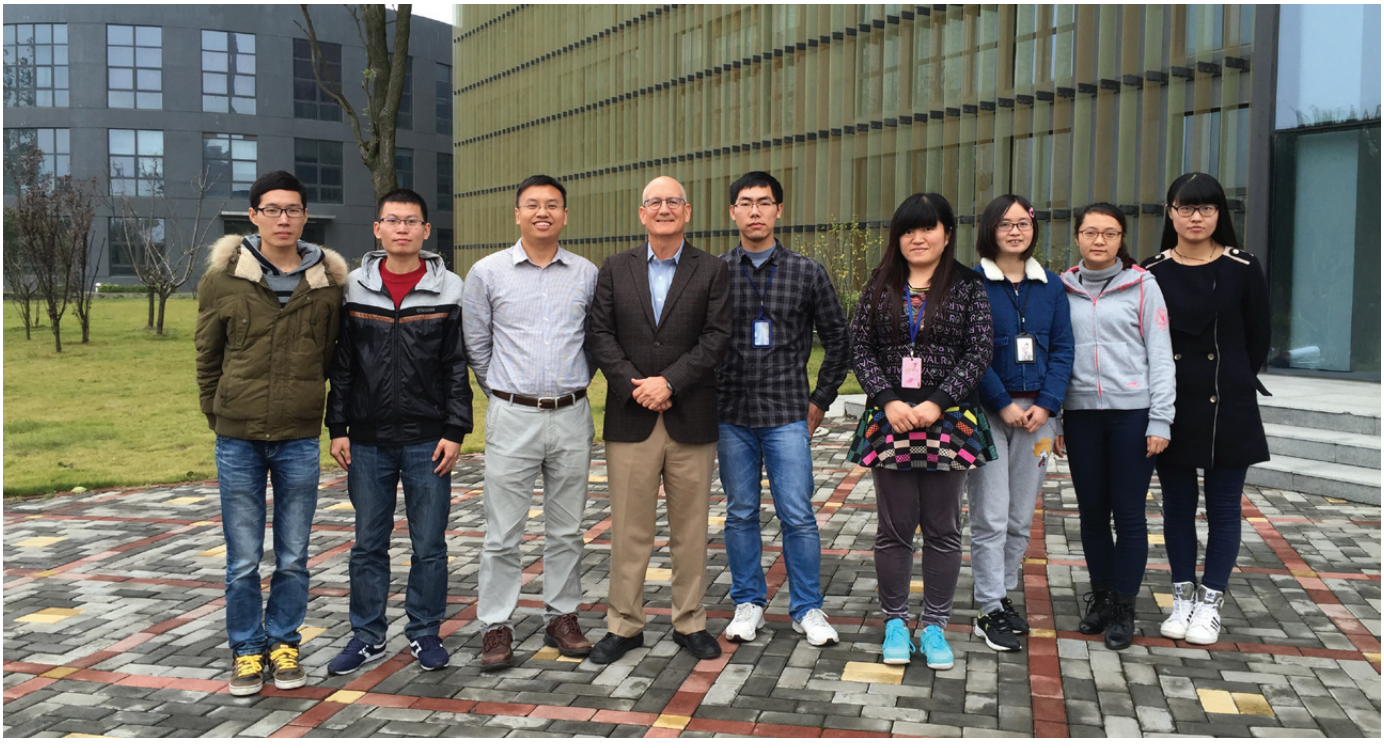


Sample	Total Reads	Overall Alignment Rate	Duplicate Rate
Input	51, 230, 532	97. 43%	1. 81%
Fed1	37, 297, 454	80. 30%	20. 23%
Fasted1	27, 401, 322	81. 28%	17. 01%
Fed2	22, 836, 892	84. 85%	8. 76%
Fasted2	26, 811, 357	88. 70%	14. 08%

Fig 3. A genome-wide approach to delineate novel transcriptional components involved in liver metabolism.

Selected publications

1. Liu J, Liang X, Gan Z. Transcriptional regulatory circuits controlling muscle fiber type switching. Sci China Life Sci. 2015;58(4):321-7.
2. Gao YQ, Chen X, Wang P, Lu L, Zhao W, Chen C, Chen CP, Tao T, Sun J, Zheng YY, Du J, Li CJ, Gan ZJ, Gao X, Chen HQ, Zhu MS. Regulation of DLK1 by the maternally expressed miR-379/miR-544 cluster may underlie callipyge polar overdominance inheritance. Proc Natl Acad Sci USA. 2015 Oct 20.
3. Gan Z, Rumsey J, Hazen BC, Lai L, Leone TC, Vega RB, Xie H, Conley KE, Auwerx J, Smith SR, Olson EN, Kralli A, Kelly DP. Nuclear receptor-microRNA circuitry links muscle fiber type to energy metabolism. J Clin Invest. 2013;123(6):2564-75.Press Release at EurekAlert: Differences between 'marathon mice' and 'couch potato mice' reveal key to muscle fitness. http://www.eurekalert.org/pub_releases/2013-05/smri-db043013.php
4. Zhang Y, Gan Z, Huang P, Zhou L, Mao T, Shao M, Jiang X, Chen Y, Ying H, Cao M, Li J, Li J, Zhang WJ, Yang L, Liu Y. A role for protein inhibitor of activated STAT 1 (PIAS1) in lipogenic regulation through SUMOylation-independent suppression of liver X receptors. J Biol Chem. 2012;287(45):37973-85.
5. Gan Z, Burkart-Hartman EM, Han DH, Finck B, Leone TC, Smith EY, Ayala JE, Holloszy J, Kelly DP. The nuclear receptor PPARb/d programs muscle glucose metabolism in cooperation with AMPK and MEF2. Genes Dev. 2011;25(24):2619-30. Press Release at EurekAlert: Super athletic mice are fit because their muscles burn more sugar. http://www.eurekalert.org/pub_releases/2011-11/smri-sam112811.php
6. Yang L, Huang P, Li F, Zhao L, Zhang Y, Li S, Gan Z, Lin A, Li W, Liu Y. c-Jun amino-terminal kinase-1 mediates glucose-responsive upregulation of the RNA editing enzyme ADAR2 in pancreatic beta-cells. PLoS One. 2012;7(11):e48611.
7. George CX, Gan Z, Liu Y, Samuel CE. Adenosine deaminases acting on RNA, RNA editing, and interferon action (invited review). J Interferon Cytokine Res. 2011;31(1):99-117.
8. Yang L, Zhao L, Gan Z, He Z, Xu J, Gao X, Wang X, Han W, Chen L, Xu T, Li W, Liu Y. Deficiency in RNA editing enzyme ADAR2 impairs regulated exocytosis. FASEB J. 2010;24(10):3720-32.



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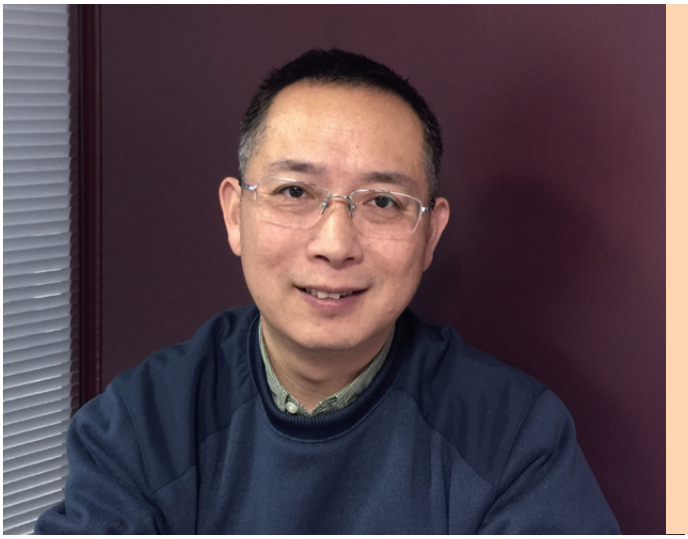

Xiang Gao, Ph.D.

Xiang received his Ph.D. degree from Thomas Jefferson University in 1994. Xiang was the founder for both MARC and National Resource Center for Mutant Mice. He is awarded Professor of Cheung Kong Scholars by Ministry of Education in 2002. He is the recipient for the National Science Fund for Distinguished Young Scholars. Xiang currently service as Director for State Key Laboratory of Pharmaceutical Biotechnology and Director for Nanjing Biomedical Research Institute of Nanjing University (NBRI).

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Metabolic homeostasis and pathogenesis

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

Recently, we found that Liver-specific Ppp2cα knockout mice (Ppp2cαloxp/loxp: Alb) exhibited improved glucose homeostasis and increased insulin sensitivity compared with littermate controls in both normal and high-fat diet conditions, despite no significant changes in body weight and liver weight under chow diet. Ppp2cαloxp/loxp: Alb mice showed enhanced glycogen deposition, serum triglyceride, cholesterol, low density lipoprotein and high density lipoprotein, activated insulin signaling, decreased expressions of gluconeogenic genes G6P and PEPCK, and lower liver triglyceride. These findings suggest that inhibition of hepatic Ppp2cα may be a useful strategy for the treatment of insulin resistance syndrome.

Another project in our lab is to dissect the potential role of Jmjd3 in control the metabolic control neurons in the brain. We found cell specific gene targeting of Jmjd3 in RIP neuron can also lead to later onset of obese, but only in female(Fig 1). Now we know it actually control the Kisspeptin gene expression. By regulating the menstrual cycle, Kisspeptin is a key mediator for female hormone release. The relationship between estrogen and body fat accumulation is well establish in previous study. Our studies added a new piece for solving the puzzle between the CNS complicated control among CNS function, reproductive function, and metabolic homeostasis.

Understanding the metabolic homeostasis may also be tackled at difference stages, the establishment, maintenance, and reset of a specific physiological status. One of the projects in my lab is try to understand the obesity "memory". It is commonly believed an obese person will obtain a tendency to get fatty again after losing weight by food restriction or excises. We confirmed this phenomenon using mouse models (Fig 2). The mice used to be fat gain body weight faster than the little mate control in both normal diet as well as high fat diet, suggesting a "memory" of old physiological status. 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 be understood. Careful studies will not only shed the light in the regulatory loops for the beauty of complicated life, but the potential cure for diseases resulting from disruption of these feedback loops.

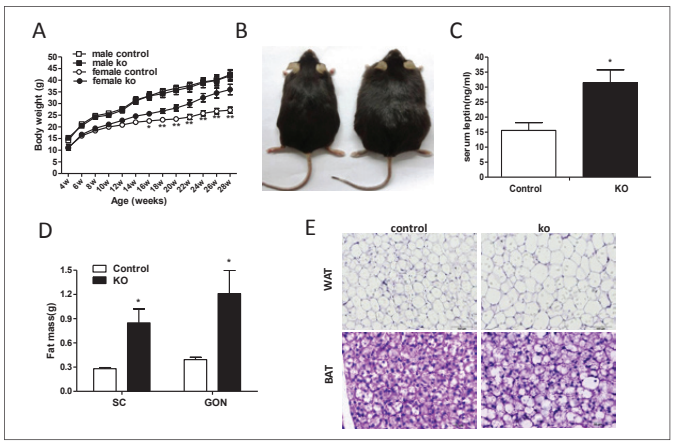

Fig.2 Female specific obesity in Rip-Cre;Jmjd3lox/lox female mice.

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

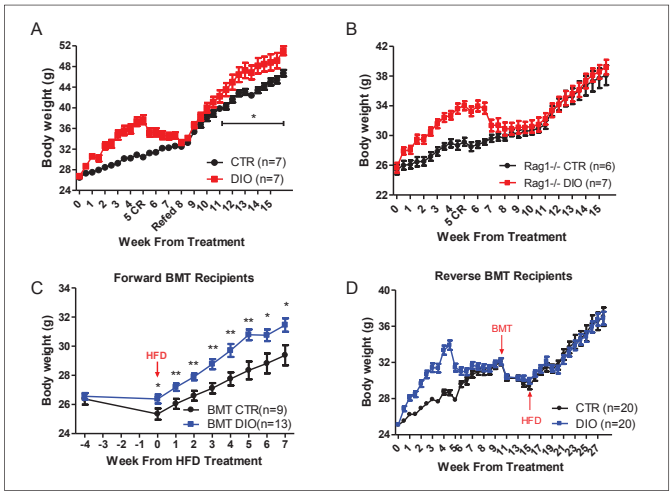

Figure2. Obesity memory relying on immune system can be revised by bone marrow transplantation.

(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.

Selected publications

1. Huang Z, Ruan HB, Xian L, Chen W, Jiang S, Song A, Wang Q, Shi P, Gu X, Gao X: The stem cell factor/Kit signalling pathway regulates mitochondrial function and energy expenditure. Nat Commun 2014, 5:4282.
2. Huang Z, Ruan HB, Zhang ZD, Chen W, Lin Z, Zeng H, Gao X: Mutation in the first Ig-like domain of Kit leads to JAK2 activation and myeloproliferation in mice. Am J Pathol 2014, 184(1):122-132.
3. Qi X, Xu J, Gu P, Yang X, Gao X: PTEN in smooth muscle cells is essential for colonic immune homeostasis. Int J Biochem Cell Biol 2014, 53:108-114.
4. Gao X: Model animals and their applications. Sci China Life Sci 2015, 58(4):319-320.
5. Qi X, Gao X: Towards a better understanding of mouse and human diseases-International Mouse Phenotyping Consortium. Sci China Life Sci 2015, 58(4):392-395.
6. Shi P, Tang A, Xian L, Hou S, Zou D, Lv Y, Huang Z, Wang Q, Song A, Lin Z, Gao X: Loss of conserved Gsdma3 self-regulation causes autophagy and cell death. Biochem J 2015, 468(2):325-336.
7. Xian L, Hou S, Huang Z, Tang A, Shi P, Wang Q, Song A, Jiang S, Lin Z, Guo S, Gao X: Liver-specific deletion of Ppp2calpha enhances glucose metabolism and insulin sensitivity. Aging (Albany NY) 2015, 7(4):223-232.
8. Zou J, Xiong X, Lai B, Sun M, Tu X, Gao X: Glucose metabolic abnormality is associated with defective mineral homeostasis in skeletal disorder mouse model. Sci China Life Sci 2015, 58(4):359-367.

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Chao-Jun Li, Ph.D

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

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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 functions. 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 including 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 Mumps infection infertility (J Exp Med, 2013); hypertrophy and heart failure (J Path, 2015a); insulin granule docked pool formation (J Path, 2015b); lipid-induced muscle insulin resistance (J Biol Chem, 2015)

1, Oocyte GGPP regulates the ovarian primary-secondary follicle transition through Rac1/Stat3 activated Gdf9 transcription

Female patients with metabolic syndrome are usually susceptible to reproductive dysfunction due to abnormal metabolites, but the underlying mechanisms are not fully understood. Here, we report that oocyte geranylgeranyl diphosphate (GGPP), a metabolic intermediate of the mevalonate pathway, is crucial for ovarian primary-secondary follicle transition and female fertility by modulating protein post-translational modification of protein lipidation. When we specifically deleted GGPP in mouse oocytes, the primary-secondary follicle transition was blocked, whereas the formation and activation of primordial follicles were unaffected. This blockade was achieved through the inhibition of granulosa cell proliferation and could be reversed by the administration of GGPP in vivo. Further examination revealed that the expression of the oocyte-secreted factor Gdf9, which can promote the proliferation of granulosa cells, was largely reduced by oocyte GGPP depletion. Mechanistically, decreased GGPP blocked Rac1 geranylgeranylation and its GTPase activity, which in turn inhibited the nuclear translocation of Stat3 and its transcriptional activation of Gdf9. Our study provides the first evidence of GGPP-regulated protein geranylgeranylation involving in regulating ovarian primary-secondary follicle transition and establishes a novel connection between intermediate metabolites, ovarian follicular development and female fertility. (Chen JIANG et al., manuscript submitting)

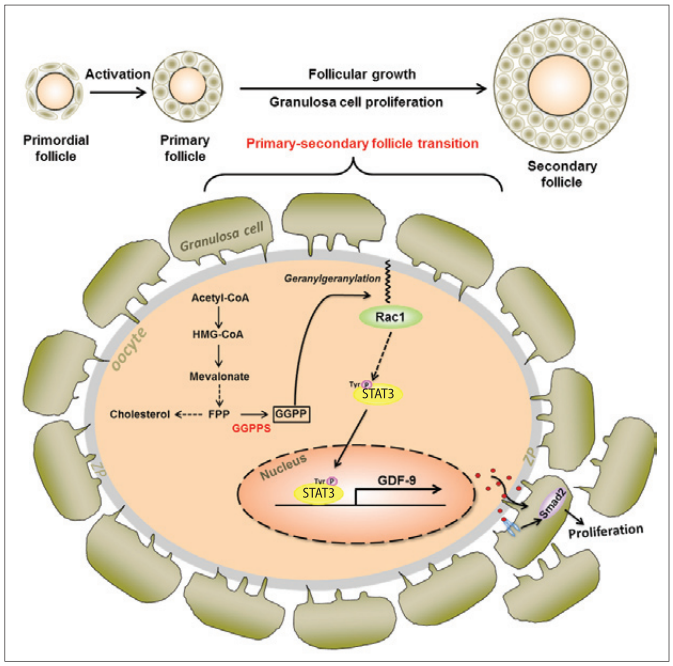

Figure 1, the working model for the regulation of primary-secondary follicle transition by protein prenylation in oocyte.

2, Protein Prenylation and heart development during mid-gestation in mice

Well-organized cardiomyocytes are important for heart to adapt to the increasing ventricular diastolic function during mid-gestation, which is regulated by protein prenylation as we reported here. Expression level, of geranylgeranyl pyrophosphate synthase (GGPPS), which regulates the balance of protein prenylation, was increased in embryonic heart at mid-gestation. When we deleted GGPPS in cardiomyocytes, mice embryos died around E13.0 associated with disorganization of cardiomyocyte. Meanwhile, cell adhesion was disrupted in GGPPS mutant heart which was evidenced by disassembly of cell junction complexes and disturbed pattern of N-cadherin and β-catenin expression. Although the protein prenylation in cardiomyocytes had been altered at E10.5, abnormal cell adhesion junction just occurred after E11.5, suggesting that protein prenylation was stage-dependent requirement for cardiomyocyte adhesion junction. Moreover, GGPP treatment could partially rescued abnormal cell junction in GGPPS mutant heart. These results implicated that GGPPS mediated protein geranylgeranylation was critical for the organization of cardiomyocytes in the developing ventricular wall by controlling cell adhesion junction at mid-gestation. (Zhong CHEN et al., manuscript preparing)

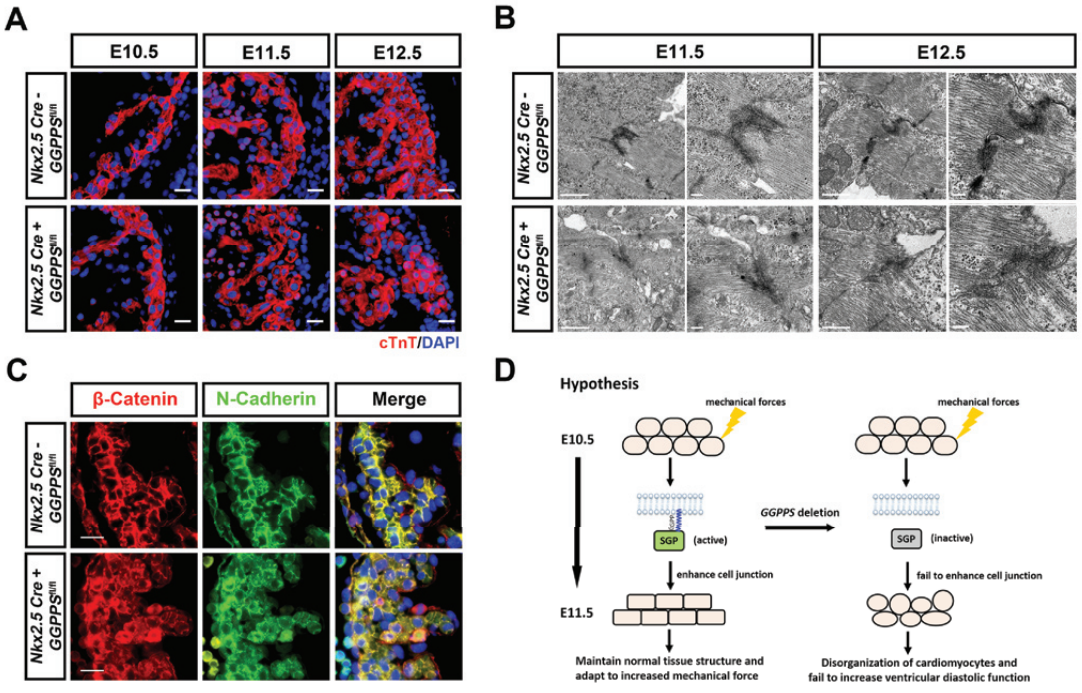

Figure 2 The working model for GGPPS regulated prenylation and insulin sensitivity in sekeletal muscle cells

3, GGPPS regulated RhoA geranylgeranylation may involve in TGF-β promoted liver hepatic stellate cell activation and liver fibrosis

Hepatic fibrosis represents the consequences of a sustained wound healing response to liver injury and the activation of quiescent hepatic stellate cell (HSC) into a fibroblast-like myofibroblast phenotype is considered as the key event in liver fibrosis. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension and often requires liver transplantation. Fortunately, hepatic fibrosis can be reversible, which raised the possibility that many chronic liver diseases could be cured. Here we found that GGPPS expression in HSCs was predominantly increased in fibrotic liver of human patients and murine models. Specific GGPPS knockdown in HSCs using VA-coupled liposome carrying siRNA Ggpps inhibited alpha-smooth muscle actin (α-SMA) synthesis and decreased liver fibrosis following carbon tetrachloride (CCl4) challenge. Further examination indicated that GGPPS was up-regulated in a TGF-β-dependent manner through a smad-independent pathway. Moreover, suppression of GGPPS blocked TGF-β-induced HSC activation, as indicated by reduced α-SMA expression, through inhibiting RhoA geranylgeranylation. Our findings suggested that inhibiting GGPPS regulated protein prenylation is able to block HSC promoted liver fibrosis process, which may represent a potential target for anti-fibrosis therapies. (Shan-Shan LAI et al., manuscript submitting)

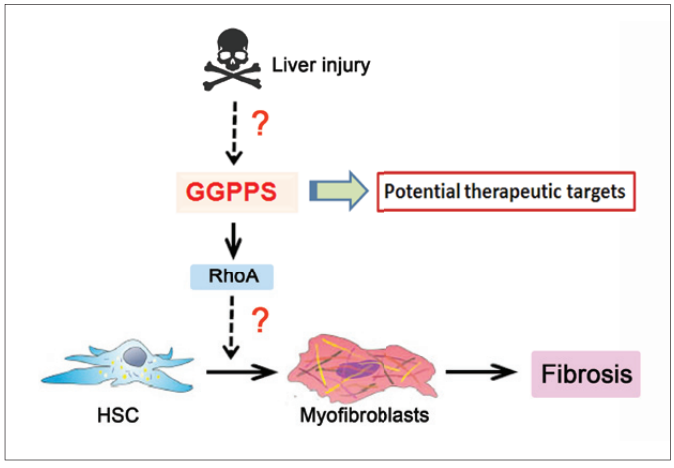

Figure 3 The working model of GGPPS regulated HSC activation and liver fibrosis.

