ANNUAL REPORT

2018

MODEL ANIMAL RESEARCH CENTER OF NANJING UNIVERSITY

MOE KEY LABORATORY OF MODEL ANIMAL FOR DISEASE STUDY

NATIONAL RESOURCE CENTER FOR MUTANT MICE

Director's Words

Since established in 2002, MARC has been devoted to biomedical research using animal models to make discoveries for a healthy life. Over the last decade, we've become stronger in developing genetically-modified animals for modeling human diseases. Through introducing state-of-art genome editing technologies, we not only accelerate generation of genetically-modified animals, but also start to develop more precise and complex models for studying human diseases. Besides this core platform for animal models, we've also expanded other facilities at MARC, including the imaging core, metabolomics core, and flow cytometry core. With these state-of-art core facilities, MARC scientists have tackled some longstanding scientific questions, and made several important discoveries this year. In a study published in Developmental Cell, a joint team led by Dr. Jiong Chen and Dr. Shuai Chen discovered a molecular mechanism coordinating cell growth and fate determination. In two other studies published in Cell Reports, Dr. Jinzhong Qin's group deciphered a new mechanism for PRC1.6 function in stem cell self-renew and pluripotency, and Dr. Zhenji Gan's team revealed a critical link between the autophagy receptor FUNDC1 and metabolic disorders, respectively. Over years, we've been expanding research fields, from genetics and developmental biology to cancer biology, metabolic biology and neurobiology. This year, we initiated a new research program focusing on regenerative biology, and recruited two new Principal Investigators Dr. Yan Li and Dr. Yohei Niikura to strengthen this direction. In the coming year, we will continue to pursue first-class science using animal models for improvement of human health.

Communications and collaborations are key to fruitful science. We, together with scientists from RIKEN BioResource Center, held a joint workshop on mouse models in Nanjing to train young scientists as well as to promote collaborations among them. This joint workshop has been held for seven successive years, and already got a very good reputation in the field. In this summer, we also organized the first CSCB Training Course on Mouse Genetics and Phenotyping, which was sponsored by Chinese Society for Cell Biology. Seventeen trainees from five Asian countries participated this training course. Through this training course, we set up collaborations with Chulalongkorn University, Thailand. Besides these training courses, we also organized a forum on Metabolism and Developmental Biology, and invited leading scientists in the field to give lectures at MARC. These events help MARC to improve our research and reputations in the world.

Elected as the director of MARC in 2018, I feel very proud to work with a great team of devoted professors, talented students and brilliant supporting personnel, who make me believe a bright future for MARC. It's a belief of every MARCer, and we are working hard to make it become true!

Shuai Chen
Director

Cover by Zhenji Gan's lab and Xuena Zhang (GE-OMX)
Landscapes by Chaojun Li

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IN DEPTH

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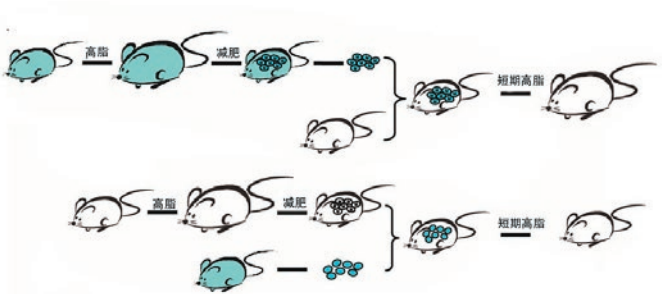
Research Highlight in 2018

Group Xiang Gao

CD4$^+$ T cells memorize obesity and promote weight regain

Jianghuan Zou[1], Beibei Lai[1], Mingzhu Zheng[2], Qin Chen[1], Shujun Jiang[1], Anying Song[1], Zan Huang[1], Peiliang Shi[1], Xin Tu[1], Di Wang[2], Linrong Lu[2], Zhaoyu Lin[1*] and Xiang Gao[1*]

Body weight regain often causes failure of obesity therapies while the underlying mechanism remains largely unknown. In this study, we report that immune cells, especially CD4+ T cells, mediate the 'memory' of previous obese status. In a weight gain-loss-regain model, we found that C57BL/6J mice with an obesity history showed a much faster rate of body weight regain. This obesity memory could last for at least 2 months after previously obese mice were kept at the same body weight as non-obese mice. Surprisingly, such obesity memory was abrogated by dexamethasone treatment, whereas immunodeficient Rag1− / − and H2A− / − mice failed to establish such memory. Rag1− / − mice repossessed the obesity memory when immune cells or CD4+ T cells isolated from previously obese mice were transferred. Furthermore, depletion of CD4+ T cells led to obesity memory ablation. Taken together, we conclude that CD4+ T cells mediate obesity memory and promote weight regain.



Group Yun Shi

Signal peptide represses GluK1 synaptic and surface expression by binding to amino-terminal domain

Gui-Fang Duan, Yaxin Ye, Sha Xu, Wucheng Tao, Shiping Zhao, Tengchuan Jin, Roger A. Nicoll, Yun Stone Shi, Nengyin Sheng

Significance:

Kainate-type glutamate receptors play critical roles in excitatory synaptic transmission and synaptic plasticity in the brain. GluK1 and GluK2 possess fundamentally different capabilities in surface trafficking as well as synaptic targeting in hippocampal CA1 neurons. Through collaboration with Sheng lab at Kunming Institute of Zoology, Shi lab find that the excitatory postsynaptic currents (EPSCs) are significantly increased by the chimeric GluK1(SPGluK2) receptor, in which the signal peptide of GluK1 is replaced with that of GluK2. Coexpression of GluK1 signal peptide completely suppresses the gain in trafficking ability of GluK1(SPGluK2), indicating that the signal peptide represses receptor trafficking in a trans manner. Furthermore, the researchers demonstrate that the signal peptide directly interacts with the amino-terminal domain (ATD) to inhibit the synaptic and surface expression of GluK1. Thus, this study uncover a novel trafficking mechanism for kainate receptors and propose that the cleaved signal peptide behaves as a ligand of GluK1, through binding with the ATD, to repress forward trafficking of the receptor.

Highlight:

GluK1 signal peptide may act as an unconventional ligand of the receptor.

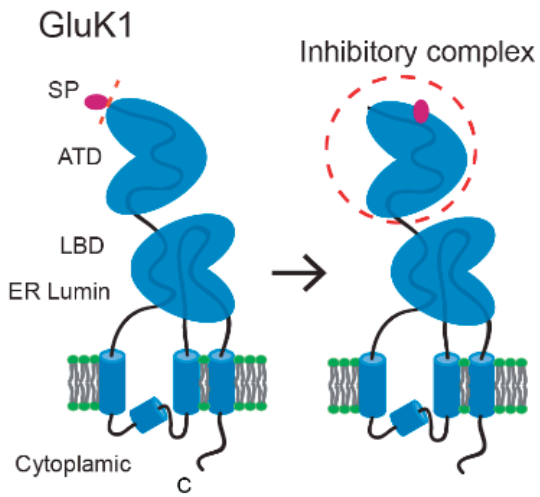


Figure 1. A schematic demonstration of signal peptide repression on GluK1 trafficking.

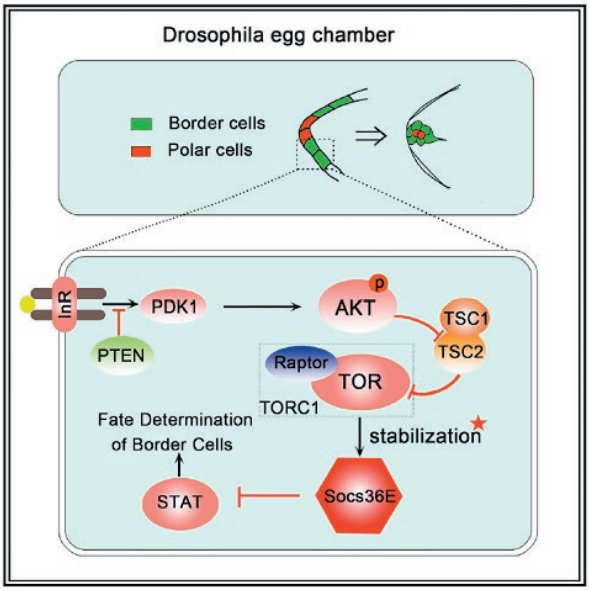
GluK1 signal peptide is excised by signal peptidase in ER, and then binds to the amino-terminal domain to form an inhibitory complex to repress GluK1 surface trafficking and synaptic targeting.