Selected Publications

1. Shan-Shan Lai, Dan-Dan Zhao, Peng Cao, Ke Lu, Ou-Yang Luo, Wei-Bo Chen, Jia Liu, En-Ze Jiang, Zi-Han Yu, Gina Lee, Jing Li, De-Cai Yu, Xiao-Jun Xu, Min-Sheng Zhu, Xiang Gao*, Chao-Jun Li*, Bin Xue* PP2Acα Positively Regulates Mice Liver Regeneration Termination through AKT/GSK3β/Cyclin D1 Pathway. J Hepatology, 2015, doi:10.1016/j.jhep.2015.09.025
2. Shan Jiang#, Di Shen#, Ning Shen, Xiao Han, 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 Path, 2015; doi: 10.1002/path.4652
3. Weiwei Tao, Jing Wu, Qian Zhang, Shan-Shan Lai, Shan Jiang, Chen Jiang, Ying Xu, Bin Xue, Jie Du & Chao-Jun Li.EGR1 regulates hepatic clock gene amplitude by activating Per1 transcription. Scientific Reports, 2015; 5:15212 DOI: 10.1038/srep15212
4. Weiwei Tao, Jing Wu, Bing-Xian Xie, Yuan-Yuan Zhao, Ning Shen, Shan Jiang, Xiu-Xing Wang, Na Xu, Chen Jiang, Shuai Chen, Xiang Gao, 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
5. Na Xu, Shan Guan, Zhong Chen, Yang Yu, Jun Xie, Fei-Yan Pan, Ning-Wei Zhao, Li Liu, Zhong-Zhou Yang, Xiang Gao, Biao Xu, 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
6. Ning Shen#, Shan Jiang#, Jia-Ming Lu, Xiao Yu, Shan-Shan Lai, Jing-Zi Zhang, Jin-Long Zhang, Wei-Wei Tao, Xiu-Xing Wang, Na Xu, 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
7. Xiu-Xing Wang, Pu Ying, Fan Diao, Qiang Wang, Dan Ye, Chen Jiang, Ning Shen, Na Xu, Wei-Bo Chen, Shan-Shan Lai, Shan Jiang, Xiao-Li Miao, Jin Feng, Wei-Wei Tao, Bing Yao, Zhi-Peng Xu, Hai-Xiang Sun, Jian-Min Li, Jia-Hao Sha, Xing-Xu Huang, Bin Xue, Hong Tang, 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
8. Ning Shen#, Yue Shao#, Long Qiao, Run-Lin Yang, Bin Xue, Fei-Yan Pan, 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)
9. Xiao Yu#, Ning Shen#, Ming-Liang Zhang Fei-Yan Pan, Chen Wang, Wei-Ping Jia, Chang Liu, Qian Gao, Xiang Gao, 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 (#:Co-author)
10. Ning Shen#, Xiao Yu#, Fei-Yan Pan, Xiang Gao, 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)
11. Ning Shen, Tao Gong, Jian-Dong Wang, Fan-Li Meng, Long Qiao, Run-Lin Yang, Bin Xue, Fei-Yan Pan, Xiao-Jun Zhou Hua-Qun Chen, Wen Ning*, Chao-Jun Li*. Cigarette-smoke induced pulmonary inflammatory responses are mediated by egr-1/ggpps/mapk signaling. Am J Pathol., 2011, 178(1):111–119

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Jia Liu	Dan-Dan Zhao	Yong-Juan Sang
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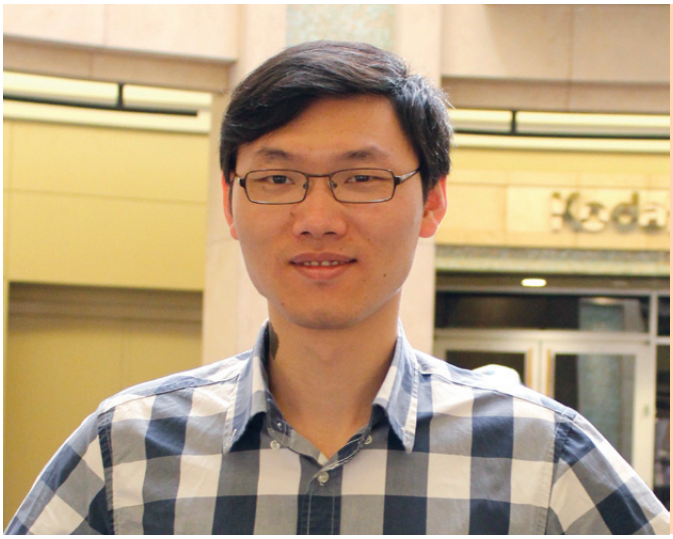

Guoqiang Wan, Ph.D.

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 now at the University of Michigan. He will be joining the MARC of Nanjing University as Principal Investigator in 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.

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.

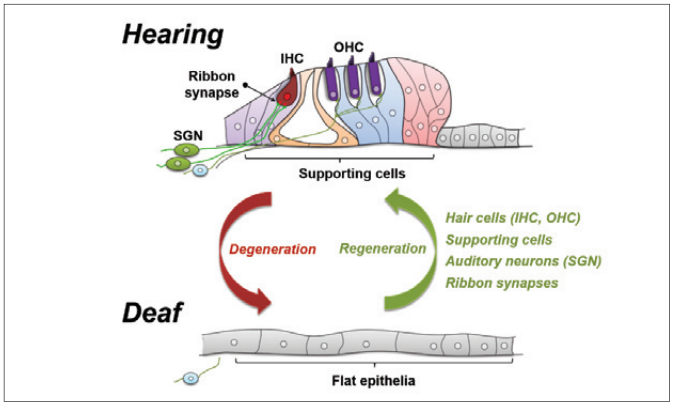

Fig 1. In mammalian cochlea, sensory hair cells (IHC and OHC), neurons (SGN), supporting cells and the auditory ribbon synapses are required for normal hearing, damage of these cells and synapses results in deafness.

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

1. Wan, G. & Corfas, G. (2015). No longer falling on deaf ears: mechanisms of degeneration and regeneration of cochlear ribbon synapses. Hearing Research, doi:10.1016/j.heares.2015.04.008
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.

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   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 and Myl-9. Our observations suggest that Ca2+/CaM-dependent MLCK and its myosin light chain phosphorylation were central to smooth muscle contraction, and MLCK is required for gut motility, asthmatic constriction and blood pressure maintenance. By use of MYPT1 knockout mice, we demonstrated MYPT1 deletion causes phenotypic transition of phasic and tonic smooth muscles, and the myogenic alteration by MYPT1 deletion is enough for generation of hypertension. With thorough analysis for two line of MYPT1 T694A and T852A mutant mice, we find that MYPT1 T694 phosphorylation is essential for sustained contraction of bladder smooth muscle, whereas MYPT T852 does not (Fig.1). Our finding provide novel mechanistic insights into the specific role of MYPT1 phosphorylation on physiological and pharmacological Ca2+ sensitization.

The polar overdominance inheritance of callipyge sheep is an unusual mode of non-Mendelian inheritance. To investigate the mechanism underlying, we established a mouse line with deletion of the microRNA (miR) 379/miR-544 cluster and assessed the role of this cluster in the inheritance (Fig.2). Our results showed that the maternally expressed miR-379/miR-544 cluster might regulate skeletal muscle growth through the imprinted Delta-like 1 homolog (Dlk1) gene. This report revealed a molecular mechanism of the polar overdominance inheritance and the working model is shown in Fig.3.

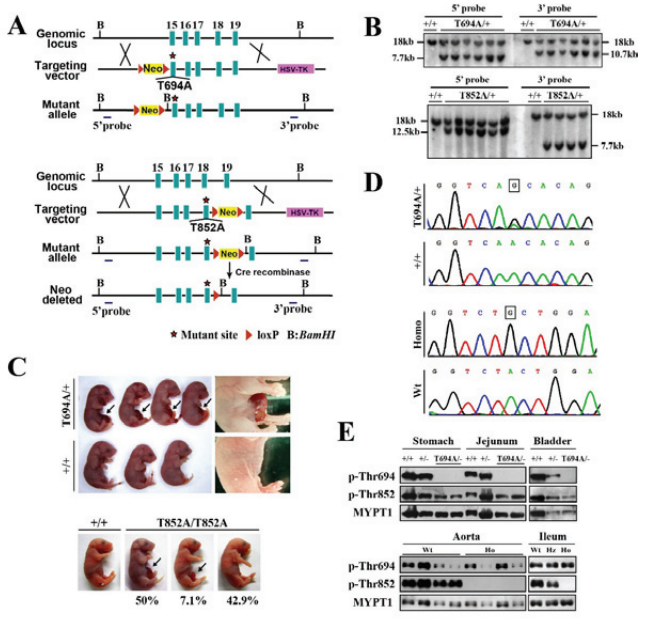

Fig1. Generation of Mypt1 knock-in mutant mice.

(A) Schematic representation of Mypt1 knock-in strategy of T694A (top) and T852A (bottom). (B) Southern blot assay for positive ES clones with homologous recombination. The extracted genomic DNA digested with BamHⅠ, electrophoresed on a 1% agarose gel, transferred to nitrocellulose and probed with 32P-labelled 5′ and 3′ probe as indicated in panel A. Wild-type allele generates a 18 kb fragment while targeted allele generates a 7.7 kb (T694A) or 12.5 kb (T852A) fragment for 5′ probe; 10.7 kb (T694A) or 7.7 kb (T852A) fragment for 3′ probe. (C) Gross appearance of mutant embryos (E18.5). Top panel (left): 100% of T694A heterozygous littermates are with severe omphalocele phenotype. Top panel (right): magnification of area containing umbilicus from T694A and control pups. The appearance of T852A homozygous was varied: the mice with severe (50%) and moderate (7.1%) omphalocele and normal appearance (42.1%) were observed as showed in the bottom panel. Arrows indicate omphalocele phenotypes. (D) Sequencing for PCR products from cDNA isolated from T694A heterozygous (T694A/+) mice, T852A homozygous (homo) mice and wild type (+/+) mice. Correct mutations at T694 and T852 were showed. (E) Western blot assay showed disappearance of MYPT1 phosphorylation at T694 site and T852 site in smooth muscle tissues from Mypt1T694A/- mice and Mypt1T852A/T852A mice. Wt: wild type; Hz: T852A heterozygous; Ho, T852A homozygous.

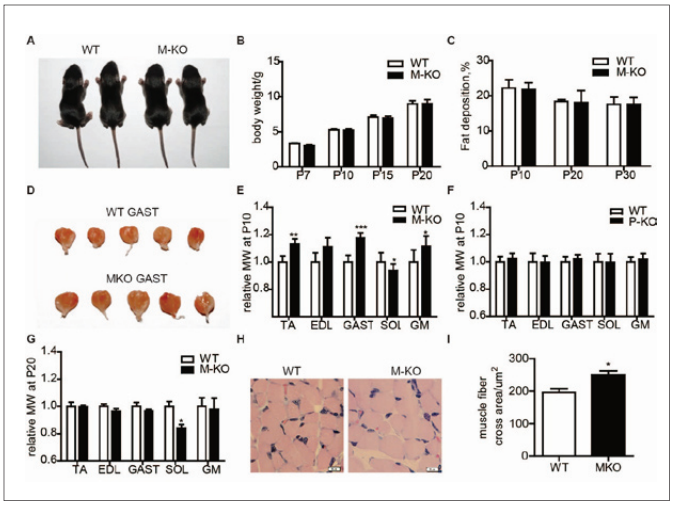

Fig.2. Maternal deletion in mir-379-544 cluster result in neonatal fast muscle hypertrophy.

(A) General appearance of WT and M-KO mice at postnatal 10th day (P10) is presented. (B) Measured body weight before weaning. (C) Measured fat deposition by PIXImus small animal dual-energy X-ray absorptiometry system. (D) General appearance of gastrocnemius muscle (GAST) from WT and M-KO mice at postnatal 10th day is presented. (E) Wet muscle weight of WT and M-KO mice at P10 is shown. (F) Wet muscle weight of WT and P-KO mice at P10 is shown. (G) Wet muscle weight of WT and M-KO mice at P20 is shown. (H) H.E. staining of GAST cross section from WT and M-KO mice at postnatal P10 is presented. (I) Cross area of GAST muscle fibers from M-KO mice is significant larger than WT mice.

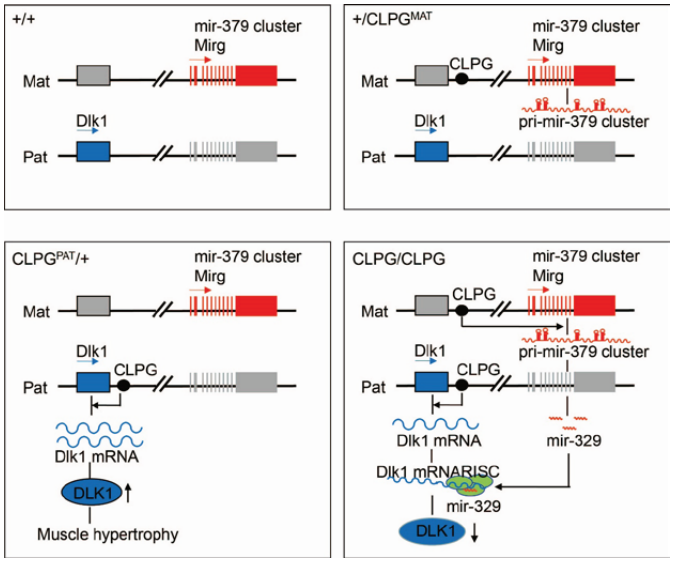

Fig3. Model for polar overdominance at the callipyge locus.

CLPG mutation acts as a long-range control element and enhances the transcript levels of the nearby imprinted genes in cis without altering their imprinted status. Thus the CLPGPAT/+ animals manifest an overexpression of DLK1 with normal expression of maternal non-coding RNAs, underlying the callipyge phenotype. Whereas in the CLPG/CLPG animals, paternally expressed DLK1 can be post-transcriptional trans inhibited by the maternally expressed miRNAs, mir-329, enhanced in cis by the CLPG mutation. So the depressed DLK1 protein cannot lead to muscle hypertrophy in CLPG/CLPG animals.

Selected Publications

1. Gao YQ, Chen X, Wang P, Lu L, Zhao W, Chen C, Chen CP, Tao T, Sun J, Zheng YY, Du Jie, Li CJ, Gan ZJ, Gao X, Chen HQ and Zhu MS*. Regulation of DLK1 by the maternally expressed miR-379-544 cluster may underlie callipyge polar overdominance inheritance. PNAS 2015 ;112(44):13627-32.
2. Chen, C.P., Chen, X., Qiao, Y.N., Wang, P., He, W. Q., Zhang, C.H., Zhao, W., Gao, Y.Q., Chen, C., Tao, T., Sun, J., Wang, Y., Gao, N., Kamm, K.E., Stull, J.T., and Zhu, M.S * Roles in vivo for Myosin Phosphatase Targeting Subunit-1 T694 and T852 Phosphorylation sites in Bladder Smooth Muscle. J. Physiology 2015;593(3):681-700
3. Lai SS, Zhao DD, Cao P, Lu K, Luo OY, Chen WB, Liu J, Jiang EZ, Yu ZH, Lee G, Li J, Yu DC, Xu XJ, Zhu MS, Gao X, Li CJ, Xue B.PP2Acα Positively Regulates Mice Liver Regeneration Termination through AKT/GSK3β/Cyclin D1 Pathway. J Hepatol. 2015 Oct 6. pii: S0168-8278(15)00673-X
4. Bu Y, Wang N, Wang S, Sheng T, Tian T, Chen L, Pan W, Zhu M, Luo J, Lu W. Myosin IIb-dependent Regulation of Actin Dynamics Is Required for N-Methyl-d-aspartate Receptor Trafficking during Synaptic Plasticity. J Biol Chem. 2015; 290(42):25395-410

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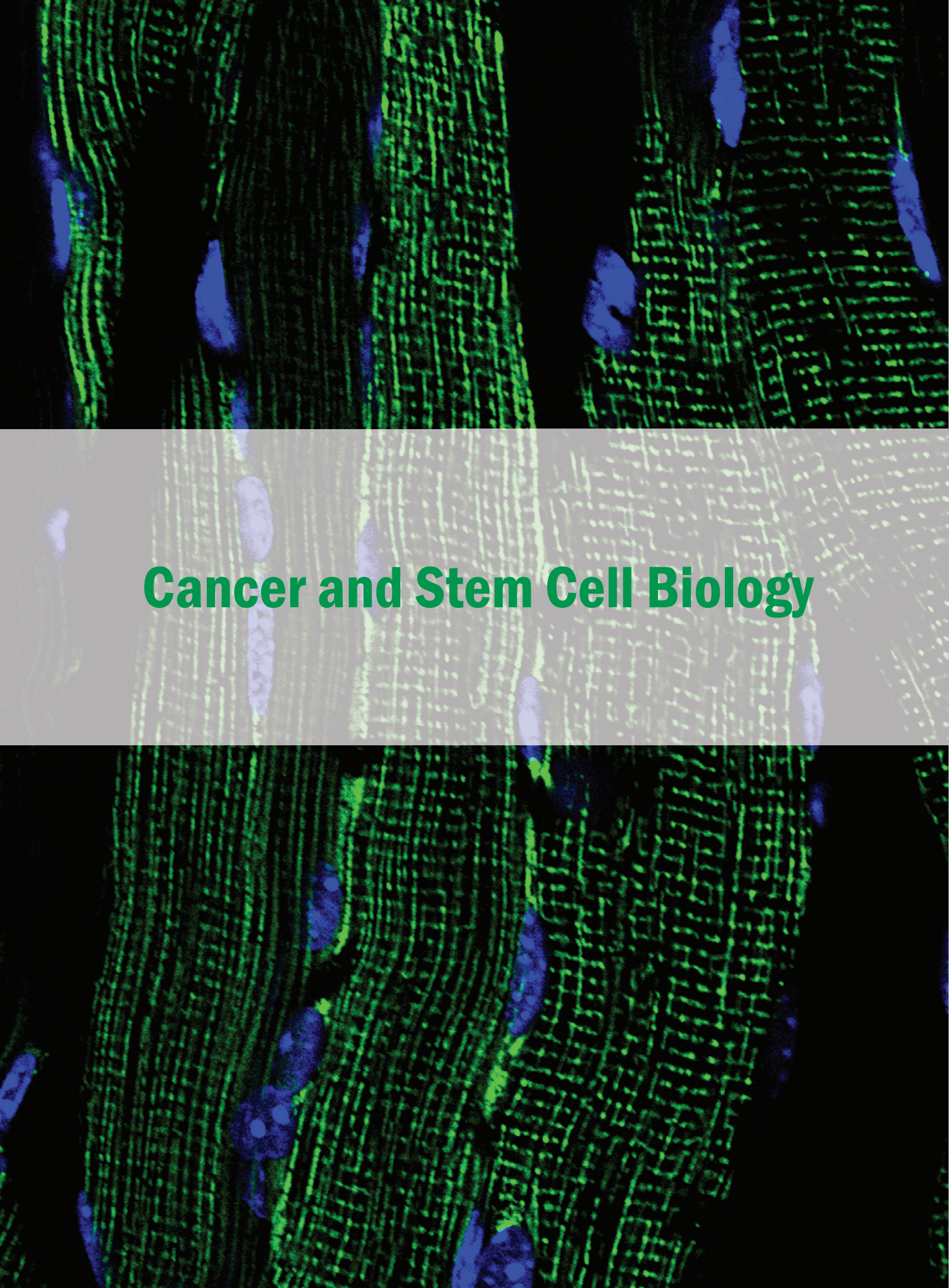
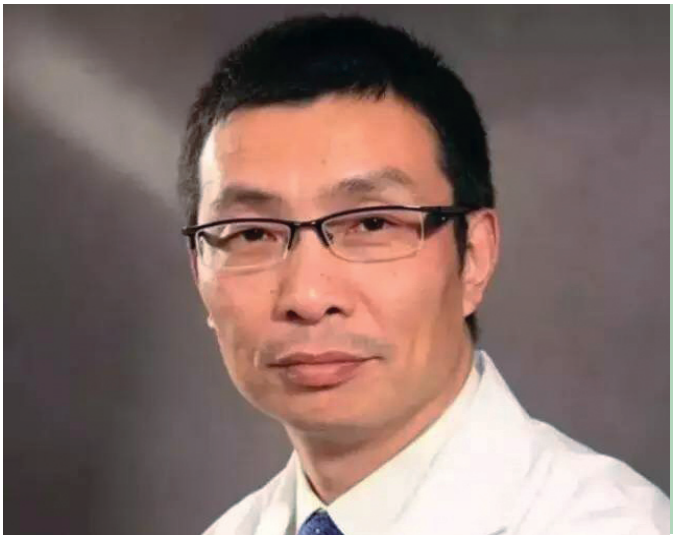


Cancer and Stem Cell Biology



Qing Jiang , Ph.D.

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

1. Brief introduction of research interests

Osteoarthritis (OA) is by far the most common type of joint disease. Epidemiological studies have shown that OA has a strong genetic component, and several susceptibility genes for OA have been reported. In 2011, a new OA susceptibility gene, HIF2a was reported by Japanese researchers. They found the association between a SNP of HIF2a and OA, and demonstrated the allelic functional differences of this SNP. But the samples size of their case-control study was few. So we carried out a meta-analysis on this association with different cohorts, and we found there was no association between this "functional" SNP and OA (Table 1) (Nakajima 2011). Our 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. In our study, we have demonstrated a clear effect of NO microbubbles on DVT resolution (Fig 1). 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.

Developmental dysplasia of the hip (DDH) is the most frequent inborn deformity of the locomotor 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, and the association between DDH and GDF5 was also found in Caucasians. 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 (Shi 2014).

We also performed an arthroscopy 2 weeks after the patient's injury. Routine anterolateral and anteromedial portals were used. The osteochondral avulsion fracture was attached to the PM bundle of the PCL and the anterolateral (AL) bundle of the PCL was intact. There were two parts to the avulsion fracture. We treated the larger avulsion with polydioxanone suture (PDS) fixation as described below using a bone bridge suture repair technique (Fig. 2).

The DNA bank for bone and joint disease we had established is still enlarging. Now we have human DNA samples for OA, DDH, DVT, ankylosing spondylitis (AS) and osteoporosis. The tissue bank for cartilage and ligament has been established and enlarged.

2. Research progress figures

Population	Genotype						Risk allele (C allele) frequency		Test for allele frequency[a]	
	Case			Control						
	CC	CT	TT	CC	CT	TT	Case	Control	*P* value	OR (95% CI)
Japanese	677	213	9	2,109	584	42	0.872	0.878	0.478	0.94 (0.80–1.11)
Chinese	657	51	1	613	32	2	0.963	0.972	0.163	0.74 (0.48–1.13)
European	557	16	0	562	11	0	0.986	0.990	0.333	0.68 (0.32–1.48)
Australian	257	1	0	513	2	0	0.998	0.998	0.999	1.00 (0.09–11.03)
Combined[b]									0.185	0.91 (0.78–1.05)

[a]Pearson's χ^2 test. [b]Mantel-Haenszel meta-analysis of all studies. OR, odds ratio; CI, confidence interval.

Table 1. Association of rs17039192 with knee osteoarthritis.

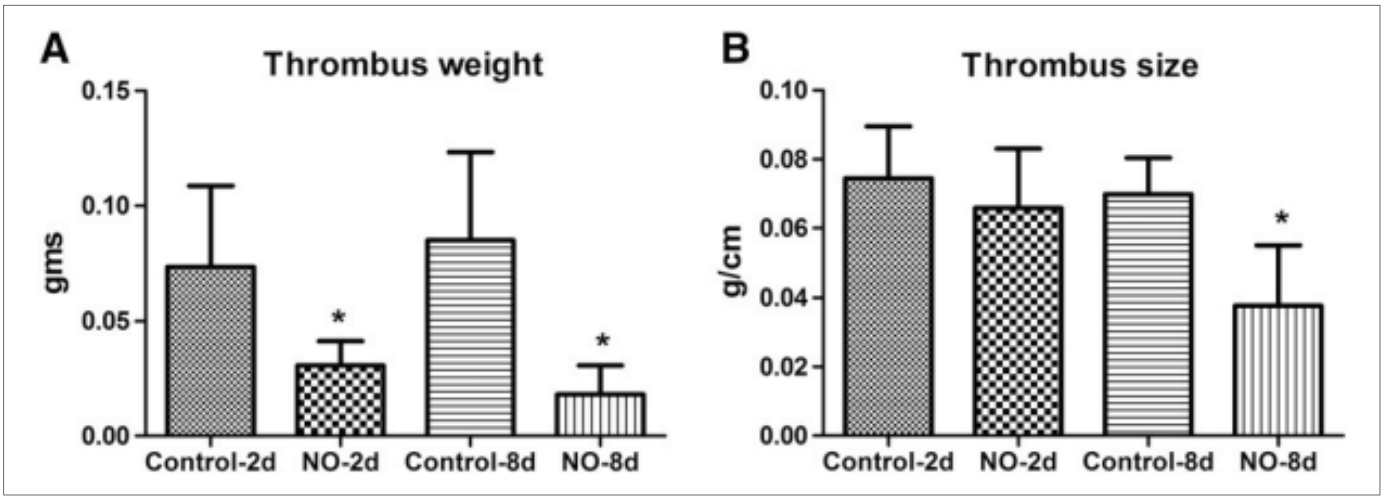

Fig. 1 NO microbubbles accelerated DVT resolution: Thrombi were isolated from IVC for fresh. (A) Thrombus weight (g) decreased significantly in NO microbubbles group compared with that in control group at 2 and 8 days (B) Thrombus size(thrombus weight/thrombus length, g/cm) was more smaller after NO microbubbles treatment at day 8. *Pb0.05 versus control..