Groups of Jiong Chen, Shuai Chen and Zhongzhou Yang

The InR/Akt/TORC1 Growth-Promoting Signaling Negatively Regulates JAK/STAT Activity and Migratory Cell Fate during Morphogenesis

Di Kang, Dou Wang, Jianbing Xu, Chao Quan, Xuan Guo, Heng Wang, Jun Luo, Zhongzhou Yang, Shuai Chen,* and Jiong Chen*

Cell growth and cell differentiation are two distinct yet coupled developmental processes, but how they are coordinated is not well understood. The groups of Jiong Chen, Shuai Chen and Zhongzhou Yang found that during Drosophila oogenesis the growth-promoting InR/Akt/TOR pathway was involved in suppressing the fate determination of the migratory border cells. The InR/Akt/TOR pathway signals through TOR and Raptor, components of TORC1, to down-regulate the JAK/STAT pathway, which is necessary and sufficient for border cell fate determination. The groups further discovered that TORC1 promotes the protein stability of SOCS36E, a conserved negative regulator of JAK/STAT signaling, through physical interaction, suggesting that TORC1 acts as a key regulator coordinating both cell growth and cell differentiation. Interestingly, their work also suggest that cell growth and cell differentiation are negatively coupled, and such relationship may also occur in other developmental contexts. (from the journal of Developmental Cell, 2018)



Group Jinzhong Qin

Combinatorial Control of Recruitment of a Variant PRC1.6 Complex in Embryonic Stem Cells

Yikai Huang[1*], Wukui Zhao[1*], Congcong Wang[1*],Yaru Zhu[1*], Mengjie Liu[1], Huan Tong[1], Yin Xia[2], Qing Jiang[3], Jinzhong Qin[1]

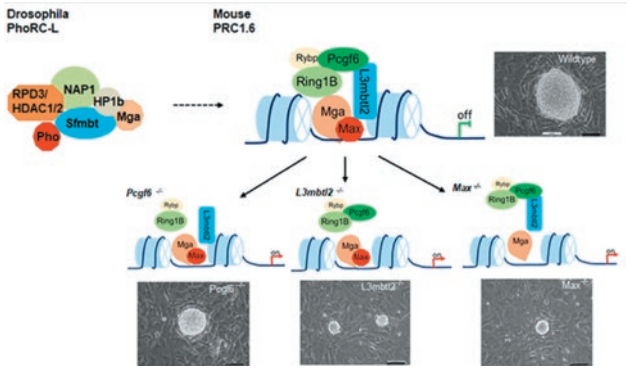
[1]MOE Key Laboratory of Model Animal for Disease Study, Model Animal Research Center, Nanjing Biomedical Research Institute, Nanjing University, Nanjing, China;

[2]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China;

[3]Department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital Affiliated to Medical School of Nanjing University, Nanjing, China

* Co- firstauthor

Despite genetic data suggesting that Polycomb group proteins (PcG) are central epigenetic transcriptional repressors that have been implicated in control of embryonic stem (ES) cell pluripotency, yet the precise mechanism of PcG complex recruitment has remained elusive, especially in mammals. We now report that the first and second MBT repeats of L3mbtl2 are important structural and functional features that are necessary and sufficient for L3mbtl2-mediated recruitment of PRC1.6 complex to targeting promoters. Interestingly, this region of L3mbtl2 harbors the evolutionarily conserved Pho-binding pocket also present in Drosophila Sfmbt and mutation of the critical residues within this pocket completely abolish its interaction with other subunits in the complex as well as target promoters. Additionally, decreased PRC1.6 chromatin occupancy was observed following loss of individual components (L3mbtl2, Pcgf6 and Max) of the complex. Our findings suggest that the recruitment of noncanonical PRC1.6 complex in ES cells might be the result of L3mbtl2's interaction with multiple components of the complex.



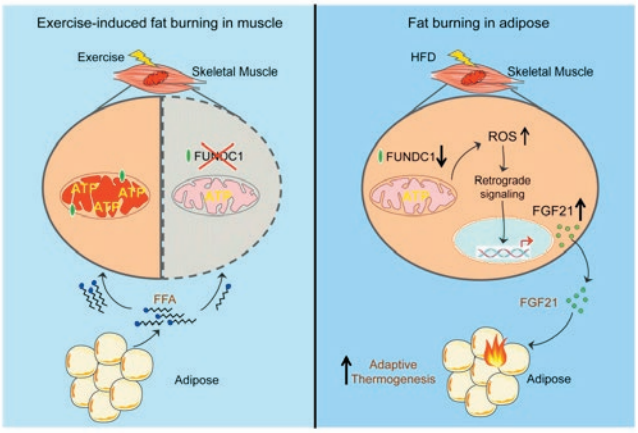
Group Zhenji Gan

Mitophagy directs muscle-adipose dialog to alleviate dietary obesity

Tingting Fu, Zhisheng Xu, Lin Liu, Qiqi Guo, Hao Wu, Xijun Liang, Danxia Zhou, Liwei Xiao, Lei Liu, Yong Liu, Min-Sheng Zhu, Quan Chen and Zhenji Gan

Skeletal muscle fitness is vital for human health and disease and is determined by its capacity for burning fuel, mitochondrial ATP production, and contraction. High quality mitochondria in skeletal muscle is essential for maintaining energy homeostasis in response to a myriad of physiologic or pathophysiological stresses. A sophisticated mitochondrial quality control system including mitochondrial autophagy (mitophagy), dynamics, and proteolysis has been identified, which maintains their functional integrity. Zhenji Gan group uncovered an essential role of mitophagy receptor FUNDC1 in controlling muscle mitochondrial quality as well as metabolic homeostasis. Ablation of FUNDC1 in skeletal muscle resulted in LC3-mediated mitophagy defect, leading to impaired mitochondrial energetics. These mice exhibited many features of deficient muscle fat utilization and endurance during exercise. Interestingly, mice lacking FUNDC1 in muscle were protected against chronic high-fat-diet-induced obesity and insulin resistance despite reduced muscle mitochondrial energetics. Mechanistically, FUNDC1 deficiency elicits a retrograde response in muscle to induce thermogenic remodeling of adipose tissue via FGF21. Together, this study uncovered a FUNDC1-dependent mitophagy signaling in skeletal muscle that communicates with adipose tissue, conferring leanness by promoting exercise-independent fat burning in adipose tissue (from the journal of Cell Reports, 2018).

Graphical Abstract



Student of the Year 2018

Chao Quan

Chao Quan received her Bachelor's degree of Biological Science in 2012 from School of Life Sciences, Hunan Normal University. She joined Dr. Shuai Chen's lab at 2012 to study the roles of insulin signaling in the heart.

In the past few years, her work has focused on investigating how insulin signaling pathway regulates cardiac function using genetic mouse models. She has uncovered novel molecular mechanisms linking insulin signaling with ion homeostasis in the heart. Impairment of insulin signaling in type 2 diabetes causes dysregulation of ion homeostasis in the heart, which consequently leads to cardiac dysfunction. These findings reveal new mechanisms underlying diabetic cardiomyopathy, and have implications for development of novel drugs to combat this disease.

Selected publications

1. 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.

Tingting Fu

Tingting Fu received her Bachelor's degree of Biological Science in 2014 from School of Life Sciences, Anhui Normal University. She joined Dr. Zhenji Gan's lab at the year of 2014 to study mitochondrial remodeling and metabolic diseases.

For the past four years, her work focused on the function of a mitophagy receptor, FUNDC1, in skeletal muscle. This year, she and her colleagues reported that loss of FUNDC1-dependent mitochondrial quality control in muscle alleviates high-fat-diet-induced obesity and improves systemic glucose homeostasis through promoting exercise-independent fat burning in adipose tissue. These findings suggest that muscle mitophagy pathways could potentially be targeted to counteract metabolic disorders, such as obesity.

Selected publications

1. Fu T, Xu Z, Liu L, Guo Q, Wu H, Liang X, Zhou D, Xiao L, Liu L, Liu Y, Zhu M, Chen Q, Gan Z. Mitophagy Directs Muscle-Adipose Crosstalk to Alleviate Dietary Obesity. Cell Reports. 2018; 23:1357–1372.
2. Gan Z, Fu T, Kelly DP, Vega RB. Skeletal muscle mitochondrial remodeling in exercise and diseases. Cell Research. 2018; 0:1–12.
3. Liang X, Liu L, Fu T, Zhou Q, Zhou D, Xiao L, Liu J, Kong Y, Xie H, Yi F, Lai L, Vega RB, Kelly DP, Smith SR, Gan Z. J Biol Chem. 2016; 291(49):25306-25318.
4. Kong Y, Li K, Fu T, Wan C, Zhang D, Song H, Zhang Y, Liu N, Gan Z, Yuan L. Quercetin ameliorates Aβ toxicity in Drosophila AD model by modulating cell cycle-related protein expression. Oncotarget. 2016; 7(42):67716-67731.
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Neurobiology

Yun Shi , Ph.D

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

Contact Information

Tel : +86-25-58641565 (Office) +86-25-58641548 (Lab)
Fax: +86-25-58641500
Email: yunshi@nju.edu.cn, shiyun@nicemice.cn

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.