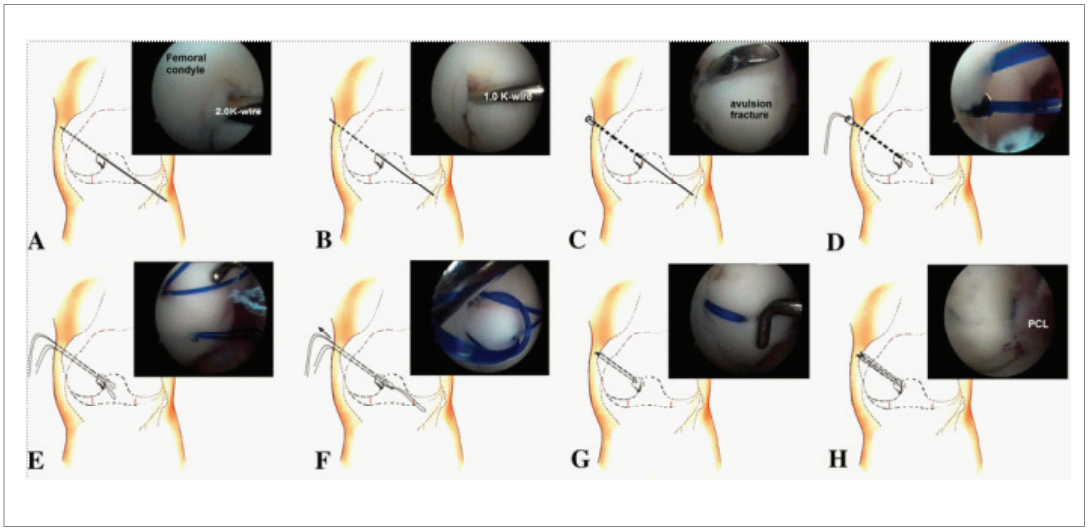

Fig. 2 A–H Sketches of the surgical technique and the arthroscopy photographs show the fixation technique of the avulsion part 1 through drill holes with the use of an epidural cannula and PDSs. (A) The hole was drilled with a 2-mm Kirschner wire (K-wire) toward the medial epicondyle. (B) The 2-mm K-wire then was exchanged for a 1-mm K-wire. (C) A straightened epidural cannula was passed through the 1-mm K-wire into the joint and the 1.0-mm K-wire was removed. (D) A double-stranded looped PDS was inserted into the joint through the epidural cannula. (E) Another doublestranded looped PDS was inserted into the joint. (F) The second PDS loop was pulled through the first PDS loop out of the anteromedial portal with a probe. (G) The first PDS was pulled out of the medial skin and the second PDS was left for fixation. (H) The avulsion fracture was tightly fixed.

Selected Publications

1. Shi D, Zheng Q, Chen D, Zhu L, Qin A, Fan J, Liao J, Xu Z, Lin Z, Norman P, Xu J, Nakamura T, Dai K, Zheng M, Jiang Q. Association of single-nucleotide polymorphisms in HLA class II/III region with knee osteoarthritis. Osteoarthritis Cartilage. 2010 Nov;18(11):1454-7.
2. Shi D, Sun W, Xu X, Hao Z, Dai J, Xu Z, Chen D, Teng H, Jiang Q*. A Replication Study for the Association of rs726252 in PAPPA2 with Developmental Dysplasia of the Hip in Chinese Han Population. BioMed Research International. 2014(2014).
3. Miyamoto Y, Shi D, Nakajima M, Ozaki K, Sudo A, Kotani A, Uchida A, Tanaka T, Fukui N, Tsunoda T, Takahashi A, Nakamura Y, Jiang Q*, Ikegawa S*. Common variants in DVWA on chromosome 3p24.3 are associated with susceptibility to knee osteoarthritis. Nat Genet. 2008 Aug;40(8):994-8.
4. 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.
5. Kerkhof HJ, Meulenbelt I, Akune T, Arden NK, Aromaa A, Bierma-Zeinstra SM, Carr A, Cooper C, Dai J, Doherty M, Doherty SA, Felson D, Gonzalez A, Gordon A, Harilainen A, Hart DJ, Hauksson VB, Heliovaara M, Hofman A, Ikegawa S, Ingvarsson T, Jiang Q, Jonsson H, Jonsdottir I, Kawaguchi H, Kloppenburg M, Kujala UM, Lane NE, Leino-Arjas P, Lohmander LS, Luyten FP, Malizos KN, Nakajima M, Nevitt MC, Pols HA, Rivadeneira F, Shi D, Slagboom E, Spector TD, Stefansson K, Sudo A, Tamm A, Tamm AE, Tsezou A, Uchida A, Uitterlinden AG, Wilkinson JM, Yoshimura N, Valdes AM, van Meurs JB. Recommendations for standardization and phenotype definitions in genetic studies of osteoarthritis: the TREAT-OA consortium. Osteoarthritis Cartilage. 2011 Mar;19(3):254-64.
6. Miyamoto Y, Mabuchi A, Shi D, Kubo T, Takatori Y, Saito S, Fujioka M, Sudo A, Uchida A, Yamamoto S, Ozaki K, Takigawa M, Tanaka T, Nakamura Y, Jiang Q*, Ikegawa S*. A functional polymorphism in the 5' UTR of GDF5 is associated with susceptibility to osteoarthritis. Nat Genet. 2007 Apr;39(4):529-33.
7. Wang C, Yang F, Xu Z, Shi D, Chen D, Dai J, Gu N, Jiang Q*. Intravenous release of NO from lipidic microbubbles accelerates deep vein thrombosis resolution in a rat model. Thrombosis research, 2013, 131(1): e31-e38.
8. Shi D, Nakamura T, Dai J, Yi L, Qin J, Chen D, Xu Z, Wang Y, Ikegawa S, Jiang Q*. Association of the aspartic acid-repeat polymorphism in the asporin gene with age at onset of knee osteoarthritis in Han Chinese population. J Hum Genet. 2007;52(8):664-7.
9. Nakamura T, Shi D, Tzetis M, Rodriguez-Lopez J, Miyamoto Y, Tsezou A, Gonzalez A, Jiang Q, Kamatani N, Loughlin J, Ikegawa S. Meta-analysis of association between the ASPN D-repeat and osteoarthritis. Hum Mol Genet. 2007 Jul 15;16(14):1676-81
10. Chapman K, Takahashi A, Meulenbelt I, Watson C, Rodriguez-Lopez J, Egli R, Tsezou A, Malizos KN, Kloppenburg M, Shi D, Southam L, van der Breggen R, Donn R, Qin J, Doherty M, Slagboom PE, Wallis G, Kamatani N, Jiang Q, Gonzalez A, Loughlin J, Ikegawa S. A meta-analysis of European and Asian cohorts reveals a global role of a functional SNP in the 5' UTR of GDF5 with osteoarthritis susceptibility. Hum Mol Genet. 2008 May 15;17(10):1497-504.

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Geng Liu, Ph.D.

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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 (Chen, et al., 2015). Further, we provided evidence suggesting that this distinct expression pattern is governed by proliferative signals acting on the cis-elements in both the p53 promoter and 3′ UTR. Importantly, p53 protein is selectively activated in the proliferating cells and tissues upon stress, highlighting the close monitoring of cellular proliferation state by p53 (Figure 1).

In the present of stress, 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.

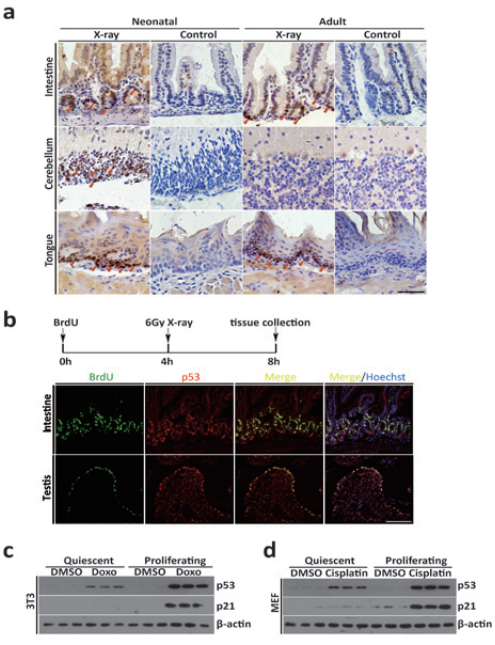

Figure 1. A direct link between cellular proliferation and p53 mediated stress response.

(a) Immunohistochemistry of p53 in small intestine, cerebellum and tongue of p7 and 2 month-old C57BL/6J control mice or mice treated with X-irradiation for 4 hours. Arrows depicted positive stained cells. Scale bar=200μm.

(b) Double immunostaining of p53 and BrdU in intestine and testis of 2 month-old C57BL/6J mice treated with X-irridiation for 4 hours.

(c) Western blot analysis of p53 and p21 expression on quiescent and proliferating 3T3 cells treated with Doxorubicin.

(d) Western blot analysis of p53 and p21 expression on quiescent and proliferating MEFs treated with Cisplatin.

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 p53 in myeloid lineage in spontaneous tumor models, we found that p53 deficiency in myeloid lineage accelerated adenoma formation in Apc^{min} 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.

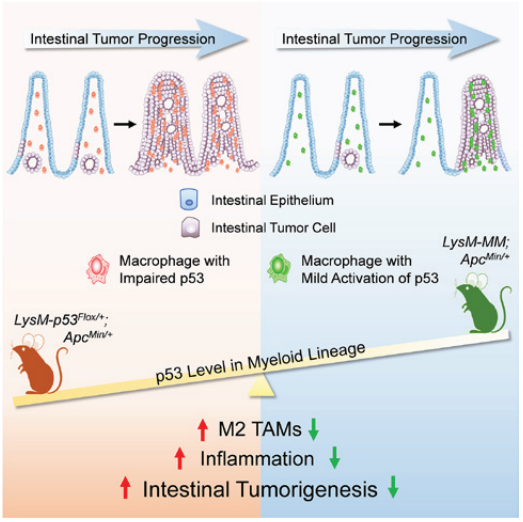

Figure 2. p53 activities critically modulate tumor microenvironment and in turn, impact on tumorigenesis.

Selected publications

1. 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
2. Chen L, Zhang G, He X, Zhang CX, Xie YY and Liu G*. (2015) BAC transgenic mice provide evidence that p53 expression is highly regulated in vivo. Cell Death and Disease 6, e1878; doi: 10.1038 /cddis. 2015.224.
3. 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.
4. 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.
5. 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.
6. Liu G, Terzian T, Xiong S, Audiffred A, Van Pelt C, and Lozano G. (2007) The p53-Mdm2 network in progenitor cell expansion during mouse postnatal development. J Pathol 213(4):360-8.
7. Barboza JA, Liu G, Ju Z, El-Naggar AK, Lozano G. (2006) p21 delays tumor onset by preservation of chromosomal stability. Proc Natl Acad Sci U S A 103(52): 19842-19847.
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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The innate immune system provides immediate defense against pathogens and helps to shape the ensuing adaptive immune responses. On the other hand, the deficiency, or more frequently, the mal-functioning of the immune system contributes to a plethora of disease states. We seek to gain more insights regarding the regulation of the innate immune system and to explore the possibility of normalizing the dys-functional immune responses in disease models. Ultimately, we hope that our bench discoveries can in some way impact management of human diseases.

1. The metabolic 'division' of innate immunity

We have been interested in studying anti-viral innate immunity, which is mediated by signaling of viral nucleic acids and the ensuring type I interferons (IFN). However, how these upstream signaling events may engage shifts in the cells' metabolic state to support the complex anti-viral programs has not been extensively investigated (Fig. 1A). We find that viral infection, by way of IFN, leads to up-regulation of glycolysis preferentially in macrophages (Fig. 1B, C). The latter metabolic effect involves increased expression of glycolytic activator phospho-fructose 2 kinase/fructose 2,6 bisphosphatase 3 (PFKFB3) (Fig. 1D, E). PFKFB3-driven glycolysis promotes the extrinsic anti-viral capacity of macrophages via enhancing their engulfment of virus-infected cells (Fig. 1F). Consistent with the data using cultured cells, in a mouse model of respiratory syncytial virus infection, PFKFB3 contributes to reducing the viral load (Fig. 1G). Therefore, our data demonstrate a previously overlooked, anti-viral aspect of glycolytic metabolism and suggest that glycolytic activation may indeed represent a novel therapeutic venue against viral infections.

In the meantime, we are also interested in examining the role of cellular metabolism in regulating the behavior of myeloid cells in other disease states such as obesity and cancer.

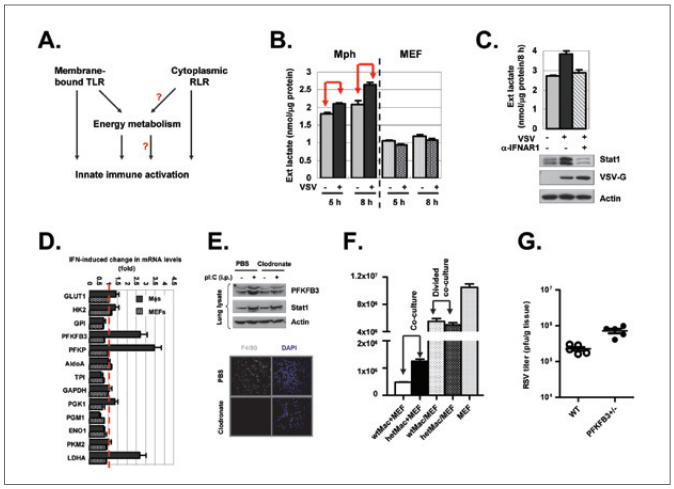

Figure 1: Metabolic aspect of anti-viral innate immune activation.

(A) RLR-mediated regulation of cellular energy metabolism was unknown. (B) VSV infection leads to increased glycolysis in macrophages (Mph), but not MEFs. (C) Type I IFN is essential for VSV-mediated glycolytic regulation in Mph. (D) Induction of glycolytic genes by IFN, preferentially in Mph. (E) Mph-preferential induction of PFKFB3 by IFN in vivo. (F) PFKFB3 promotes cell-extrinsic anti-viral activity of Mph. (G) PFKFB3 protects mice from RSV infection.

2. Transcriptional 're-wiring' of inflammation

Innate immune activation triggers robust transcriptional activation of pro-inflammatory cytokines. From a synthetic biology point of view, the transcriptional regulatory information for various cytokine genes can be used to classify the inflammatory responses. Artificial transcriptional circuits can subsequently be designed to specifically 're-wire' a given inflammatory condition.

Transcriptional regulation is mediated by transcription factors and their cis-regulatory elements. However, functional annotation of cis-regulatory elements at genomic loci was still technically challenging. In collaboration with Dr. Xing-xu Huang previously at MARC, we recently established a CRISPR-based, highly effective and non-mutational method to specifically interrogate cis-element function in human cells (Fig. 2). Furthermore, we hope to extend such cutting-edge technology to develop toolkits to purposefully 'rewire inflammation'.

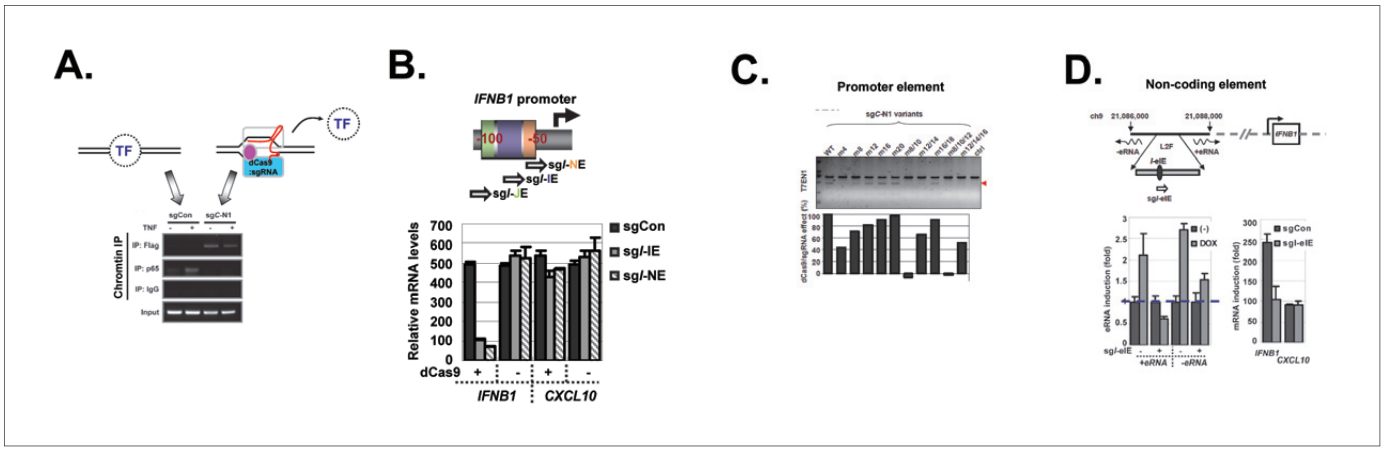

Figure 2: Functional annotation of cis-regulatory elements in human cells.

(A) sgRNA-guided nuclease-deficient Cas9 protein (dCas9) can physically block TF recruitment to a cis-regulatory element. (B) dCas9/sgRNA mediated functional analysis of cis-elements in IFNB1 promoter. (C) Specificity of dCas9/sgRNA-mediated cis-element targeting is similar to the specificity of Cas9-mediated indel induction. (D) dCas9/sgRNA can be used to target an enhancer-born cis-regulatory element.

Selected publications: (*corresponding author)

1. Zhang Y, Wang Y, Zhang C, Wang J, Pan D, Liu J* and Feng F*. Targeted Gene Delivery to Macrophages by Biodegradable Star-Shaped Polymers. In press (DOI: 10.1021), ACS Appl Mater Inter.
2. Du Y, Meng Q, Zhang J, Sun M, Shen B, Jiang H, Kang Y, Gao J, Huang X* and Liu J*. Functional annotation of cis-regulatory elements in human cells by dCas9/sgRNA. Cell Res 2015, 25(7):877-80.
3. Tong, Y., Li, F., Lu, Y., Cao, Y., Gao, J.* and Liu, J.*, Rapamycin-sensitive mTORC1 signaling is involved in physiological primordial follicle activation in mouse ovary. Mol. Reprod. Dev., 80: 1018–1034, 2013.
4. HuangFu, W. C., Qian, J., Liu, C., Liu, J., Lokshin, A. E., Baker, D. P., Rui, H., and Fuchs, S. Y. Inflammatory signaling compromises cell responses to interferon alpha. Oncogene, 31(2): 161-72, 2012.
5. Jiang, H., Lu, Y., Yuan, L., and Liu, J.* Regulation of Interleukin-10 Receptor Ubiquitination and Stability by Beta-TrCP-Containing Ubiquitin E3 Ligase. PLoS ONE, 6: e27464, 2011.
6. Liu, J., Carvalho, L. P., Bhattacharya, S., Carbone, C. J., Kumar, K. G., Leu, N. A., Yau, P. M., Donald, R. G., Weiss, M. J., Baker, D. P., McLaughlin, K. J., Scott, P., and Fuchs, S. Y. Mammalian Casein Kinase 1 Alpha and Its Leishmanial Ortholog Regulate Stability of IFNAR1 and Type I Interferon Signaling. Mol Cell Biol, 29: 6401-6412, 2009.
7. Liu, J., HuangFu, W. C., Kumar, K. G., Qian, J., Casey, J. P., Hamanaka, R. B., Grigoriadou, C., Aldabe, R., Diehl, J. A., and Fuchs, S. Y. Virus-induced unfolded protein response attenuates antiviral defenses via phosphorylation-dependent degradation of the type I interferon receptor. Cell Host Microbe, 5: 72-83, 2009.

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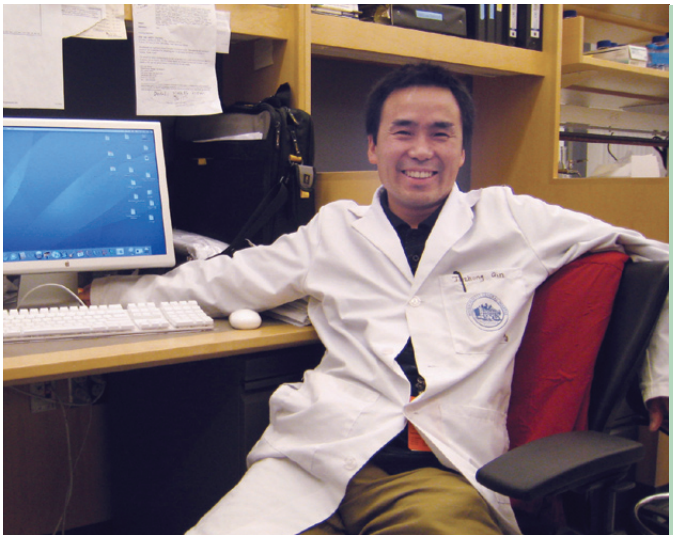

Jinzhong Qin , Ph.D.

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.

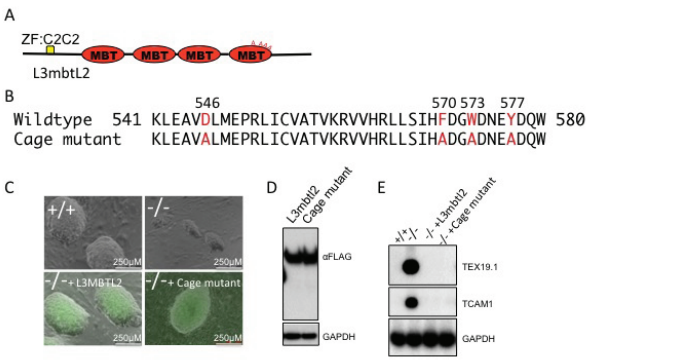

Figure 1. Mutant with four point mutations in the aromatic cage rescued similar to wild-type L3mbtl2. A. Illustration of L3mbtl2 domain organization. B. L3mbtl2 aromatic cage mutant. C. Aromatic cage mutant kept the ability to rescue colony growth. D. Western blot analysis confirmed protein expression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

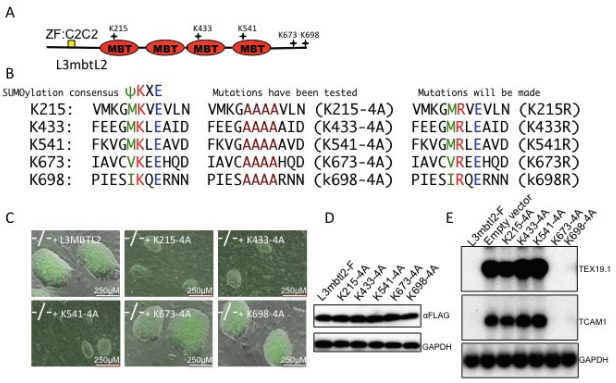

Figure 2. Crosstalk between summoylation and L3mbtl2 in ES cells. A. Cartoon depicting zinc finger and MBT domains in L3mbtl2 (including five consensus SUMOylation sites). B. Generation of L3mbtl2 putative summoylation mutants. C. Ability of these mutants in colony growth rescue. D. Western blot analysis 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.
3. Qin, J., D. Van Buren, H. S. Huang, L. Zhong, R. Mostoslavsky, S. Akbarian, and H. Hock. 2010. Chromatin protein L3MBTL1 is dispensable for development and tumor suppression in mice. J Biol Chem 285:27767-27775.
4. Schindler, J. W., D. Van Buren, A. Foudi, O. Krejci, J. Qin, S. H. Orkin, and H. Hock. 2009. TEL-AML1 corrupts hematopoietic stem cells to persist in the bone marrow and initiate leukemia. Cell Stem Cell 5:43-53.
5. Bulek, K., S. Swaidani, J. Qin, Y. Lu, M. F. Gulen, T. Herjan, B. Min, R. A. Kastelein, M. Aronica, M. Kosz-Vnenchak, and X. Li. 2009. The essential role of single Ig IL-1 receptor-related molecule/Toll IL-1R8 in regulation of Th2 immune response. J Immunol 182:2601-2609.
6. Xiao, H*., M. F. Gulen*, J. Qin, J*. Yao, K. Bulek, D. Kish, C. Z. Altuntas, D. Wald, C. Ma, H. Zhou, V. K. Tuohy, R. L. Fairchild, C. de la Motte, D. Cua, B. A. Vallance, and X. Li. 2007. The Toll-interleukin-1 receptor member SIGIRR regulates colonic epithelial homeostasis, inflammation, and tumorigenesis. Immunity 26:461-475.(*Co-first)

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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

We are studying the functional regulation of tumor-associated macrophages (TAMs) because of their central roles in the development and progression of cancer. A major focus of our group has been to explore the possible mechanisms that control differentiation, polarization and immunological actions of TAMs. With this as our goal, we are currently studying the effects of certain molecules and underlying molecular pathways such as PPARγ signaling, metabolic reprogramming mediated by MAE's action (Fig1) etc. By employing various cellular and molecular methods combining with proteomics, metabolomics and knock-out/knock-in techniques, we have identified certain key nodes such as MAE in TAMs that are related with tumor-promoting inflammation. Consequently, the phenotypes of TAMs are altered by blocking the MAE activity. In collaboration with 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 leukemia treatment (Fig 2).

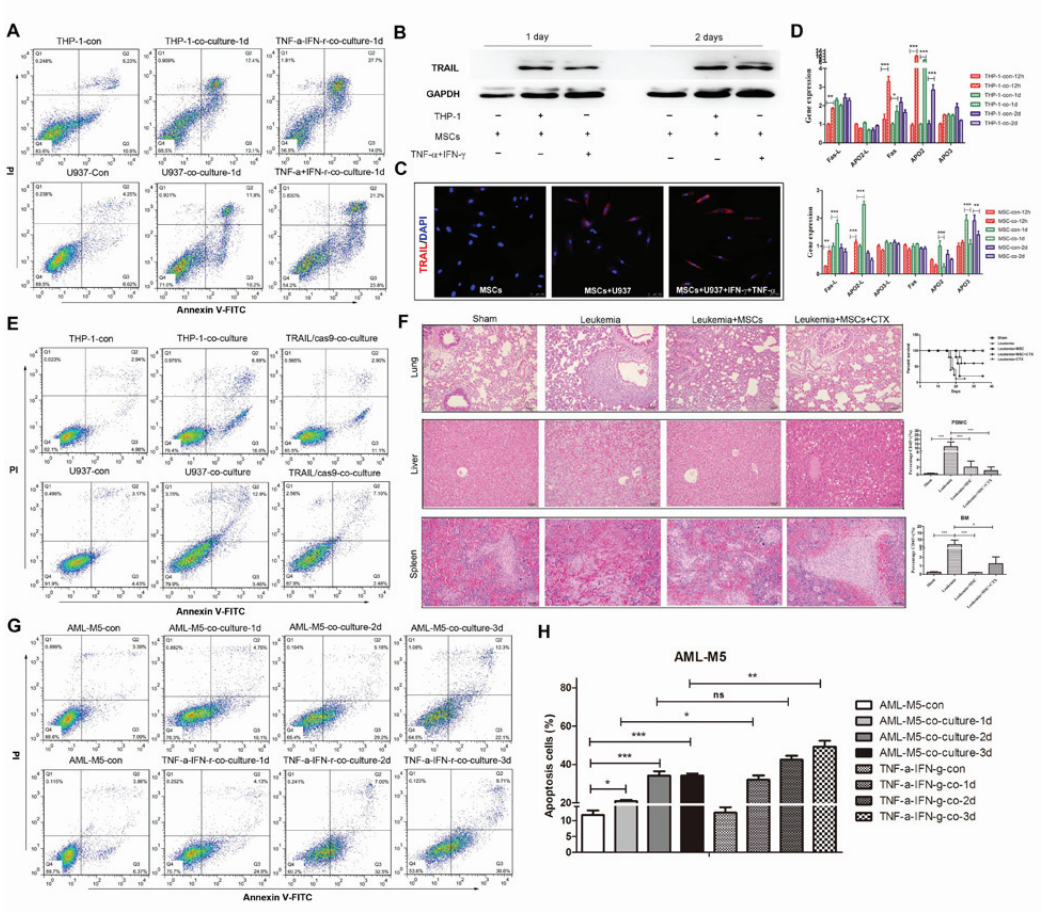


Fig1. tPPARγ promotes TAMs differentiation by inactivating MAE

A. MAE selectively interacts with tPPARγ

B. tPPARγ translocates into the mitochondria and inactivates MAE.

C. tPPARγ causes a metabolic reprogramming in TAMs.

D.The tumor-infiltrating efficiency of MAE-overexpressing BMDMs.

E. BMDMs genetically engineered to overexpress MAE suppresses tumor growth.

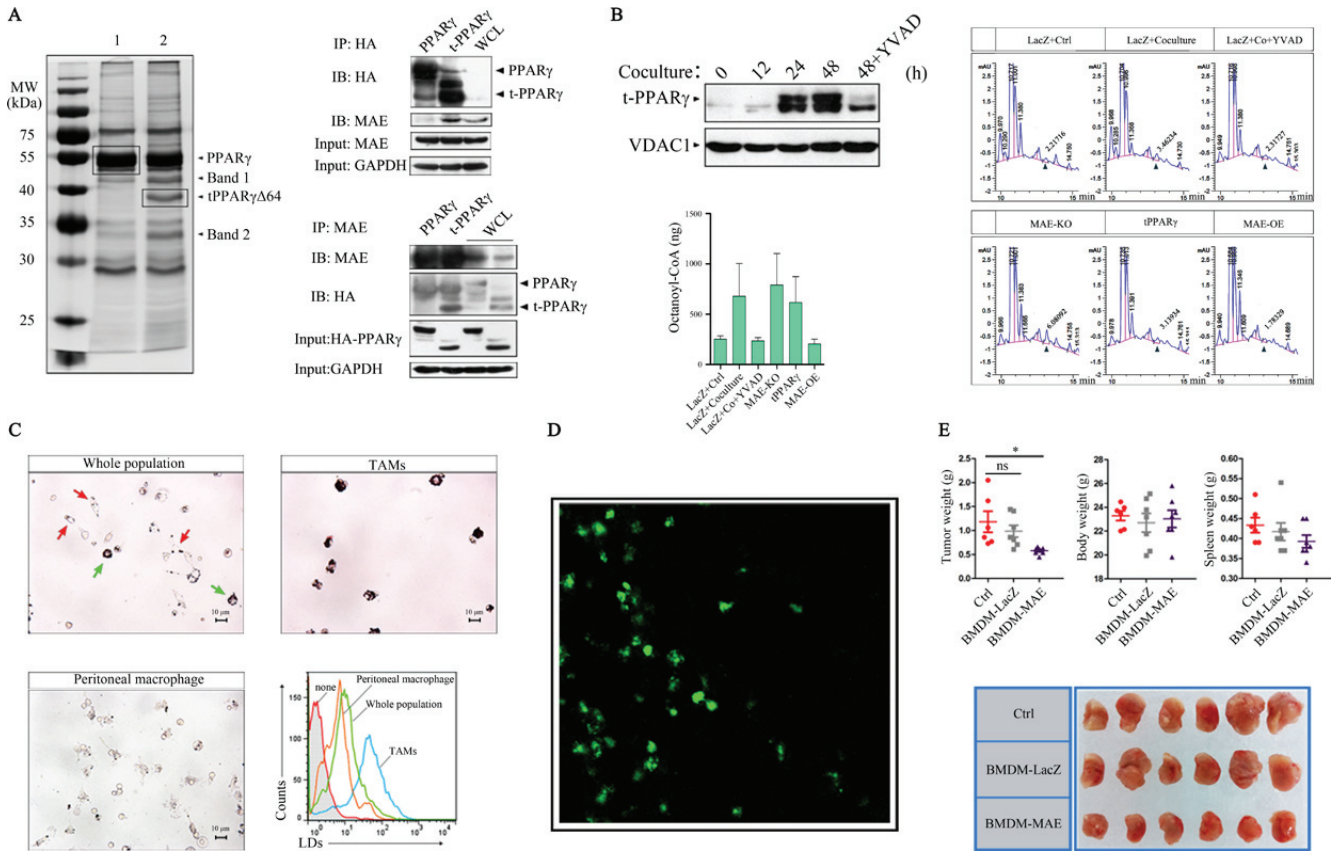


Fig2. The efficacy of MSCs in the treatment of acute monocytic leukemia

A. MSCs could induce apoptosis of leukemia cells.

B-D. TRAIL was up-regulated in MSCs after co-culture with leukemia cells and pre-activated MSCs.

E. MSCs induced apoptosis of leukemia cells by TRAIL;

F. The efficacy of MSCs transplantation in treatment of leukemia, in vivo;

G. MSCs could induce apoptosis of human AML-M5 leukemia cells;

H. Percentage of apoptotic AML-M5 leukemia cells from experiment as in (G).

Nonresolving chronic inflammation

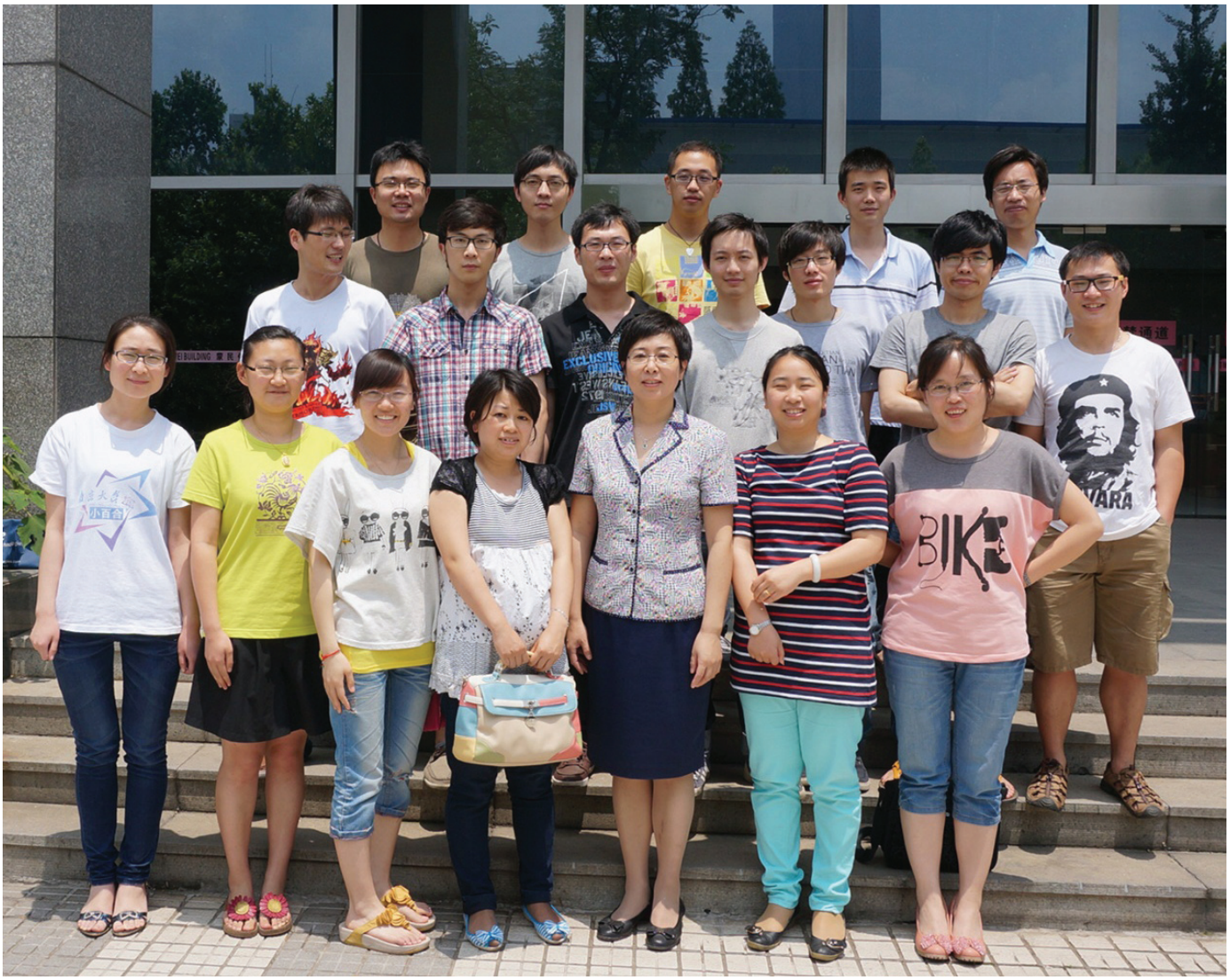
Nonresolving inflammation is a major driver of many diseases. Macrophages are now regarded as prominent players in metabolic disorder and associated nonresolving inflammation. We are investigating how macrophages interact with adipocytes to determine the phenotype of macrophages, and how macrophages 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 drugs and have caught several natural products acting as modulators or ligands of PPARγ displaying inflammatory resolving activities.

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 is to be used to detect DNA or RNA with no need for amplification, hence, revealing the actual number of live viruses. We have succeeded in detecting specimens from HPV (Human Papillomavirus) infected skin and mucous membrane. Additionally, we are interested in antigen modification and antibody production and have produced polyclonal and monoclonal antibodies against HPV E6/E7 recombinant protein etc. 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.

Selected publications and awards

1. Ding S., Qian Steven Y., Zhang Y., Wu W.L., Lu G.S., Lu Y., Feng X.J., Li L., Shen P.P.*. Establishment of immunoassay for detecting HPV16 E6 and E7 RNA. Scientific Reports, 2015, 5:13686. DOI: 10.1038/srep13686.
2. Lu Y., Zhang Y., Li L., Feng X.J., Ding S., Zheng W., Li J.X., Shen P.P.*. TAB1: a target of triptolide in macrophages, Chemistry & Biology, 2014,21(2):246-256.
3. Feng X.J., Qin H.H., Shi Q., Zhang Y., Zhou F.F., Wu H.C., Ding S., Niu Z.Y., Lu Y., Shen P.P.*. Chrysin Attenuates Inflammation by Regulating M1/M2 Status via Activating PPARγ. Biochemical Pharmacology, 2014,89:503-514.
4. Lin X.Z., Zheng W., Liu J., Zhang Y., Qin H.H., Wu H.C., Xue B., Lu Y., Shen P.P. *. Oxidative stress in malignant melanoma enhances TNF-α secretion of tumor-associated macrophages that promote cancer cell invasion. Antioxidants & Redox Signaling. 2013, 19(12):1337-1355.
5. Feng X.J., Sun T.Z., Bei Y.C., Zheng W., Lu Y., Shen P.P.*. S-nitrosylation of ERK inhibits ERK phosphorylation and induces apoptosis. Scientific Reports, 2013, 3:1814. DOI: 10.1038/srep01814.
6. Yang W.W., Lu Y., Xu Y.C., Xu L.Z., Zheng W., Wu Y.Y., Li L., Shen P.P.*. Estrogen represses Hepatocellular Carcinoma (HCC) growth via inhibiting alternative activation of tumor-associated macrophages (TAMs). Journal of Biological Chemistry. 2012, 287(48):40140-40149.
7. Xu H.W., Wei Y.N., Zhang Y., Xu Y.C., Li F., Liu J., Zhang W., Han X.D., Tan R.X., Shen P.P.*. Oestrogen attenuates tumor malignancy in hepatocellular carcinoma progression. Journal of Pathology, 2012, 228: 216–229
8. Shen Pingping etc. The first prize of Science and Technology, Jiangsu Province, 2014 (20140356)



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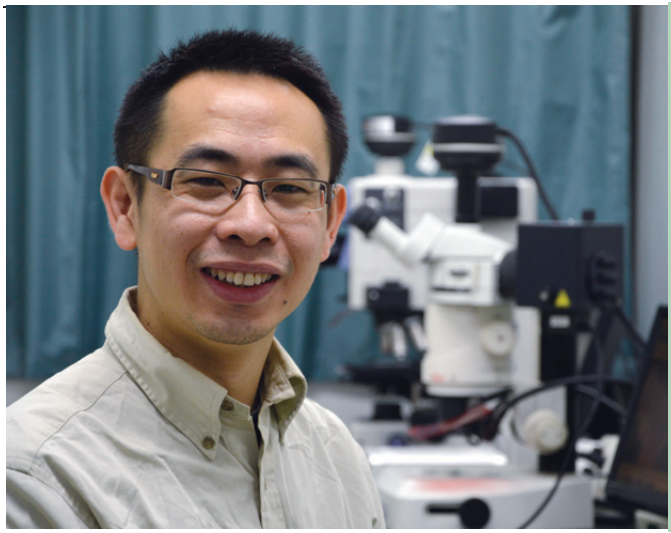
Xiujing Feng

Technician

Wei Zheng



Jun Yan, Ph.D.

Jun received his Bachelor in Genetics at Fudan University in Shanghai in 1997 and his Ph.D. degree in Cell Biology at Institute of Biochemistry and Cell Biology, Shanghai Institutes for Life Sciences, Chinese Academy of Sciences in 2003. Afterwards, he pursued his postdoctoral training at Baylor College of Medicine in Houston and 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, cancer 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 potnetial therapeutic agents.

A) Tumor microenvironment play 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 recapitulat human cancer development (Fig. 2). These mouse models will provide excellent platforms for pre-clinical study in near future.

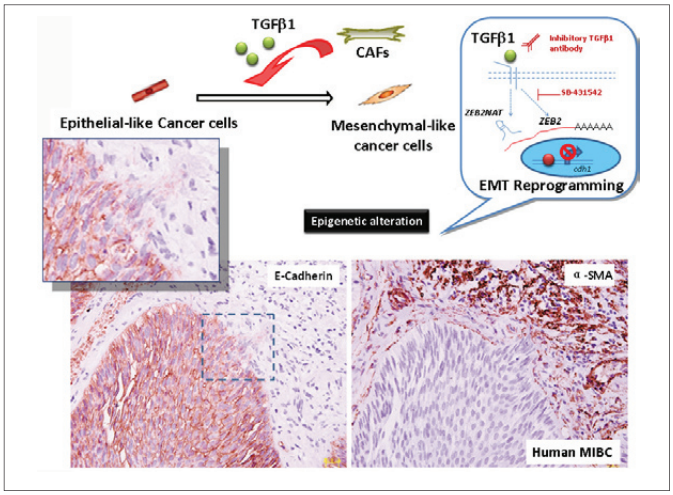


Fig 1. Graphic illustration of CAFs induces EMT of human bladder cancer cells on the boundary of epithelial and stromal compartments. CAFs secreted TGFβ1 to induces lncRNA-ZEB2NAT and ZEB2, leading to EMT reprograming and cancer cell invasion.

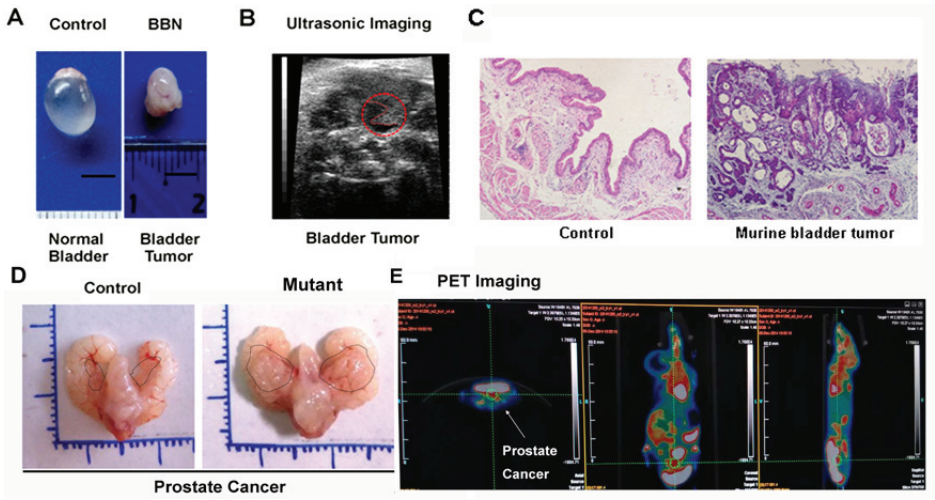


Fig 2. Bladder and prostate cancer mouse model.

A) Photograph of mouse bladder.

B) In vivo US imaging.

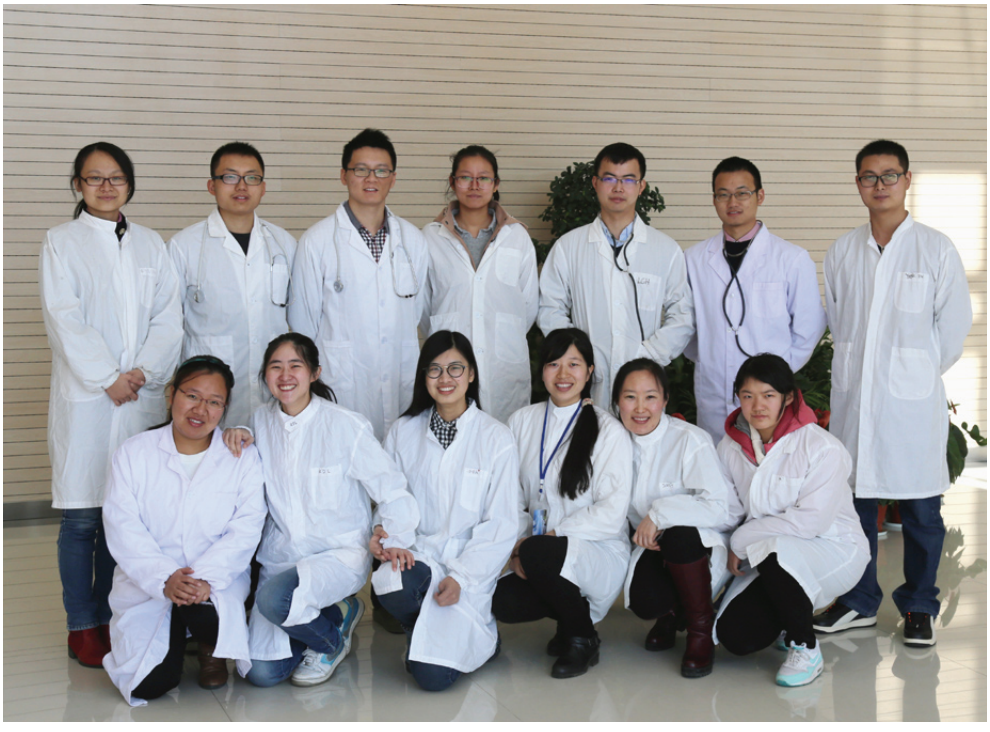
C) Histologic analysis of normal bladder and bladder tumor.