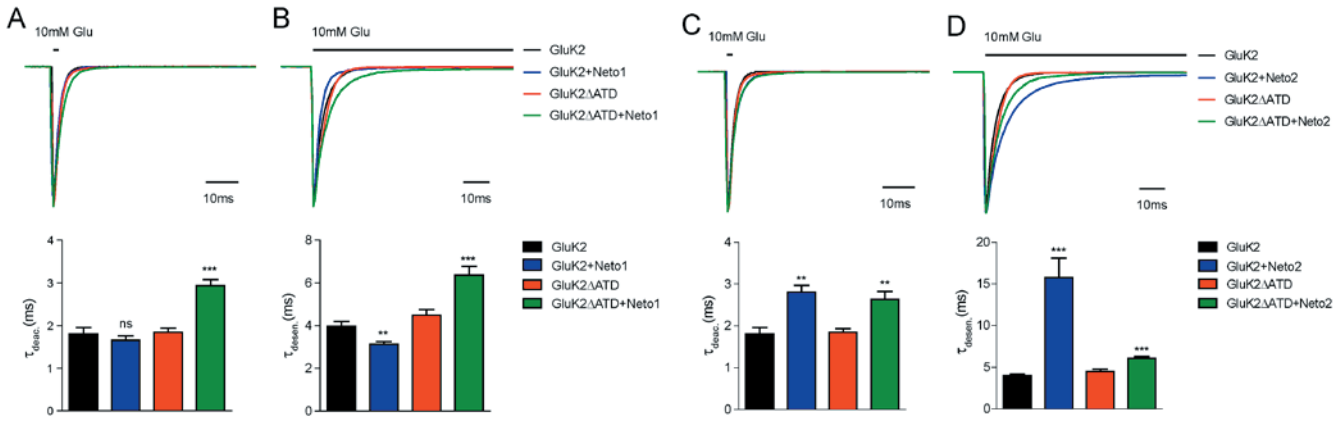

Figure 1. GluK2 ATD is critical for Neto regulation of KAR function.

A.The deactivation of GluK2 was not affected by ATD truncation. Neto1 had no effect on the deactivation time course of full-length GluK2, whereas slowed that of GluK2ΔATD. B. The desensitization of GluK2 was not affected by ATD truncation. Neto1 facilitated the desensitization of full-length GluK2 while significantly slowed the desensitization of GluK2ΔATD. C. Neto2 had similar effect on the deactivation of full-length and ATD-deleted GluK2. D. Neto2 dramatically slowed the desensitization of full-length GluK2, while this effect was significantly reduced on GluK2ΔATD.

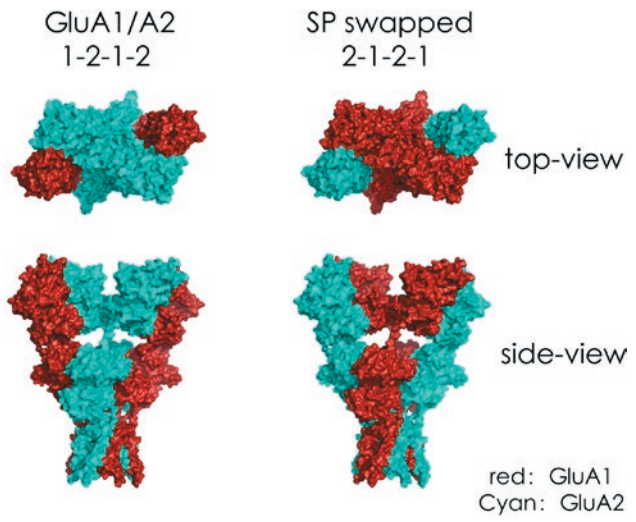

Figure 2. Signal peptides determine the spatial assembly of heteromeric AMPA receptors.