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

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

Selected publications(* corresponding author)

1. 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; doi: 10.18632/oncotarget.6135.
2. 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
3. Li L, Zhang D, Wang X, Yao X, Ye C, Zhang S, Wang H, Xia H, Wang Y, Fang J, Yan J*, Ying H*. Hypoxia-inducible miR-182 enhances HIF1α signaling via targeting PHD2 and FIH1 in prostate cancer. Sci Rep 2015;5:12495.
4. Zhao W, Chang C, Cui Y, Zhao X, Yang J, Shen L, Zhou J, Hou Z, Zhang Z, Ye C, Hasenmayer D, Perkins R, Huang X, Yao X, Yu L, Huang R, Zhang D, Guo H*, Yan J*. Steroid receptor coactivator-3 regulates glucose metabolism in bladder cancer cells through coactivation of hypoxia inducible-factor 1α. J Biol Chem. 2014;289:11219-11229
5. 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 celluar senescence by degrading the SENP7 deSUMOylase. Cell Rep (Accepted)
6. 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(6):904-16. (# Co-senior author)
7. Liu C, Zhou Z, Yao X, Chen P, Sun M, Su M, Chang C, Yan J, Jiang J, Zhang Q. Hedgehog signaling downregulates Suppressor of Fused through the HIB/SPOP-Crn axis in Drosophila. Cell Res. 2014;24:595-609.
8. Ye C‡, Zhao W‡, Li M‡, Zhuang J, Yan X, Lu Q, Chang C, Huang X, Zhou J, Xie B, Zhang Z, Yao X, Yan J*, Guo H*. δ-Tocotrienol induces human bladder cancer cell growth arrest, apoptosis and chemosensitization through inhibition of STAT3 pathway. PLoS One 2015;10(4):e0122712.
9. Hou Z, Zhao W, Zhou J, Shen L, Zhan P, Xu C, Chang C, Bi H, Zou J, Yao X, Huang R, Yu L*, Yan J*. A long noncoding RNA Sox2ot regulates lung cancer cell proliferation and is a prognostic indicator of poor survival. Int J Biochem Cell Biol. 2014;53:380-388.
10. Liu W, Cao H, Yan J, Huang R, Ying H. 'Micro-managers' of hepatic lipid metabolism and NAFLD. Wiley Interdiscip Rev RNA. 2015;6(5):581-93. Review



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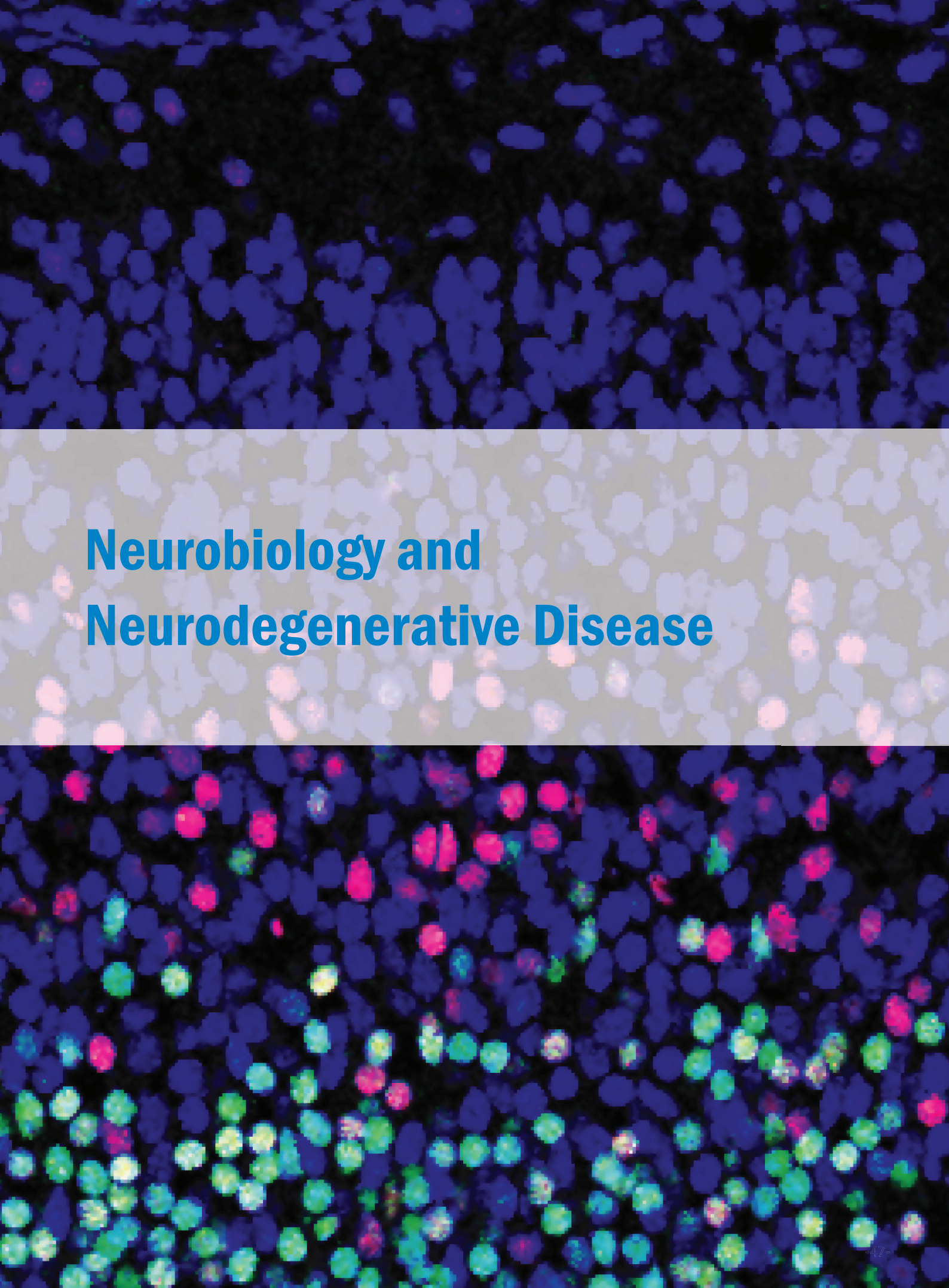
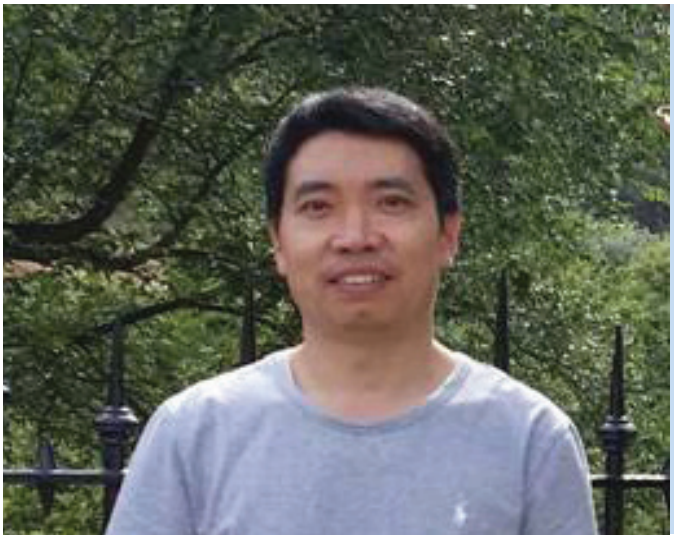


Neurobiology and Neurodegenerative Disease



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 neurodegenerative disease

Alzheimer's disease (AD) is the most common form of dementia. It is well-documented that Alzheimer's brain displays abnormal tau phosphorylation and altered activities of protein kinases. A number of recent studies have shown that two key players in the insulin signaling pathway, e.g. Akt and its upstream kinases, are down-regulated in AD. However, the roles of the insulin signaling in tau phosphorylation and neurodegeneration are poorly understood. Previous evidence has indicated that deletion of Akt three isoforms causes early embryonic lethality in mice. To investigate whether dysfunction of Akt affects tau phosphorylation and neuron survival, Cre-loxP technology was employed to generate a viable Akt three isoforms conditional knockout (Akt cTKO) mouse model in which total Akt levels were dramatically reduced in the adult brain.

Western blotting data showed significantly increased levels of p-tauT205, p-tauT231 and p-tauS396 in the cortex of Akt cTKO (Fig. 1A). IHC results were consistent with biochemical data, as evidenced by increased p-tauT205 immuno-reactivity in the brain of Akt cTKO (Fig. 1B:a-p).

To dissect molecular mechanisms, GSK3α/β was examined. Levels for p-GSK3αY279 but not p-GSK3βY216 were increased in Akt cTKO mice (Fig. 2A). In contrast, levels for p25, p-Erk1/2 and p-p38 were not increased (Fig. 2B-2D). Since the tyrosine phosphorylation of GSK3 requires the cAMP-PKA signaling, PKA was examined. The activity of PKA seemed enhanced (Figure 3. A: Levels of p-PKA substrates were increased in Akt cTKOs. B: Levels of p-VASP were increased. C: Levels of p-tauS214 and p-tauS356 were increased.).

TUNEL assay was performed to examine apoptotic neuronal death, but no difference in the total number of TUNEL+ cells was observed in Akt cTKO mice (Fig. 4A). Moreover, IHC analyses showed comparable immuno-reactivity of NeuN, GFAP and Iba1 in the brain of control and mutant mice (Fig. 4B-4C), suggesting no neurodegeneration in Akt cTKO mice. Currently, a big effort has been made to investigate whether neuron loss observed in various types of neurodegenerative mouse models could be prevented using both pharmacological and genetic manipulations.

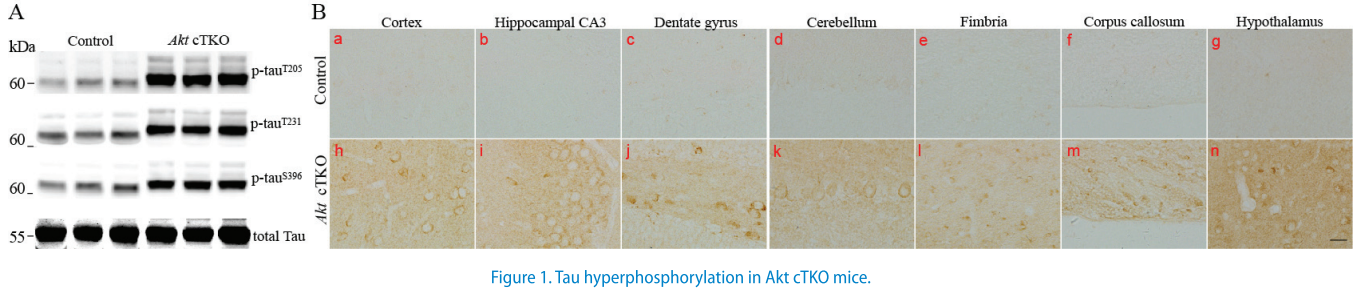

Figure 1. Tau hyperphosphorylation in Akt cTKO mice.

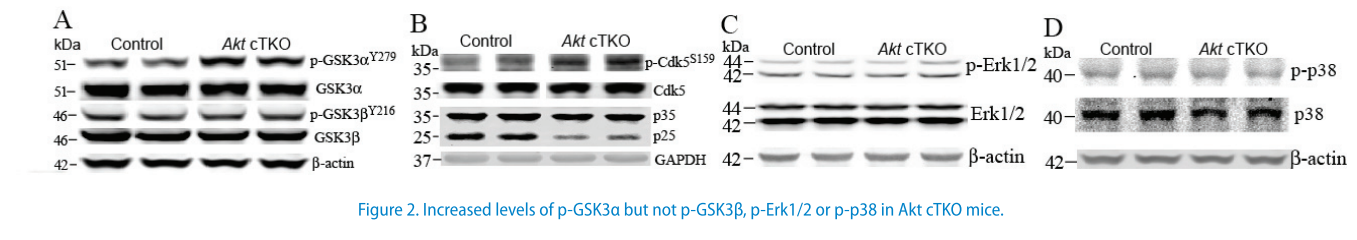

Figure 2. Increased levels of p-GSK3α but not p-GSK3β, p-Erk1/2 or p-p38 in Akt cTKO mice.

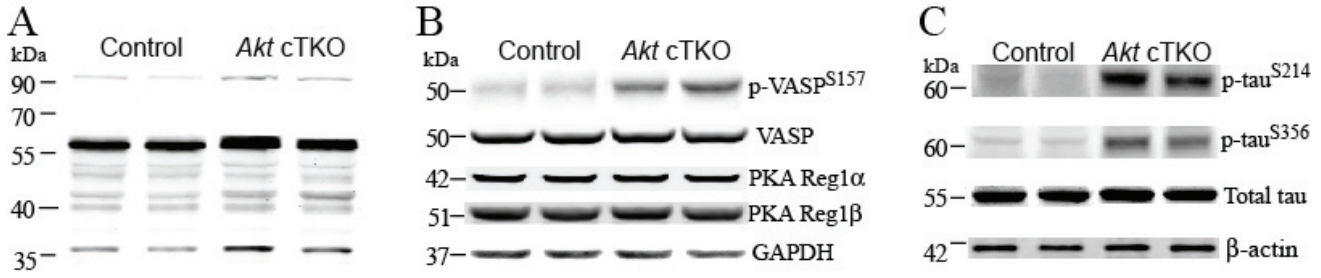

Figure 3. Enhanced PKA activity in Akt cTKO mice.

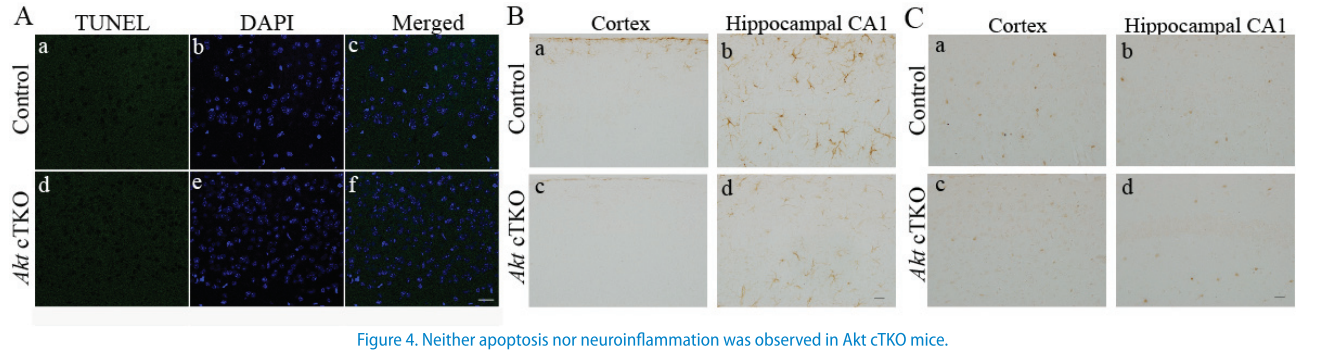

Figure 4. Neither apoptosis nor neuroinflammation was observed in Akt cTKO mice.

Recent publications (*, Corresponding author)

1. Hou J, Cheng S, Chen L, Wang Q, Shi Y, Xu Y, Yin Z, Chen G*. Astroglial activation and tau hyperphosphorylation precede to neuron loss in a neurodegenerative mouse model. Accepted.
2. Wang L, Cheng S, Yin Z, Xu C, Lu S, Hou J, Yu T, Zhu X, Zou X, Peng Y, Xu Y, Yang ZZ, Chen G*. Conditional inactivation of Akt three isoforms causes tau hyperphosphorylation in the brain. Molecular Neurodegeneration, 2015; DOI: 10.1186/s13024-015-0030.
3. Cheng S, Hou J, Zhang C, Xu C, Wang L, Zou X, Yu, H, Shi Y, Yin Z, Chen G*. Minocycline reduces neuroinflammation but does not ameliorate neuron loss in a mouse model of neurodegeneration. Scientific Reports, 2015; 5,10535, DOI:10.1038.
4. Holland PR, Searcy JL, Salvadores N, Scullion G, Chen G, Lawson G, Scott F, Bastin ME, Ihara M, Kalaria R, Wood ER, Smith C, Wardlaw JM, Horsburgh K. Gliovascular disruption and cognitive deficits in a mouse model with features of small vessel disease. Journal of Cerebral Blood Flow & Metabolism, 2015; DOI:10.1038
5. Cheng S, Zhang C, Xu C, Wang L, Zou X, Chen G*. Age-dependent neuron loss is associated with impaired adult neurogenesis in forebrain neuron-specific Dicer conditional knockout mice. The International Journal of Biochemistry & Cell Biology, 2014; 57:186-96.
6. Chen G, Zou X, Watanabe H, van Deursen JMA, Shen J. CREB binding protein is required for both short-term and long-term memory. The Journal of Neuroscience, 2010; 30: 13066-13077.
7. Tabuchi K[#], Chen G[#], Südhof, T, Shen J. Conditional forebrain inactivation of nicastrin causes progressive memory impairment and neurodegeneration. The Journal of Neuroscience 2009; 29: 7290-7301. ([#]co-first author.)
8. Chen G, Chen KS, Knox J, Inglis J, Bernard A, Martin SJ, Justice A, McConlogue L, Games D, Freedman SB, Morris RGM. A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. Nature 2000; 408: 975-979.

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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 self-defensive attempt by the brain to remove harmful stimuli and to initiate the healing process. The neuroinflammatory response must be tightly regulated and actively terminated to prevent unnecessary tissue destruction. Persistent injurious stimuli (e.g., toxins, pathogens, and autoimmunogens) and failed resolution of acute neuroinflammation can flip a protective immune response to chronic destruction to brain tissues.

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). Autism is characterized by social deficits, communication difficulties, repetitive/restricted/stereotyped behaviors and interests, and language impairment. 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.

Endotoxin tolerance (ET) is a reduced responsiveness of innate immune cells like macrophages/monocytes to an endotoxin challenge following a previous encounter with the endotoxin. Although ET in peripheral systems has been well studied, little is known about ET in the brain. We recently identified a distinct non-cell autonomous mechanisms in ET formation in brain immune cells, microglia. Specifically, neurons and astroglia were indispensable for microglial ET (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 when microglia encountered repeated lipopolysaccharide (LPS) treatments. 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, this study demonstrated that neurons and astroglia govern microglial ET through M-CSF—MCSFR—Erk1/1 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.

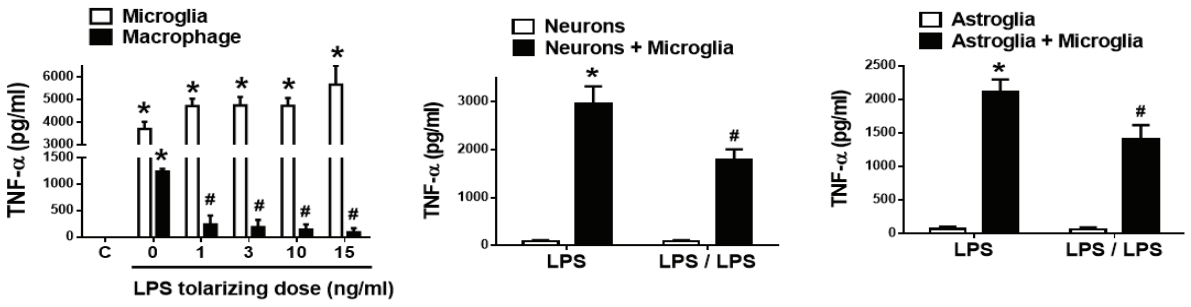

Figure 1. Neurons and astroglia are required for microglial endotoxin tolerance.

(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.

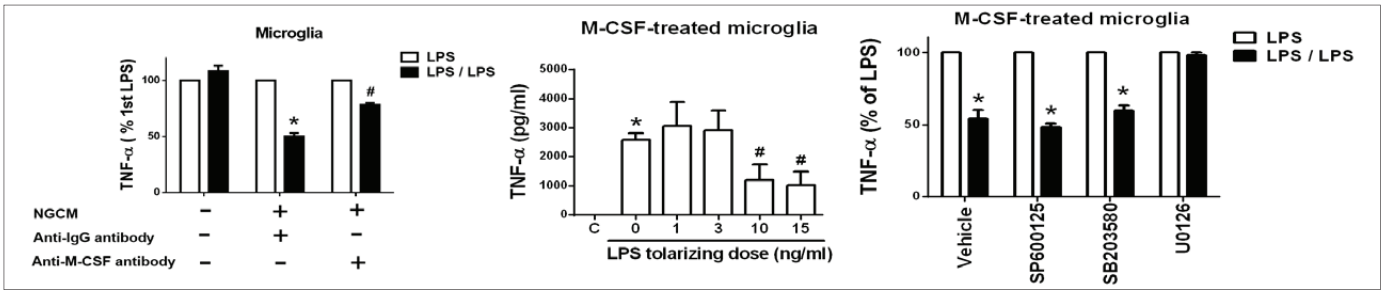

Figure 2. M-CSF secreted by neurons and astroglia governs microglial endotoxin tolerance.

(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.

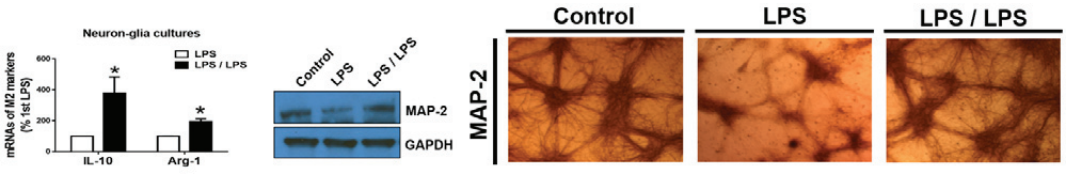

Figure 3. Endotoxin-tolerant microglia displayed M2-like polarized phenotypes and were neuroprotective.

Selected publications

1. Chu CH, Wang S, Chen SH, Wang Q, Lu RB, Gao H-M*, and Hong JS (2015) Neurons and astroglia govern microglial endotoxin tolerance through macrophage colony-stimulating factor receptor-mediated ERK1/2 signals. Brain Behavior and Immunity (in press)
2. Chu CH, Chen SH, Wang Q, Langenbach R, Li H, Zeldin D, Chen SL, Wang S, Gao H-M*, Lu RB*, Hong JS (2015) PGE2 Inhibits IL-10 Production via EP2-Mediated β-Arrestin Signaling in Neuroinflammatory Condition. Molecular Neurobiology. 52(1):587-600.
3. Zhou H, Liao J, Aloor J, Nie H, Wilson B, Fessler M, Gao H-M*, Hong J-S (2013) CD11b/CD18 is a novel receptor mediating extracellular dsRNA-induced immune responses. Journal of Immunology 190:115-125 (SCI citations: 8); NIEHS Paper of the month; Faculty 1000 recommends (4 stars); Featured in "In This Issue" of The Journal of Immunology.
4. Gao H-M*, Zhou H, Hong J-S (2012) NADPH oxidases: novel therapeutic targets for neurodegenerative diseases. Trends in Pharmacological Sciences 33(6): 295-303 (SCI citations: 40)
5. Zhou H, Zhang F, Chen S-H, Zhang D, Wilson B, Hong J-S, Gao H-M* (2012) Rotenone activates phagocyte NADPH oxidase through binding to its membrane subunit gp91phox. Free Radical Biology & Medicine 52: 303–313. (SCI citations: 18); NIEHS Paper of the month
6. Gao H-M* and Hong J-S (2011) Gene–environment interactions: key to unraveling the mystery of Parkinson's disease. Progress in Neurobiology 94:1–19. (SCI citations: 48)
7. Gao H-M*, Zhou H, Zhang F, Wilson B, Kam W, Hong J-S (2011) HMGB1 acts on microglia Mac1 to mediate chronic neuroinflammation that drives progressive neurodegeneration J. Neurosci. 31(3):1081–1092 (SCI citations: 70); NIEHS Paper of the month; Faculty 1000 recommends
8. Gao H-M*, Zhang F, Zhou H, Kam W, Wilson B, Hong J-S (2011) Neuroinflammation and alpha-synuclein dysfunction potentiate each other driving chronic progression of neurodegeneration in a mouse model of Parkinson's disease. Environmental Health Perspectives 119 (6): 807-814 (SCI citations: 55); NIEHS Paper of the month
9. Gao H-M, Kotzbauer PT, Uryu K, Leight S, Trojanowski JQ, Lee VM. (2008) Neuroinflammation and consequent oxidation/nitration of alpha-synuclein directly linked to dopaminergic neurodegeneration. J. Neurosci. 28(30):7687–7698 (SCI citations: 152)
10. Gao H-M*, Hong J-S (2008) Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends in Immunology 29: 357-365 (SCI citations: 203); ** cover illustration

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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.