A non-reducing western assay was designed to examine the assembly and stoichiometry of GluA1/A2 heteromeric AMPA receptors. The conclusion was depicted with following models. Left. The structural model of GluA1/A2. In the model the 2 GluA1s and 2 GluA2s form a functional assembly of AMPA receptor with 1-2-1-2 architecture. Right. Swapping signal peptides switched the spatial position of GluA1 and GluA2, so that the receptors were assembled with 2-1-2-1 pattern.

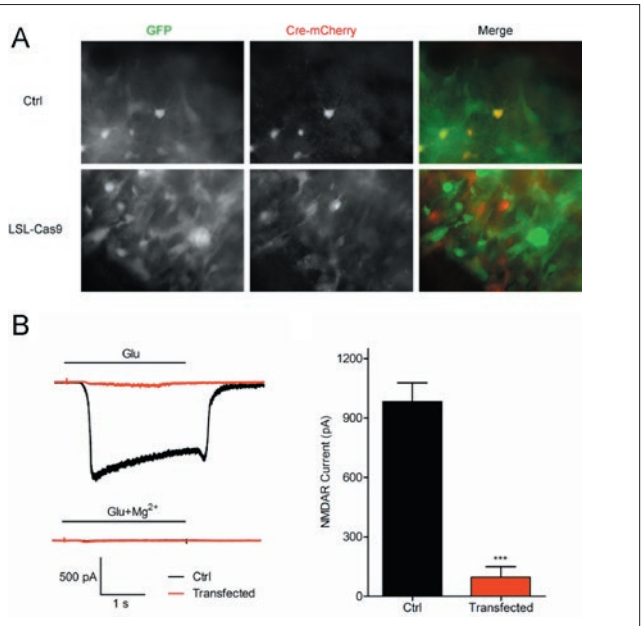

Figure 3. Targeted gene deletion in neurons using a Cas9 transgenic mouse.

A. Hippocampus slices from sgRNAs^{EGFP}-LSL-Cas9; EGFP mouse was infected by lentivirus expressing Cre recombinase fused with mCherry. The GFP expression was eliminated in the Cre positive neurons. B. In primary culture of cerebellar granule neurons, expression of Cre recombinase and Grin1-sgRNAs removed the NMDA receptor mediated glutamate currents.

Selected publications

1. Duan GF, Ye Y, Xu S, Tao W, Zhao S, Jin T, Nicoll RA, Shi YS#, Sheng N# (2018). Signal peptide represses GluK1 surface and synaptic trafficking through binding to amino-terminal domain. Nat Commun. 9(1):4879.
2. Yang Y, Chen J, Guo Z, Deng S, Du X, Zhu S, Ye C, Shi YS and Liu JJ.(2018) Postsynaptic Endophilin A1 Regulates Synaptic Plasticity and Long-term Memory. Front Mol Neusci. 11:177
3. Sheng N, Bemben MA, Díaz-Alonso J, Tao W, Shi YS, Nicoll RA. (2018) LTP requires postsynaptic PDZ-domain interactions with glutamate receptor/auxiliary protein complexes. Proc Natl Acad Sci U S A. 115(15):3948-3953.
4. Chen J*, Du Y*, He X, Huang X#, Shi YS#. (2017) A Convenient Cas9-based Conditional Knockout Strategy for Simultaneously Targeting Multiple Genes in Mouse. Sci Rep. 7(1):517.
5. Niu Y*, Dai Z*, Liu W*, Zhang C, Yang Y, Guo Z, Li X, Xu C, Huang X, Wang Y, Shi YS#, Liu JJ#. (2017) Ablation of SNX6 leads to defects in synaptic function of CA1 pyramidal neurons and spatial memory. Elife. 6. pii: e20991.
6. Sheng N, Shi YS, Nicoll RA. (2017) The amino-terminal domains of kainate receptors determine the differential dependence on Neto auxiliary subunits for trafficking. Proc Natl Acad Sci U S A. 114(5):1159-1164.
7. He XY*, Li YJ*, Kalyanaraman C, Qiu LL, Chen C, Xiao Q, Liu WX, Zhang W, Yang JJ, Chen G, Jacobson MP, Shi YS#. (2016) GluA1 signal peptide determines the spatial assembly of heteromeric AMPA receptors. Proc Natl Acad Sci U S A.113(38):E5645-54.
8. Wei M, Zhang J, Jia M, Yang C, Pan Y, Li S, Luo Y, Zheng J, Ji J, Chen J, Hu X, Xiong J, Shi Y, Zhang C.(2016) α/β-Hydrolase domain-containing 6 (ABHD6) negatively regulates the surface delivery and synaptic function of AMPA receptors. Proc Natl Acad Sci U S A. 113(19):E2695-704
9. Sheng N, Shi YS, Lomash RM, Roche KW, Nicoll RA. (2015) Neto auxiliary proteins control both the trafficking and biophysical properties of the kainate receptor GluK1. Elife. 4, pii: e11682