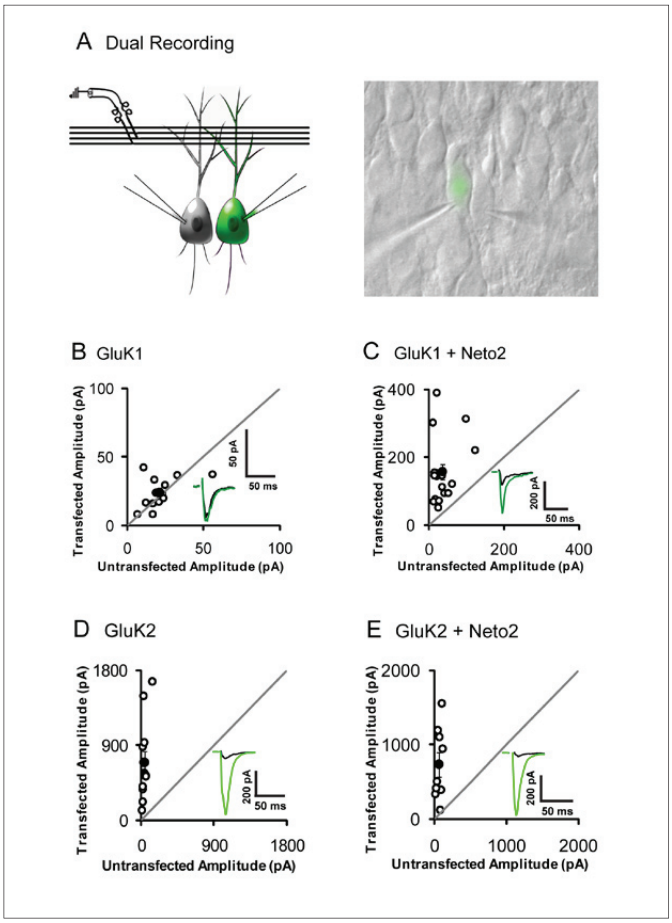

Figure 1. Synaptic response mediated by GluK1 and GluK2 expressed in CA1 neurons.

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.

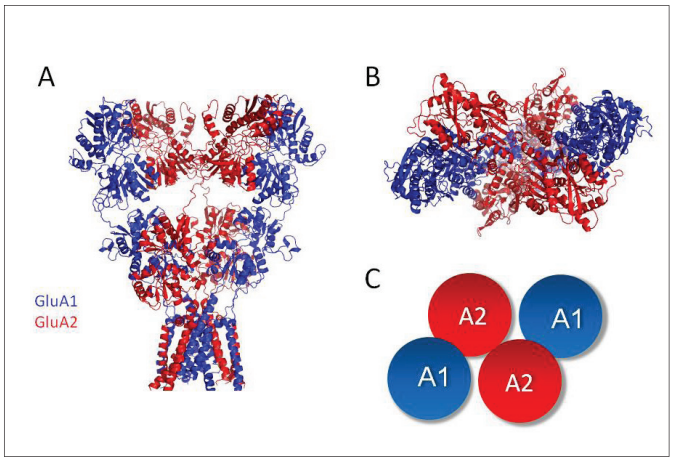

Figure 2. the Model of Heteromeric AMPA receptors.

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

Selected publications

1. Granger AJ, Shi Y, Lu W, Cerpas M, Nicoll RA. (2013) LTP requires an extrasynaptic pool of glutamate receptors independent of subunit type. Nature. 493, 495-500
2. Herring B*, Shi Y*, Suh YH, Zheng CY, Schmid SM, Roche KW, Nicoll RA. (2013) Cornichon proteins determine the subunit composition of synaptic AMPA receptors. Neuron 77:6,1083-96
3. Gray JA, Shi Y, Usui H, During MJ, Sakimura K, Nicoll RA. (2011) Distinct modes of AMPA receptor suppression at developing synapses by GluN2A and GluN2B: analysis of single-cell GluN2 subunit deletion in vivo. Neuron. 71, 1085-1101
4. Shi Y, Suh YH, Milstein AD, Isozaki K, Schmid SM, Roche KW, Nicoll RA. (2010) Functional comparison of the effects of TARPs and cornichons on AMPA receptor trafficking and gating. Proc Natl Acad Sci U S A 107, 16315-16319
5. Shi Y, Lu W, Milstein AD, Nicoll RA. (2009) The stoichiometry of AMPA receptors and TARPs varies by neuronal cell types. Neuron. 62, 633-40
6. Lu W, Shi Y, Jackson A, Bjorgan K, During MJ, Sprengel R, Seeburg PH, Nicoll RA. (2009) Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. Neuron. 62, 254-68
7. Shi Y, Chen X, Wu Z, Shi W, Jiang C, Harrison R. (2008) cAMP-dependent protein kinase phosphorylation produces interdomain movements in SUR2B leading to activation of vascular KATP channel. J Biol Chem. 283, 7523-30
8. Shi Y, Cui N, Shi W, Jiang C. (2008) A short motif in Kir6.1 consisting of 4 phosphorylation repeats underlies vascular KATP channel inhibition by protein kinase C. J Biol Chem. 283, 2488-94

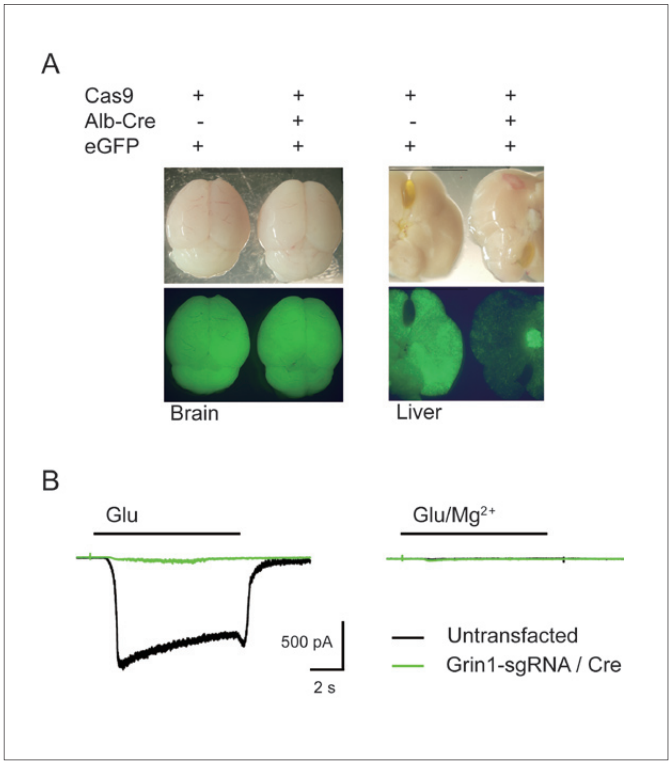

Figure 3. Characterization of a Cas9 transgenic mouse.

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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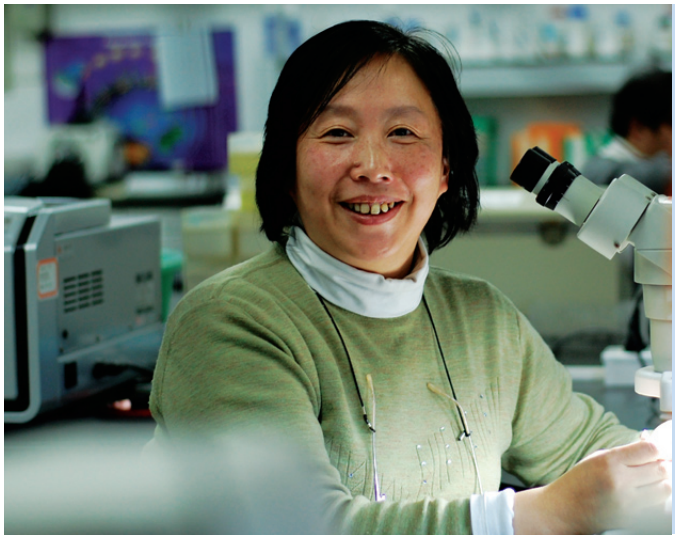

Ying Xu , Ph.D.

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

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

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

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

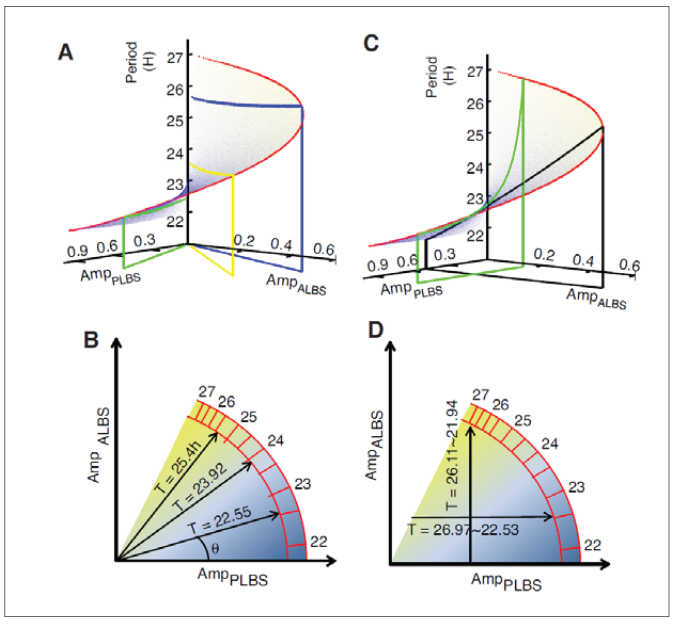


Figure 1 An intensity ratio of interlocking loops regulates circadian period length

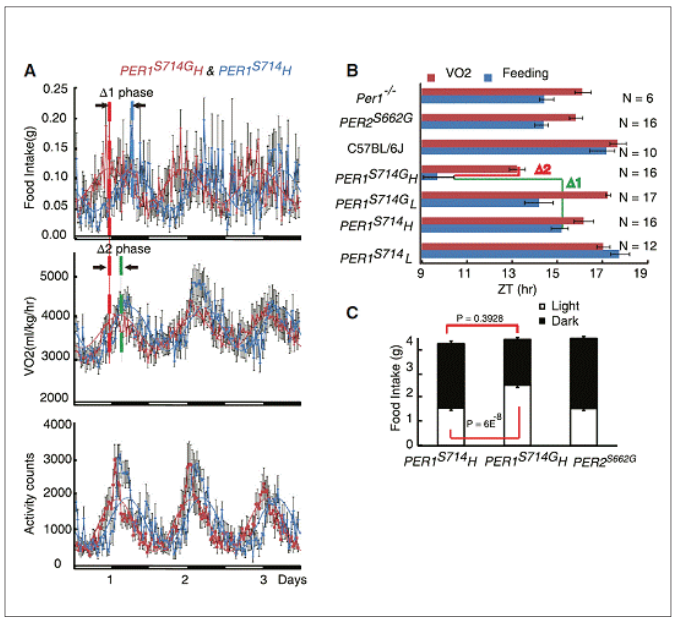


Figure 2 PER1 and PER2 regulates feeding and Sleep behavior separately

Selected publications

1. Guangsen Shi, Pancheng Xie, Zhipeng Qu, Zhihui Zhang, Zhen Dong, Yang An, Lijuan Xing, Zhiwei Liu, Yingying Dong, Guoqiang Xu, Ling Yang, Yi Liu and Ying Xu (2015) Distinct roles of HDAC3 in the core circadian negative feedback loop are critical for clock function. Cell Report, Accepted.
2. 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.
3. 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 Report, 7(5) 1509-1520.
4. 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.
5. 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. Proceedings of the National Academy of Sciences,110 (12) 4750-4755.
6. 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.
7. 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 PER2S662G Is Linked to Cell Cycle Progression and Tumorigenesis. Cell Death and Differentiation 19(3):397-405.
8. Weiwei Tao, Siyu, Guangsen Shi, Jinhu, Ying Xu and Chang Liu (2011) SWItch/Sucrose NonFermentable (SWI/SNF) Complex Subunit BAF60a Integrates Hepatic Circadian Clock and Energy Metabolism. Hepatology, 54(4):1410-20.
9. Xi Wu, Zhiwei Liu, Guangsen Shi, Lijuan Xing, Xiaohan Wang , Xiwen Gu , hipeng Qu Zhen Dong, Jing Xiong, Xiang Gao, Chenyu Zhang, &Ying Xu (2011) The Circadian Clock Influences Heart Performance. Journal of Biological Rhythms, 26 (5): 402-411.
10. Xiaohan Wang, Xiang Gao & Ying Xu 2011 MAGED1: Molecular Insights and Clinical Implications. Annals of Medicine, 43:347-355.
11. 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

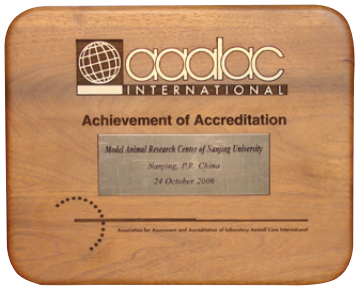
Nanjing Biomedical Research Institute of Nanjing University (NBRI)

NBRI is recognized as a leading mammalian genetics research center and a national resource platform, which is dedicated to providing high-quality model mice and professional services. Based on Model Animal Research Center of Nanjing University (MARC) and National Resource Center for Mutant Mice(NRCMM), NBRI built advanced and reliable platforms of mouse models that provide mouse model , customized animal model services, and mouse phenotype analysis services.

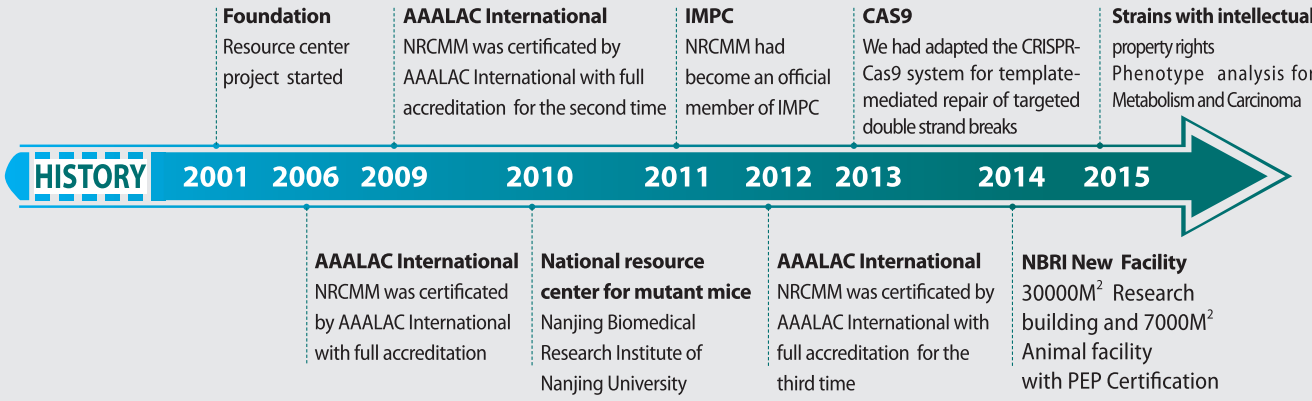
NBRI has harbored 3,000 mouse strains and has distributed ~300,000 genetically engineered mice including cardiovascular diseases, tumor, metabolic diseases, immunodeficiency and neurodegenerating diseases models. With breading services and cryopreservation , laboratory animal pathogen detection , and agency services, we strive to offer One Step Service and comprehensive solution for model mice to worldwide.

NBRI has developed a series of cooperations with the following organizations and company: Eli Lilly, GSK, Pfizer, UC Davis and the Beatson Institute for Cancer Research, the University of Western Australia, MRC, Schepens Eye Research Institute, Harvard Medical School.

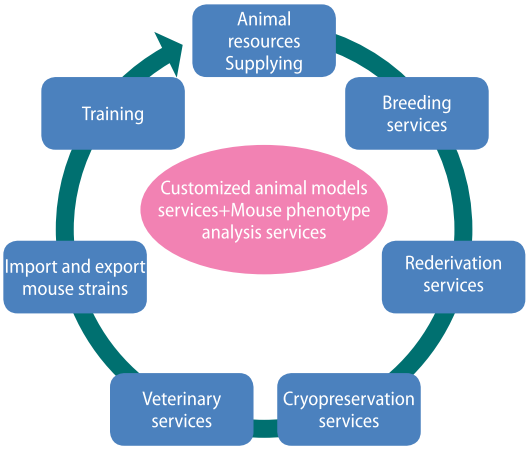
- The **only** national resource center for mutant mice authorized by the Ministry of Science and Technology of China
- The **first** research center certificated by AAALAC International Institution in China
- The **largest** mouse preservation center and the **largest** gene knockout service organization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



HISTORY OF NBRI



Service System of NBRI



One-Step Services
Providing a Complete Solution for Model Mice

Nanjing Biomedical Research Institute of Nanjing University

Sales : +86-25-58641520/58641550

Technical Service : +86-25-58641559/ 58641580

Phenotype Analysis : +86-25-58641551

Office : +86-25-58641533

Fax : +86-25-58641551

QQ Enterprise : 8000899984

Email : service@nbri-nju.com

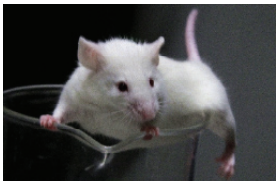
Web : www.nbri-nju.com

MarcMice, Better Science.

2015 Highlights

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

NCG mice developed by NBRI (**with independent property right**), through CRISPR/Cas9, do not express the Prkdc gene nor the X-linked Il2rg gene, are the most highly immunodeficient mice.



ID: T001475

Background : NOD/ShiLtJNju

Strains type: Cas9-KO

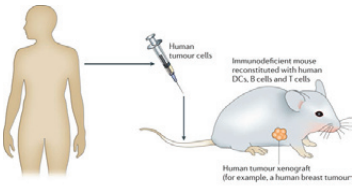
Phenotype:

- NO mature lymphocytes, NO serum Ig and LOW NK cell activity.
- Abnormal structures in the thymus / spleen / lymph nodes.
- Resistant to lymphoma development even after irradiation treatment.
- Readily support engraftment of human CD34+ hematopoietic stem cells and represent a superior, long-lived model suitable for studies employing xenotransplantation strategies.
- Enables transplantation of human pancreatic islets and the autoimmune lymphocytes that cause type 1 diabetes

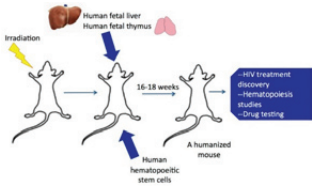
Application of NCG mice

Applications:

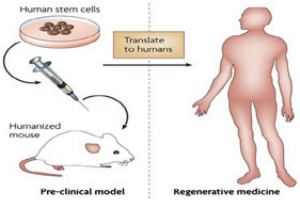
- Humanized mice model
- Human cells and tissue transplatation
- Tumor reseach
- Stem cell research
- Infectious disease research



Human-derived tumor model



Humanized mice model/hematopoiesis and immune system researching tumor model/human infectious disease model



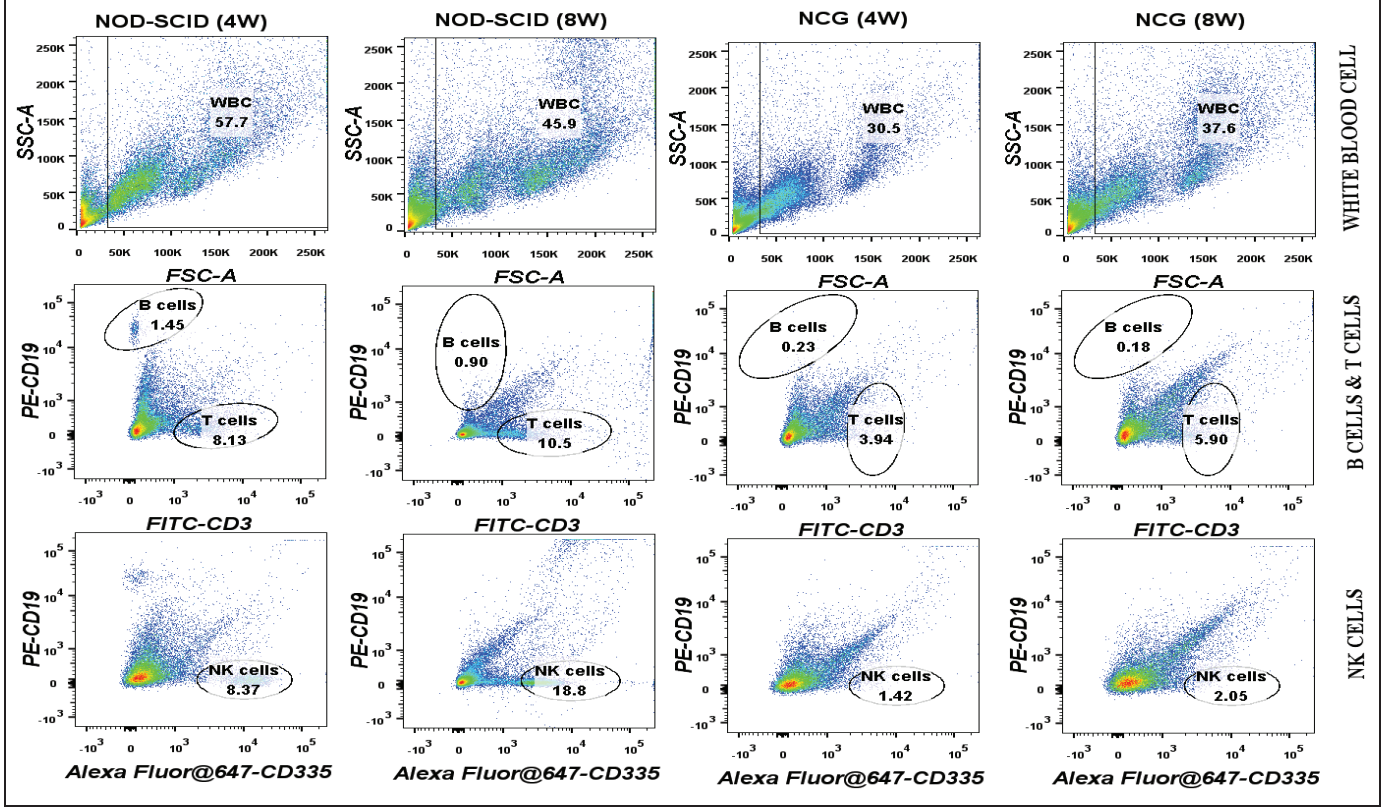
Stem cells and iPS cells

Application of NCG mice in Human-derived tumor model

Advantages in CDX and PDX:

- Highly immunodeficient, almost no rejection for human cells or tissue.
- Life span as normal mice, can be used for long term observation.
- Keep characteristics of histology , molecular marker and drug-sensitivity of primary tumor.
- Clear genetic background, similar experiment data between biological repeats.

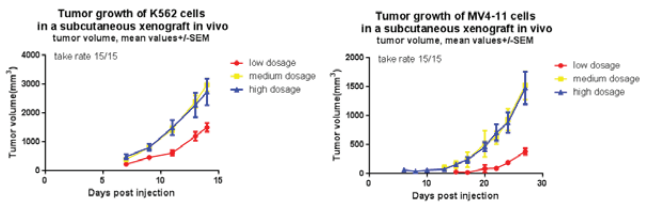
The Immnuophenotyping Analysis of NCG Mice



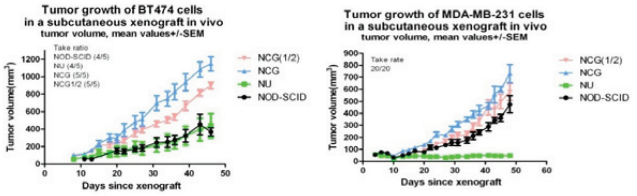
Application of NCG mice in Cell-line-derived Xenograft

- High success rate of molding, 100% success rate in leukemia xenograft model and breast cancer xenograft model.
- Short term for molding, only half month needed for molding according to 100-150mm^3 forming tumor standard.
- Low cell injection dose.

Tumor-bearing result of human leukemia cell in NCG mice



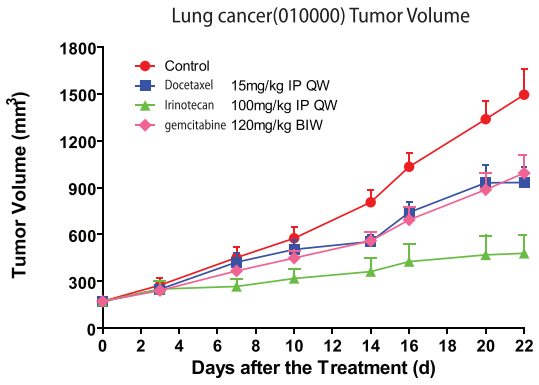
Application comparison of human mammary gland cells in NCG mice

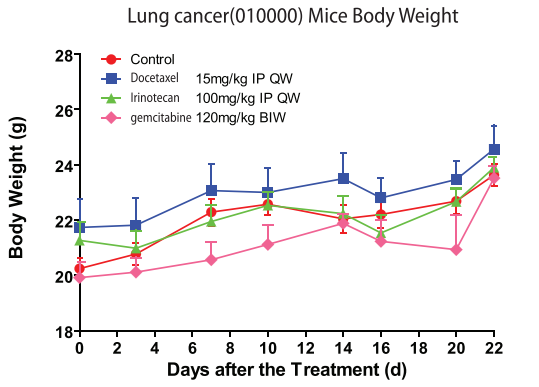


Application of NCG mice in Patient-derived Xenograft

- High success rate and Short time of molding(1-3 months).
- Puncture and microscopy samples can be used for molding.

The project of NCG mice application in PDX---chemotherapeutics efficiency screening



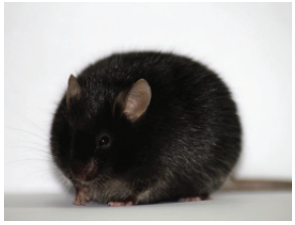


1.Supplying SPF Laboratory Mouse Strains

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

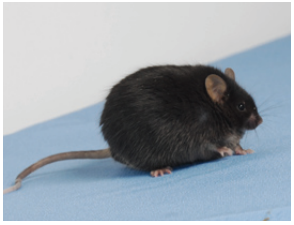
Throughout the year of 2015, NBRI has accomplished an output of around 75,000 model mice including commercial purchases and exporting service. Up to now, we have produced 402 independent intellectual property rights of the strains and received 436 donations lines added to our Resource Centre.

Star mice



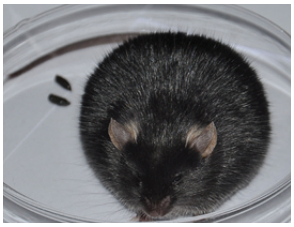
B6.Cg-*Lep*^{ob}/JNju

Type II Diabetic mouse model



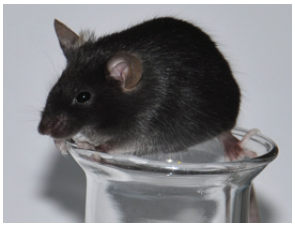
B6.BKS(D)-*Lepr*^{db}/JNju

Type II Diabetic mouse model



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

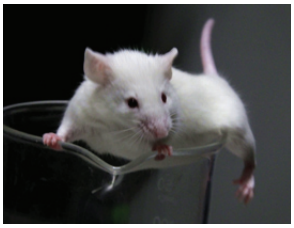
Type II diabetic mice model



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

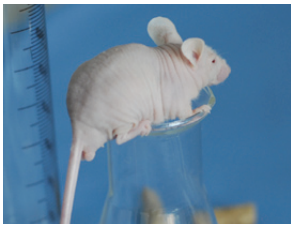
Atherosclerosis

Immune deficiency mouse model

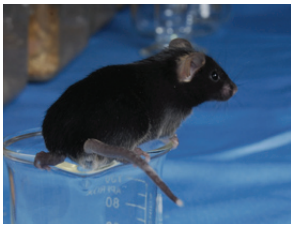


NOD-*Prkdc*^{em26}*Il2rg*^{em26}Nju

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



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

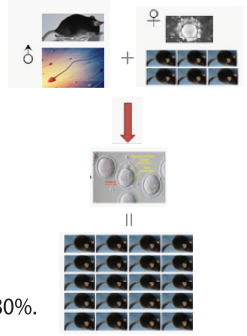


B6.129S7-*Rag1*^{tm1Mom}/JNju

2.Breeding services and cryopreservation

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

In 2015, NRCMM have finished approximately 350 strains for cryopreservation. We can supply the cryopreservation services. We can also help customers to breed, house, genotype and maintain mouse colonies. Furthermore, we also provide custom breeding services to meet your specific requirements. Below are some routine services provided by our animal facilities: Ideal for strains whose males have short lifespan or breeding windows.



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

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

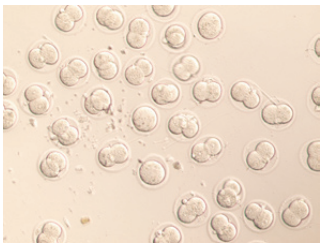
Embryos cryopreservation is suited to homozygous, multiple unlinked mutation unique genetic backgrounds.

Sperm cryopreservation is suited to single mutations on a common inbred background, most transgenic and knockouts.

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

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

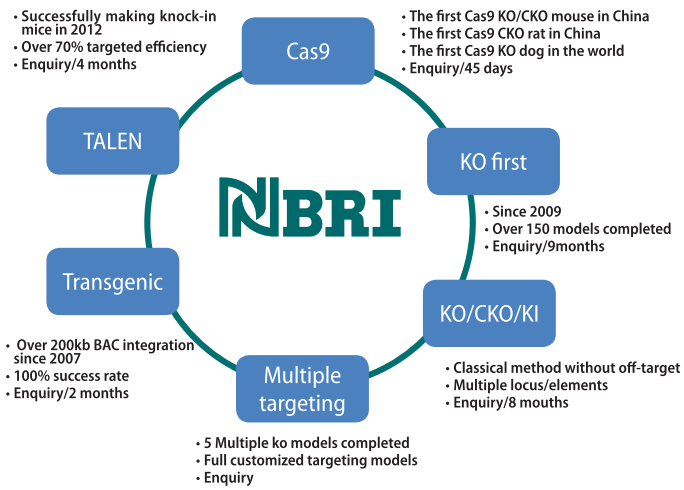
5) Strain Rescue Service: Through IVF or ovary transfer, we can further check the state of the sperm. Our success rate is over 80%.



3.Transgenic/Knockout Mice Services

NBRI provides services for generating transgenic mice and knockout mice. Nowadays, CRISPR/Cas9 has become the leading tool for genome engineering by using RNA-guided DNA endonuclease Cas9, the type II (clustered regularly interspaced short palindromic repeat) .CRISPR–Cas system uses CRISPR RNA (crRNA) as a guide to locate the DNA target and the Cas9 protein to cut DNA then generates DSBs in the target DNA. Easy programmability of the Cas9 endonuclease using customizable RNAs brings unprecedented flexibility and versatility for targeted genome modification.

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

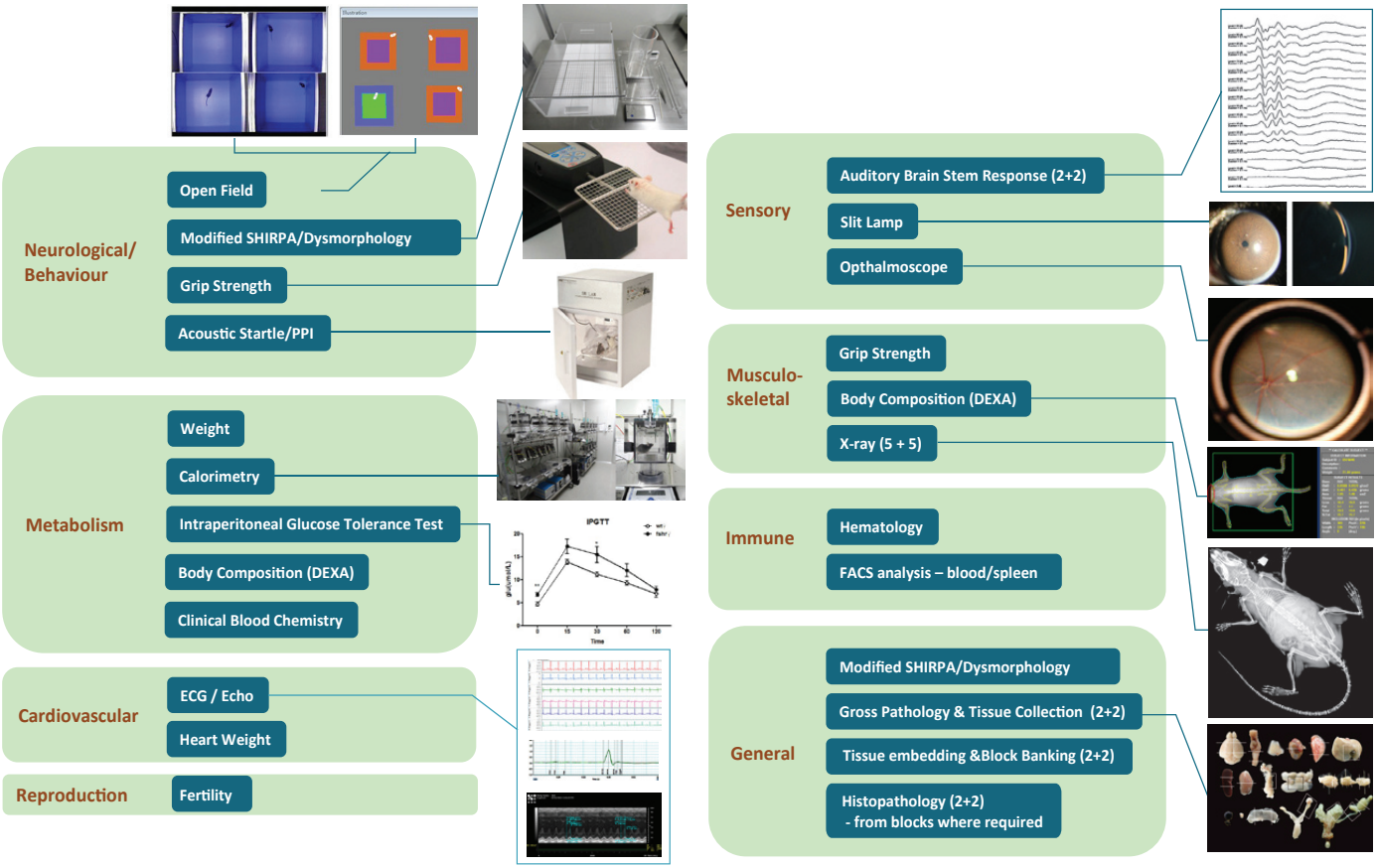


4.International standardization phenotypic analysis platform

NRCMM has set up a high-qualified standardized phenotype analysis platform which is the unique member of the International Mouse Phenotyping Consortium (IMPC) in China. This year we have established 107 knockout mouse strains based on ES cell targeted clones and 143 strains by Crisp-Cas9 technology. Up till now, our IMPC phenotyping platform has completed the core analysis pipeline on more than 80 knockout strains. And the sets of data were uploaded to the IMPC database which will be shared to every science researcher.

Our services include:

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



Oncology Platform---- Xenograft Models and Services

Basic Study Design

- Standardized Cell line
- High Cell Viability
- SOP

Powerful Mice models

- Nude
- Nod-scid
- NCG

Experimental Methods

- Caliper measurements
- Blood hematology
- Clinical chemistry
- Histological services

Results and Analysis

- Tumor growth inhibition (TGI)
- Increased life span (ILS)
- Combination approaches

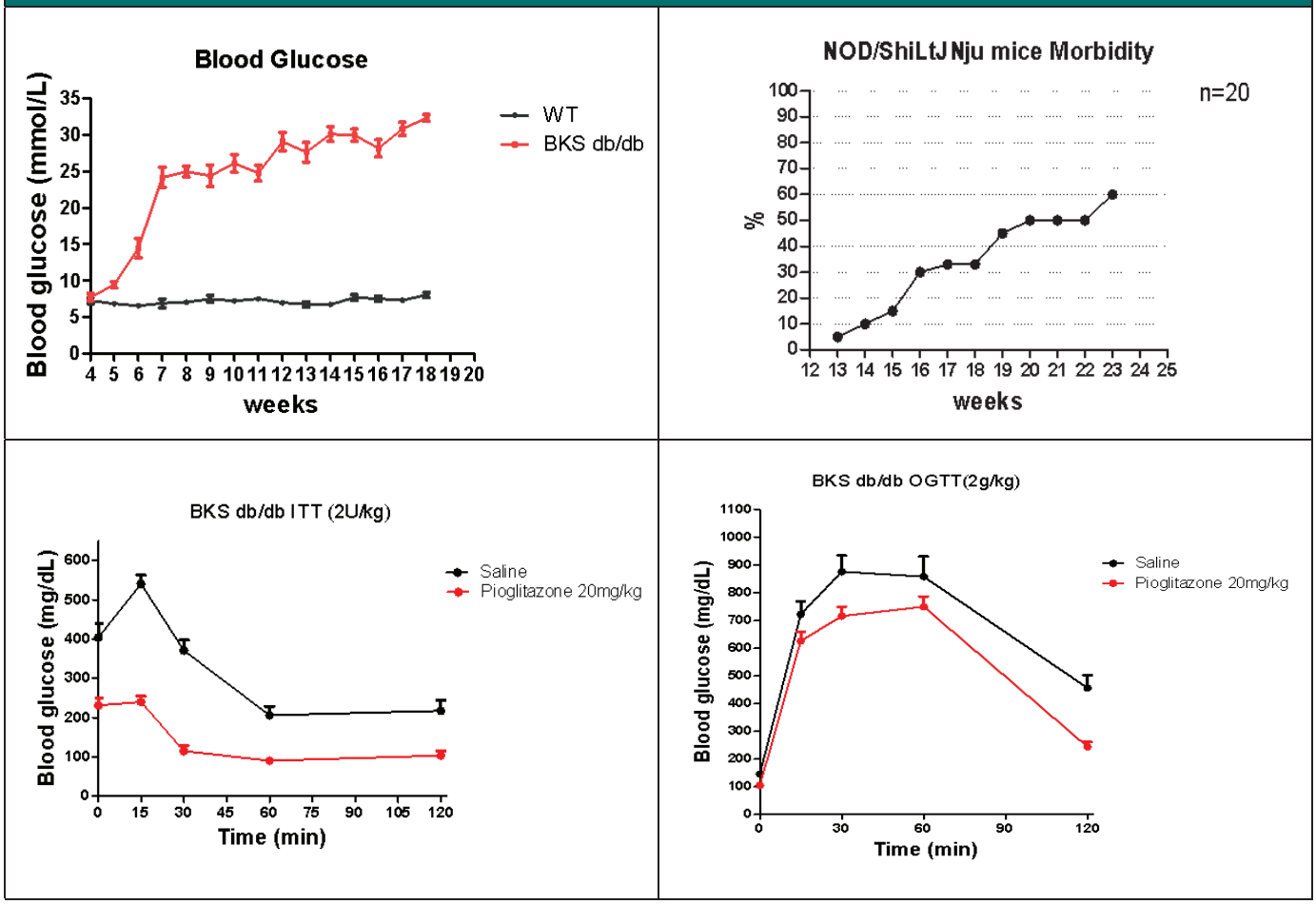
Advantages of Xenografts Models

- Fast
- Models can be reproduced easily
- Considered adequate as a preclinical test of anti-cancer drugs
- Provide visual evidence that mice really do have the tumors prior to the administration of test therapies
- Provide a visual method of reviewing tumor reaction or increase over a period of time

Metabolic Platform ---- Metabolic Disease Models and Services

Disease	Model	Species
Diabetes	STZ-induced	Mouse/Rat
	Genetically engineered	Db/db, ob/ob Mouse
	High fat diet-induced obesity	DIO Mouse
Obesity	Genetically engineered	Db/db, ob/ob Mouse
	High fat diet-induced obesity (growing/established)	DIO mouse

Oral Glucose Tolerance Test



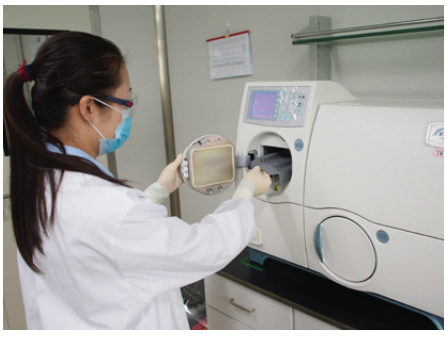
5.laboratory animal pathogen detection and veterinary services

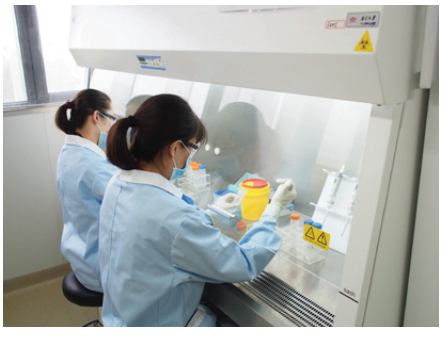
The veterinaries ensure the NBRI's animal welfare and health by supervising all animal health report review and approval program, by assisting in establishment and/or monitoring animal quarantine procedures, by in charge of Health Monitoring Program which include disease detection and surveillance, prevention, diagnosis, treatment,and resolution.

In 2014 we have participated in the ICLAS (International Council for Laboratory Animal Science) Performance Evaluation Program and obtained the certificates, which proved the ability of our Diagnostic Laboratory. And we became to be the ICLAS membership in2015.

This year The Veterinary department introduced a VITEK 2 COMPACT which can identify a wide range of clinical common bacteria with shorter detection time.

Veterinary services include serological, microbiological, parasitological testing services, as well as facility inspection program and training.





6.Agency Services

We have a high-quality service team, with expertise and technical capability, which has successfully imported mice more than 100 times from USA, Britain, Germany, France, Japan, Netherlands, Austria, Australia, Hongkong and other countries, at the same time, exported ells, embryos, sperm, tissues etc.

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

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

The Jackson Laboratory

Taconic

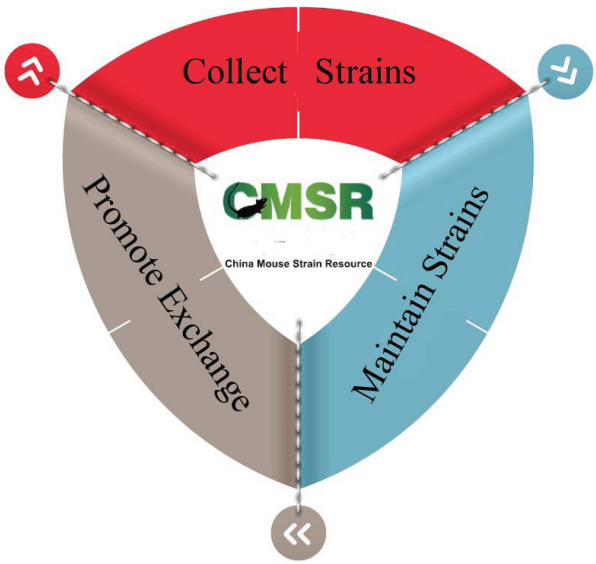
MMRRC
Mutant Mouse Regional Resource Centers supported by NIH

NATIONAL CANCER INSTITUTE

UCDAVIS
KOMP Repository
KNOCKOUT MOUSE PROJECT

Bioresources of RIKEN BRC

Resource Sharing Alliance



Http://CMSR.NRCMM.CN

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

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

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

The first sharing alliance meeting was held on Jun 18th, 2014. At this meeting, the members reached an agreement on the alliance statutes, got the meeting resolutions, and elected MARC (NRCMM) to be unit director, the experimental animal center of Tsinghua University to be vice unit director, Pro. Xiang Gao to be the first chairman. In the past one year , 18 researchers had shared 85 kinds of strains to CMSR and NRCMM.

Publications in 2015

1	Zhang Y, Wang Y, Zhang C, Wang J, Pan D, Liu J, Feng F (2015) Targeted Gene Delivery to Macrophages by Biodegradable Star-Shaped Polymers. ACS Appl Mater Interfaces.
2	Xian L, Hou S, Huang Z, Tang A, Shi P, Wang Q, Song A, Jiang S, Lin Z, Guo S, Gao X (2015) Liver-specific deletion of Ppp2c alpha enhances glucose metabolism and insulin sensitivity. Aging-Us 7: 223-232.
3	Shen N, Jiang S, Lu J-M, Yu X, Lai S-S, Zhang J-Z, Zhang J-L, Tao W-W, Wang X-X, Xu N, Xue B, Li C-J (2015) The Constitutive Activation of Egr-1/C/EBPa Mediates the Development of Type 2 Diabetes Mellitus by Enhancing Hepatic Gluconeogenesis. American Journal of Pathology 185: 513-523.
4	Cai D, Yin S, Yang J, Jiang Q, Cao W (2015) Histone deacetylase inhibition activates Nrf2 and protects against osteoarthritis. Arthritis Research & Therapy 17: 269.
5	Wang X, Jiang C, Fu B, Zhu R, Diao F, Xu N, Chen Z, Tao W, Li C-J (2015) MILI, a PIWI family protein, inhibits melanoma cell methylation of LINE1. Biochemical and Biophysical Research Communications 457: 514-519.
6	Shi P, Tang A, Xian L, Hou S, Zou D, Lv Y, Huang Z, Wang Q, Song A, Lin Z, Gao X (2015) Loss of conserved Gsdma3 self-regulation causes autophagy and cell death. Biochemical Journal 468: 325-336.
7	Hou H, Zheng K, Wang G, Ikegawa S, Zheng M, Gao X, Qin J, Teng H, Jiang Q (2015) Influence of Intra-Articular Administration of Trichostatin A on Autologous Osteochondral Transplantation in a Rabbit Model. Biomed Research International: 470934.
8	Shi D, Xu X, Guo A, Dai J, Xu Z, Chen D, Jiang Q (2015) Bone Cement Solidifiliation Influence the Limb Alignment and Gap Balance during TKA. Biomed Research International: 109402.
9	Rong Z, Xu Z, Sun Y, Yao Y, Song K, Chen D, Shi D, Dai J, Zheng M, Jiang Q (2015) Deep venous thrombosis in the nonoperated leg after primary major lower extremity arthroplasty: a retrospective study based on diagnosis using venography. Blood Coagulation & Fibrinolysis 26: 762-766.
10	Xu Z, Li L, Shi D, Chen D, Dai J, Yao Y, Teng H, Jiang Q (2015) The level of red cell distribution width cannot identify deep vein thrombosis in patients undergoing total joint arthroplasty. Blood Coagulation & Fibrinolysis 26: 298-301.
11	Lou X, Burrows JT, Scott IC (2015) Med14 cooperates with brg1 in the differentiation of skeletogenic neural crest. BMC Dev Biol 15: 41.
12	Zong W, Liu S, Wang X, Zhang J, Zhang T, Liu Z, Wang D, Zhang A, Zhu M, Gao J (2015) Trio gene is required for mouse learning ability. Brain Research 1608: 82-90.
13	Chen L, Zhang GX, Zhou Y, Zhang CX, Xie YY, Xiang C, He XY, Zhang Q, Liu G (2015) BAC transgenic mice provide evidence that p53 expression is highly regulated in vivo. Cell death & disease 6: e1878.
14	Xu J, Wan P, Wang M, Zhang J, Gao X, Hu B, Han J, Chen L, Sun K, Wu J, Wu X, Huang X, Chen J (2015) AIP1-mediated actin disassembly is required for postnatal germ cell migration and spermatogonial stem cell niche establishment. Cell death & disease 6: e1818.
15	Zhao X, Fu J, Xu A, Yu L, Zhu J, Dai R, Su B, Luo T, Li N, Qin W, Wang B, Jiang J, Li S, Chen Y, Wang H (2015) Gankyrin drives malignant transformation of chronic liver damage-mediated fibrosis via the Rac1/JNK pathway. Cell death & disease 6: e1751.
16	He XY, Xiang C, Zhang CX, Xie YY, Chen L, Zhang GX, Lu Y, Liu G (2015) p53 in the Myeloid Lineage Modulates an Inflammatory Microenvironment Limiting Initiation and Invasion of Intestinal Tumors. Cell Rep 13: 888-897.
17	Nie J, Jiang M, Zhang X, Tang H, Jin H, Huang X, Yuan B, Zhang C, Lai JC, Nagamine Y, Pan D, Wang W, Yang Z (2015) Post-transcriptional Regulation of Nkx2-5 by RHAU in Heart Development. Cell reports 13: 723-732.
18	Chen Y, Cui Y, Shen B, Niu Y, Zhao X, Wang L, Wang J, Li W, Zhou Q, Ji W, Sha J, Huang X (2015) Germline acquisition of Cas9/RNA-mediated gene modifications in monkeys. Cell Research 25: 262-265.
19	Du Y, Meng Q, Zhang J, Sun M, Shen B, Jiang H, Kang N, Gao J, Huang X, Liu J (2015) Functional annotation of cis-regulatory elements in human cells by dCas9/sgRNA. Cell Research 25: 877-880.
20	Luo W, Zhao X, Jin H, Tao L, Zhu J, Wang H, Hemmings BA, Yang Z (2015) Akt1 signaling coordinates BMP signaling and beta-catenin activity to regulate second heart field progenitor development. Development 142: 732-742.
21	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 beta-Catenin. Developmental Cell 33: 660-674.
22	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. Developmental Cell 34: 58-72.