Group members

Graduated students

Yanjun Li

Graduate students

Dan Wu
Jiang Chen
Han Du
Guifang Duan
Chang Ye
Jiahui Sun
Pengshi Xiao
Chaohua Jiang
Qingqing Li
Yueying Wang
Xiaoyu Teng

Visiting students

Min Jia
XiaoHui Tang

Techanicians

Yanyu Zang

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 γ–secretase 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.

Contact Information

Tel : +86-25-58641541
Fax: +86-25-58641500
Email: chengq@nicemice.cn

Molecular and cellular mechanisms for neurodevelopmental and neurodegenerative diseases

γ-Secretase functions as a protease to cleave amyloid precursor protein and Notch receptors. It is composed of presenilin, presenilin enhancer 2 (Pen-2), nicastrin and Aph-1. Recent evidence has shown that Pen-2 is implicated in neurodevelopmental disease, but it remains unknown whether Pen-2 is essential for the maintenance of neural progenitor cells (NPCs). A previous study reported that germ-line deletion of Pen-2 causes embryonic lethal effect in mice (Bammens et al., 2011), precluding the possibility to use Pen-2 straight knockout mice to study its role in cortical development. To address this question, we generated NPC specific Pen-2 cKO mice. Quantitative real-time PCR and Western analyses revealed significantly decreased levels of Pen-2 (RNA and protein) in Pen-2 cKO mice as compared to controls, indicating high inactivation efficiency on Pen-2 via Cre-mediated recombination. Nissl staining showed that the cortex size was relatively small in Pen-2 cKO mice as compared to controls.

To examine the effects of loss of Pen-2 on neural progenitor cells (NPCs), we first conducted BrdU pulse-labeling experiment. We observed significant reduction on the immuno-reactivity of BrdU in the VZ/SVZ of the dorsal telencephalon in Pen-2 cKO mice (Figure 1). Cell counting results confirmed markedly decreased number of BrdU+ cells in Pen-2 cKO mice (Figure 1). Second, we carried out IHC on Pax6 and observed less number of Pax6+ cells in Pen-2 cKO mice at E13.5 than in controls (Figure 1). Third, IHC on PH3 revealed less number of PH3+ cells in Pen-2 cKO mice at E13.5 than in controls (Figure 2). There was more than 40% reduction on the PH3+ cell number in Pen-2 cKO at E13.5 or E14.5, respectively. Moreover, there was significantly reduced number of PH3+ cells on the apical surface of the VZ in Pen-2 cKO embryos (Figure 2).

We further examined the cell cycle re-entry. BrdU was injected to pregnant Pen-2 cKO mice at E12.5 or E13.5 and brain sections were collected 24 hours after. Cell counting results showed that the ratio of the number of BrdU+/Ki67+ cells to that of total BrdU+ cells was significantly decreased in Pen-2 cKO as compared to control (Figure 3), suggesting that more NPCs had exited the cell cycle in Pen-2 cKO mice than in controls. It is likely that impairment on cell cycle re-entry may serve as cellular mechanisms for loss of dividing NPCs in Pen-2 cKO mice.

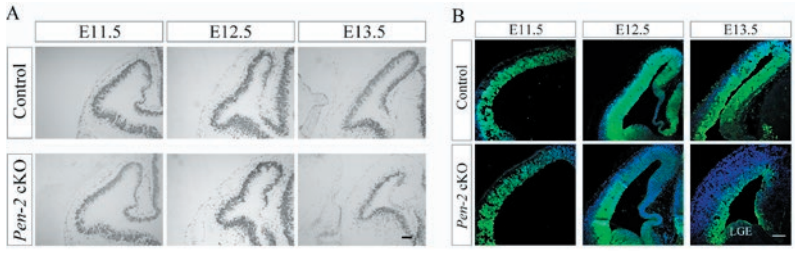

Figure 1. Decreased number of NPCs in Pen-2 cKO mice. Immunohistochemistry for BrdU (A) and Pax6 (B).