23	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. Developmental Dynamics 244: 1328-1346.
24	Chen W-B, Lai S-S, Yu D-C, Liu J, Jiang S, Zhao D-D, Ding Y-T, Li C-J, Xue B (2015) GGPPS deficiency aggravates CCl4-induced liver injury by inducing hepatocyte apoptosis. Febs Letters 589: 1119-1126.
25	Xu Z, Dai X, Yao Y, Shi D, Chen D, Dai J, Teng H, Jiang Q (2015) Higher Levels of Serum Triglycerides Were Associated With Postoperative Deep Vein Thrombosis After Total Hip Arthroplasty in Patients With Nontraumatic Osteonecrosis of the Femoral Head. Int J Low Extrem Wounds.
26	Lai S, Yuan J, Zhao D, Shen N, Chen W, Ding Y, Yu D, Lie J, Pan F, Zhu M, Li C, Xue B (2015) Regulation of mice liver regeneration by early growth response-1 through the GGPPS/RAS/MAPK pathway. International Journal of Biochemistry & Cell Biology 64: 147-154.
27	Li M, Quan C, Toth R, Campbell DG, MacKintosh C, Wang HY, Chen S (2015) Fasting and systemic insulin signaling regulate phosphorylation of brain proteins that modulate cell morphology and link to neurological disorders. J Biol Chem.
28	Lai SS, Zhao DD, Cao P, Lu K, Luo OY, Chen WB, Liu J, Jiang EZ, Yu ZH, Lee G, Li J, Yu DC, Xu XJ, Zhu MS, Gao X, Li CJ, Xue B (2015) PP2Acalpha Positively Regulates Mice Liver Regeneration Termination through AKT/GSK3beta/Cyclin D1 Pathway. J Hepatol.
29	Jiang S, Shen D, Jia WJ, Han X, Shen N, Tao W, Gao X, Xue B, Li CJ (2015) GGPPS mediated Rab27A geranylgeranylation regulates beta-cell dysfunction during type 2 diabetes development via affecting insulin granule docked pool formation. J Pathol.
30	Li J, Yue Y, Dong X, Jia W, Li K, Liang D, Dong Z, Wang X, Nan X, Zhang Q, Zhao Q (2015) Zebrafish foxc1a Plays a Crucial Role in Early Somitogenesis by Restricting the Expression of aldh1a2 Directly. Journal of Biological Chemistry 290: 10216-10228.
31	Tao W, Wu J, Xie B-X, Zhao Y-Y, Shen N, Jiang S, Wang X-X, Xu N, Jiang C, Chen S, Gao X, Xue B, Li C-J (2015) Lipid-induced Muscle Insulin Resistance Is Mediated by GGPPS via Modulation of the RhoA/Rho Kinase Signaling Pathway. Journal of Biological Chemistry 290: 20086-20097.
32	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. Journal of Biological Chemistry 290: 20273-20283.
33	Yu P, Zhang Y, Li C, Li Y, Jiang S, Zhang X, Ding Z, Tu F, Wu J, Gao X, Li L (2015) Class III PI3K-mediated prolonged activation of autophagy plays a critical role in the transition of cardiac hypertrophy to heart failure. Journal of Cellular and Molecular Medicine 19: 1710-1719.
34	Xu N, Guan S, Chen Z, Yu Y, Xie J, Pan F-Y, Zhao N-W, Liu L, Yang Z-Z, Gao X, Xu B, Li C-J (2015) The alteration of protein prenylation induces cardiomyocyte hypertrophy through Rheb-mTORC1 signalling and leads to chronic heart failure. Journal of Pathology 235: 672-685.
35	Chen C-P, Chen X, Qiao Y-N, Wang P, He W-Q, Zhang C-H, Zhao W, Gao Y-Q, Chen C, Tao T, Sun J, Wang Y, Gao N, Kamm KE, Stull JT, Zhu M-S (2015) In vivo roles for myosin phosphatase targeting subunit-1 phosphorylation sites T694 and T852 in bladder smooth muscle contraction. Journal of Physiology-London 593: 681-700.
36	Qin J, Liang H, Shi D, Dai J, Xu Z, Chen D, Chen X, Jiang Q (2015) A panel of microRNAs as a new biomarkers for the detection of deep vein thrombosis. Journal of Thrombosis and Thrombolysis 39: 215-221.
37	An J, Ren S, Murphy SJ, Dalangood S, Chang C, Pang X, Cui Y, Wang L, Pan Y, Zhang X, Zhu Y, Wang C, Halling GC, Cheng L, Sukov WR, Karnes RJ, Vasmatzis G, Zhang Q, Zhang J, Cheville JC, Yan J, Sun Y, Huang H (2015) Truncated ERG Oncoproteins from TMPRSS2-ERG Fusions Are Resistant to SPOP-Mediated Proteasome Degradation. Mol Cell 59: 904-916.
38	Chu C-H, Chen S-H, Wang Q, Langenbach R, Li H, Zeldin D, Chen S-L, Wang S, Gao H, Lu R-B, Hong J-S (2015) PGE(2) Inhibits IL-10 Production via EP2-Mediated beta-Arrestin Signaling in Neuroinflammatory Condition. Molecular Neurobiology 52: 587-600.
39	Yang J, Lai B, Xu A, Liu Y, Li X, Zhao Y, Li W, Ji M, Hu G, Gao X, Gao J (2015) Maged1 Co-interacting with CREB Through a Hexapeptide Repeat Domain Regulates Learning and Memory in Mice. Molecular Neurobiology 51: 8-18.
40	Wang L, Cheng S, Yin Z, Xu C, Lu S, Hou J, Yu T, Zhu X, Zou X, Peng Y, Xu Y, Yang Z, Chen G (2015) Conditional inactivation of Akt three isoforms causes tau hyperphosphorylation in the brain. Molecular Neurodegeneration 10: 33.
41	Zhang Q, Zhao W, Ye C, Zhuang J, Chang C, Li Y, Huang X, Shen L, Cui Y, Song J, Shen B, Eliaz I, Huang R, Ying H, Guo H, Yan J (2015) Honokiol inhibits bladder tumor growth by suppressing EZH2/miR-143 axis. Oncotarget.
42	Quan C, Xie B, Wang HY, Chen S (2015) PKB-Mediated Thr(649) Phosphorylation of AS160/TBC1D4 Regulates the R-Wave Amplitude in the Heart. Plos One 10: e0124491.
43	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 (2015) A Common Variant Of Ubiquinol-Cytochrome c Reductase Complex Is Associated with DDH. Plos One 10: e0120212.
44	Ye C, Zhao W, Li M, Zhuang J, Yan X, Lu Q, Chang C, Huang X, Zhou J, Xie B, Zhang Z, Yao X, Yan J, Guo H (2015) delta-Tocotrienol Induces Human Bladder Cancer Cell Growth Arrest, Apoptosis and Chemosensitization through Inhibition of STAT3 Pathway. Plos One 10: e0122712.

45	Gao Y-Q, Chen X, Wang P, Lu L, Zhao W, Chen C, Chen C-P, Tao T, Sun J, Zheng Y-Y, Du J, Li C-J, Gan Z-J, Gao X, Chen H-Q, Zhu M-S (2015) Regulation of DLK1 by the maternally expressed miR-379/miR-544 cluster may underlie callipyge polar overdominance inheritance. Proceedings of the National Academy of Sciences of the United States of America 112: 13627-13632.
46	Cao Y (2015) Germ layer formation during Xenopus embryogenesis: the balance between pluripotency and differentiation. Science China-Life Sciences 58: 336-342.
47	Gao X (2015) Model animals and their applications. Science China-Life Sciences 58: 319-320.
48	Lan J, Zhang X, Chen D (2015) Molecular mechanisms of dietary restriction in aging-insights from Caenorhabditis elegans research. Science China-Life Sciences 58: 352-358.
49	Liu J, Liang X, Gan Z (2015) Transcriptional regulatory circuits controlling muscle fiber type switching. Science China-Life Sciences 58: 321-327.
50	Luo J, Zuo J, Wu J, Wan P, Kang D, Xiang C, Zhu H, Chen J (2015) In vivo RNAi screen identifies candidate signaling genes required for collective cell migration in Drosophila ovary. Science China-Life Sciences 58: 379-389.
51	Qi X, Gao X (2015) Towards a better understanding of mouse and human diseases-International Mouse Phenotyping Consortium. Science China-Life Sciences 58: 392-395.
52	Qu Z, Wang X, Liu D, Gao X, Xu Y (2015) Inactivation of Cipc alters the expression of Per1 but not circadian rhythms in mice. Science China-Life Sciences 58: 368-372.
53	Sun Y, Mao P, Lu J, Dai J, Teng H, Jiang Q (2015) ABO blood type and ABO gene with susceptibility to deep vein thrombosis following orthopedic surgery: a case-control study in Chinese Han population. Science China-Life Sciences 58: 390-391.
54	Tao T, Chen C, Sun J, Peng Y, Zhu M (2015) A bacterial artificial chromosome transgenic mouse model for visualization of neurite growth. Science China-Life Sciences 58: 373-378.
55	Xu N, Shen N, Wang X, Jiang S, Xue B, Li C (2015) Protein prenylation and human diseases: a balance of protein farnesylation and geranylgeranylation. Science China-Life Sciences 58: 328-335.
56	Zou J, Xiong X, Lai B, Sun M, Tu X, Gao X (2015) Glucose metabolic abnormality is associated with defective mineral homeostasis in skeletal disorder mouse model. Science China-Life Sciences 58: 359-367.
57	Cheng S, Hou J, Zhang C, Xu C, Wang L, Zou X, Yu H, Shi Y, Yin Z, Chen G (2015) Minocycline reduces neuroinflammation but does not ameliorate neuron loss in a mouse model of neurodegeneration. Scientific Reports 5: 10535.
58	Ding S, Qian SY, Zhang Y, Wu W, Lu G, Lu Y, Feng X, Li L, Shen P (2015) Establishment of immunoassay for detecting HPV16 E6 and E7 RNA. Scientific Reports 5: 13686.
59	Li Y, Zhang D, Wang X, Yao X, Ye C, Zhang S, Wang H, Chang C, Xia H, Wang Y-c, Fang J, Yan J, Ying H (2015) Hypoxia-inducible miR-182 enhances HIF1 alpha signaling via targeting PHD2 and FIH1 in prostate cancer. Scientific Reports 5: 12495.
60	Tao W, Wu J, Zhang Q, Lai S-S, Jiang S, Jiang C, Xu Y, Xue B, Du J, Li C-J (2015) EGR1 regulates hepatic clock gene amplitude by activating Per1 transcription. Scientific Reports 5: 15212.
61	Wang Y, Du Y, Shen B, Zhou X, Li J, Liu Y, Wang J, Zhou J, Hu B, Kang N, Gao J, Yu L, Huang X, Wei H (2015) Efficient generation of gene-modified pigs via injection of zygote with Cas9/sgRNA. Scientific Reports 5: 8256.
62	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. Scientific Reports 5: 12709.
63	Zhuang J, Lu Q, Shen B, Huang X, Shen L, Zheng X, Huang R, Yan J, Guo H (2015) TGF beta 1 secreted by cancer-associated fibroblasts induces epithelial-mesenchymal transition of bladder cancer cells through lncRNA-ZEB2NAT. Scientific Reports 5: 11924.
64	Sun Y, Mao P, Lu J, Li L, Lu W, Jiang Q, Teng H (2015) Localized lower extremity ischemic preconditioning prevents against local thrombus formation. Vasa-European Journal of Vascular Medicine 44: 285-288.

Seminar

	Date	Speaker	Title	Unit
1	2015/1/6	Qin Ma Ph.D.	Computational Methods in Bioinformatics	University of Georgia
2	2015/1/6	Chao Xie M.D.	The role of intravenous transplanted human mesenchymal stem cell during bone repair and its molecular imaging evaluation	University of Rochester School of Medicine
3	2015-03-24	Xiaowei Chen Ph.D.	The Secretory Pathway in Metabolic Control: A Tale of the COPII Complex	Pecking University
4	2015-03-26	Zhengfan Jiang Ph.D.	Natural immunity and cell signal transduction	Beijing University
5	2015-04-16	Peng Li Ph.D.	Obesity an adipocyte biology	Tsinghua University
6	2015-04-22	Eric N.Olson Ph.D.	Mechanisems of muscle development, disease and regeneration.	UT Southwestern Medical Center
7	2015-04-28	Tim Hunt Ph.D.	Getting in and out of mitosis	The Francis Crick Institute, UK
8	2015-04-30	Zhiheng Xu Ph.D.	JNKsignaling pathway regulation and related diseases	Heritage and Development Institute
9	2015-05-04	Yi Sun Ph.D.	Anti-lung tumorigenesis by targeting Sag/Rbx2 E3 ubiquitin ligase: Translational Application	Zhejiang University
10	2015-05-06	Ming-hui Zou Ph.D.	Blooming of the French Liliac: Mechanisms and Therapeutics of Metformin	Oklahoma University
11	2015-05-06	Randy Levinson Ph.D.	Navigating the Publishing Process at High-Impact Journals	Nature Medicine
12	2015-05-06	Kai Ge PhD	Epigenetic Regulation of PPARγ and Adipogenesis by Histone Methylation	National Institutes of Health
13	2015-05-08	Jingjing Zhang Ph.D.	Modulation of zebrafish tissue barrier by Clostridium perfringens enterotoxin fragments	Affiliated hospital of Guangdong Medical College
14	2015-05-15	Aibin He Ph.D.	Epigenetic insight into heart development and diseases	Peking University
15	2015-06-11	Yongqing Zhang Ph.D.	Synapse development, amentia and infantile autism	Institute of Genetics and Developmental Biology,CAS
16	2015-06-12	Guoqiang Wan Ph.D.	Regeneration of cochlear cells and synapses for hearing restoration	Kresge Hearing Research Institute University of Michigan
17	2015-06-16	Jian Ding Ph.D.	Abrogation of Trbp reveals a novel linear miRNA-mediated regulatory pathway in the heart	Department of Cardiology, Boston Children's Hospital, Harvard Medical School
18	2015-06-25	Wei Liu Ph.D.	The molecular mechanism and function of cell autophagy	Zhejiang University
19	2015-06-25	Mei Xin Ph.D.	Hippo signaling in heart development and repair	University of Cincinnati
20	2015-08-11	Ren Xu Ph.D.	Extracellular matrix, a network in and out of the cell	University of Kentucky School of Medicine
21	2015-08-13	Zhen Zhu Ph.D.	Microfluidics and Electrical Impedance Spectroscopy for Single-Cell Study	Southeast University
22	2015-11-16	Daniel P. Kelly, M.D.	Deciphering the Metabolic Origins of Heart Failure	Sanford-Burnham Medical Research Institute
23	2015-12-08	Deqiang Li Ph.D.	The business of cardiomyocyte: commitment, differentiation and proliferation	University of Pennsylvania

Courses and Teachers

The MARC, as an institute of the University of Nanjing, is home to approximately 170 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 2015, the following lecture series and courses were given by MARC staff at Nanjing University (unless indicated otherwise).

Bioinformatics

Mingshen ZHU

Cell Biology and Molecular Biology

Shuai CHEN

Di CHEN

Chaojun LI

Cell signaling

Geng LIU

Jianghuai LIU

Jun YAN

Chaojun LI

Zhongzhou YANG

Genetics

Qing ZHANG

Jinzhong QIN

Di CHEN

Xin LOU

Doctoral qualification exam I&II

All PI in MARC

Frontier of Cell Biology

Zhengfan JIANG (Beijing University)

Yongqing ZHANG (Institute of Genetics and Developmental Biology, CAS)

Wei LIU (Zhejiang University)

Peng LI (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

The Fourth RIKEN BRC/Nanjing University MARC International Summer Intensive Course of the Mouse

Xiang GAO

Ying XU

Shuai CHEN

Zhongzhou YANG

Jing ZHAO

PhD Theses

MARC students successfully defended the following PhD theses in 2015

PhD Theses:

Group Xiang Gao

An Tang

PP2Acs are essential for the completion of meiosis I in mouse oocyte

Xin Tu

Functional Analysis of PP2A in Cardiac Hypertrophy and Glucose Homeostasis

Qinghua Wang

Disruption of Maged1 enhance adiposity via preadipocyte proliferation and differentiation

Anying Song

H3K27 demethylase, JMJD3 controls female reproduction and energy homeostasis by regulating kisspeptin signaling in hypothalamus

Group Minsheng Zhu

Yunqian Gao

Maternal expression of miRNA 379 cluster mediates polar overdominance of callipyge/mir-379

Xin Chen

Molecular Mechanism of Muscle Growth of Broiler Chicken: A Role of miR-199-5p in Regulating Differentiation of Skeletal Myoblasts

Pei Wang

TMEM16A Participates in Inflammatory Agonists mediated Airway Smooth Muscle Contractility and Airway Hyperresponsiveness in Asthma

Wei Zhao

Myogenic Pathogenesis of Hirschsprung Disease

Group Zhongzhou Yang

Baiyin Yuan

WDR1-Mediated Actin Dynamics Plays Essential Roles in Mouse Myocardial Growth, Maintenance and Second Heart Field Progenitor Cells Development

Group Chaojun Li

Weiwei Tao

Egr-1 regulates skeletal muscle insulin signaling pathway

Shan Jiang

Study of GGPPS Function in Non-alcoholic Fatty Liver

Fan Diao

The function of protein prenylation in spermatogonial stem cell self-renewal and differentiation balance regulation

Group Qingshun Zhao

Wenshuang Jia

The role of miR-210-5p in zebrafish primitive myelopoiesis

Xiaohua Dong

Zebrafish znfl1s play essential roles in patterning hindbrain anterior-posterior axis and embryonic left-right asymmetry

Group Geng Liu

Guoxin Zhang

Study of p53 gene promoter activity and p53-mediated cell competition during mouse embryogenesis

Group Jianghuai Liu

Hui Jiang

Type I interferon activates PFKFB3-driven glycolysis to promote bystander anti-viral effects in macrophages

Group Jiong Chen

Di Kang

Beyond Metabolic Regulation: Roles of Insulin Signaling Pathway in Cell Fate Determination in Border Cells of Drosophila Ovary

Group Hongyang Wang

Xiaofang Zhao

Activity and mechanism of Gankyrin in CCl4 induced hepatic fibrosis and hepatocarcinogenesis

Group Ying XU

Zhen Dong

Loss of HBP1,a key regulator of mitochondrial function, preserves follicle reserve in mammalian ovary

Group Ying Cao

Lei Lu

Kdm2a/b regulate canonical Wnt signaling via mediating the stability of nuclear β-Catenin

Yan Gao

The lysine demethylases Kdm2a/Kdm2b regulate Xenopus body axis patterning via modulating the stability of nuclear β-Catenin

Xuena Zhang

Jmjd6 regulates the function of the transcriptional repressor Tcf7l1 and is required for germ-layer differentiation and body axis patterning during Xenopus embryogenesis

Group Qing Zhang

Zizhang Zhou

Deubiquitination of Ci/Gli by Usp7/HAUSP regulates Hedgehog signaling

Group Xingxu Huang

Yinan Du

Using CRISPR/Cas9 System for Gene-Modified Pigs Generation and Cis-elements Annotation in Human Cells

2015 Annual Conference of MARC

The 2015 MARC Annual Conference was held in Nantong University from November 4th to 6th .This conference was organized by Dr. Shuai Chen and Dr. Jun Yan. More than 180 scientists and students from Nanjing University, Nantong University, Shandong University and Suzhou University attended the conference.

Dr. Jun Yan, one of the co-organizers 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 74 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, Zizhang Zhou from Dr. Qing Zhang's laboratory, was awarded the 2015 Student of MARC for his excellent research. Shan Jiang from Dr. Chaojun Li's laboratory and Lei Lu from Dr. Ying Cao's laboratory were nominated. In addition, ten students received 2015 Outstanding Poster Prize.

2015 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 6th Summer Camp from July 13–17 This summer. 47 excellent undergraduates were selected from a pool of 218 applicants from 15 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, Zebrafish, and Caenorhabditis elegans.

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, Peiliang Shi and Qi Xiao, 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.

The fourth RIKEN BRC/NANJING UNIVERSITY MARC MOUSE RESOURCE WORKSHOP

Model Animal Research Center (MARC), Nanjing University, China and RIKEN BioResource Center (BRC), Japan have been co-organizing a short educational course focusing on mouse genetics and related experimental technologies for young scientists. The 1st course was held from August 27 to 29, 2012 in Tsukuba , the 2nd from July 29 to 31, 2013 in Nanjing and the 3rd course from July 28 to 30 in Tsukuba . This year, MARC took its turn again and hosted the 4th course from July 27(Mon) to 29 (Wed) in Nanjing University.

Periods: From 9:00 Monday June 27, 2015 to 16:00 Wednesday June 29, 2015

Place: MARC, Nanjing University

Students: 93 students from China, Japan and India

Speakers: 19 lectures from China and Japan. Academy Heping Cheng, Prof. Weinian Shou, Prof. Bin Zhou, and six from Nanjing University,
Fudan University and Suzhou University in China, and 10 from RIKEN BRC including Dr. Yuichi Obata (Director)

Contents: Lectures and experimental training courses

Co-organizers: Dr. Xiang Gao (Founding Director of MARC) Dr. Yuichi Obata (Director of RIKEN BRC)

Themes of Lectures:

1. History and Basic Principles of Mouse Genetics
2. Management and Quality Control of Mouse Husbandry and Facilities
3. Standardization of Mouse Phenotyping
4. Technologies in Mouse Research
5. Development and Embryology in the Mouse
6. Mutant Mice and Rats as Models for Human

Diseases(Cardiovascular, Metabolic, Circadian, Tumor and Neurodegeneration)

During July 27 to 28, 19 speakers gave lectures focusing on the themes.

During the sessions for open discussion, participants actively asked questions to the lecturers and extensively engaged in discussions. At last, Dr. Gao, Founding Director of Nanjing University MARC and Dr. Yuichi Obata, Director of RIKEN BRC awarded certificates to participants.

Experimental training courses:

On July 29, the experimental courses arranged five courses: Pathology, Animal Health and Veterinary Care, Sperm Cryopreservation, Microinjection and SHIRPA, and the students all devoted to the courses and benefited a lot.

Looking forward, The Fifth RIKEN BRC-Nanjing University MARC Mouse Resource Workshop 2016 will take place in Tsukuba in Japan focusing on Biological Imaging of Disease Models.

2015 Developmental Biology Conference

In order to promote basic research on developmental biology in China , MARC and Chinese Society for Cell Biology(CSCB) organized the 2015 Developmental Biology Conference which was held in Nanjing from September 23th-26th. Among 60 participants are professors from such as Nanjing University , Tsinghua University, Tongji University, Zhejiang University, Suzhou University, Xiamen University, Beijing Normal University, Sichuan University, PIs form such as CAS, staff of National Natural Science Foundation of China and administrative staff.

Dr. Xiang Gao, chairman of the conference, presided at the opening ceremony, then gave a summary talk about the status of developmental research in China. Dr. Naihe Jing , investigator of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences(CAS), gave the first talk titled 3D transcriptome of early mouse embryo.

31 talks from domestic scientists, were divided into five session: Genetics and Genomics of Development, Panel discussion, Development and Diseases, Stem Cells and Regeneration, Organogenesis and Signal Transduction in Development.These sessions were chaired by Dr. Lijian Hui(investigator at SIBS, CAS), Dr. Xiang Gao(Nanjing University), Dr.Steven Y. Cheng(Nanjing Medical University),Dr. Hengyu Fan(Zhejiang University), Dr. Chaojun Li(Nanjing University). The speaker presented latest research progress and scientific ideas from their laboratories, which were followed by heated discussion between the speaker and the audience.

2015 Students Union

The student activities are always rich and colorful in MARC. Beside studying and researching, the 150 MARC students may also found themselves cultivated by a culture promoting humanity, critical thinking, and social well-being here. Thanks to the generous financial support guaranteed by both MARC and the government, we have adequate resources to enrich our lives here.

In the year of 2015, the annual badminton and table tennis game came at the appointed time in May and December this year, respectively, and being attractive as usually. Moreover, our basketball team played the basketball game of Pukou New District in August. Sport activities of these will not only spice up our life, but 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, including Nobel Laureate Tim Hunt, have given lectures here. And more speakers from non-science background, such as experts of literature, arts and social science were invited as well.

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