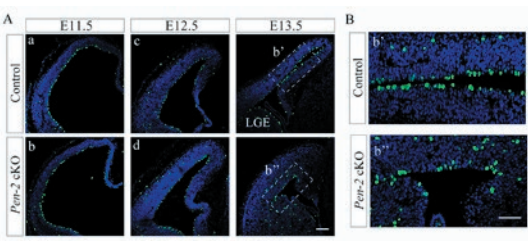

Figure 2. Decreased number of PH3+ cells in Pen-2 cKO mice.

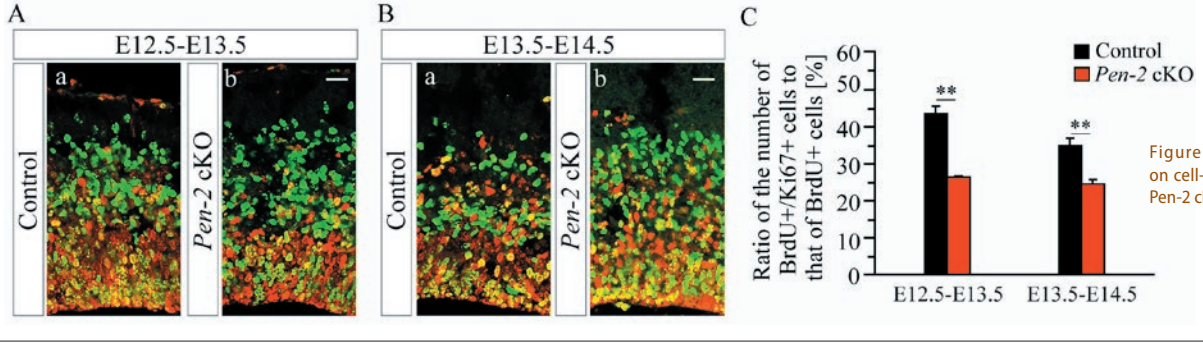

Figure 3. Impairment on cell-cycle re-entry in Pen-2 cKO mice.

Recent publications (*, Corresponding author)

1. Cheng S, Liu T, Xia Y, Hou J, Huang C, Zou X, Shi Y, Zheng Y, Hu Y, Lu J and Chen G*. Conditional inactivation of Pen-2 in the developing neocortex leads to rapid switch of apical progenitors to basal progenitors. In submission, 2018.
2. Yang S, Chang Y, Zhang H, Yu X, Shang W, Chen G, Chen DY and Gu Z. Enrichment of phosphorylated peptides with metal–organic framework nanosheets for serum profiling of diabetes and phosphoproteomics analysis. Analytical Chemistry, 2018; 90:13796–13805.
3. Wu DM, Wen X, Wang YJ, Han XR, Wang S, Shen M, Fan XH, Zhuang J, Zhang ZF, Shan Q, Li MQ, Hu B, Sun CH, Lu J*, Chen G* and Zheng YL*. Effect of microRNA-186 on oxidative stress injury of neuron by targeting IL 2 through the JAK-STAT pathway in a rat model of Alzheimer's disease. Journal of Cellular Physiology Doi:10.1002/jcp.26297.
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5. Yang S, Yu H, Wang Z, Liu H, Zhang H, Yu X, Shang W, Chen G and Gu Z. An exfoliated 2-D egyptian blue nanosheet for highly selective enrichment of multi-phosphorylated peptides in mass spectrometric analysis. European Journal of Chemistry, 2018; 24: 2109-2116.DOI:10.1002/chem.201704138.
6. Zhou J, Wang X, Zhou L, Zhou L, Xu F, Su L, Wang H, Jia F, Xu F, Chen G, De Zeeuw C and Shen Y. Ablation of TFR1 in Purkinje cells inhibits mGlu1 trafficking and impairs motor coordination, but not autistic-like behaviors. Journal of Neuroscience, 2017.
7. Liu T, Ye X, Zhang J, Yu T, Cheng S, Zou X, Xu Y, Chen G* and Yin Z* Increased adult neurogenesis associated with reactive astrocytosis occurs prior to neuron loss in a mouse model of neurodegenerative disease. CNS Neuroscience & Therapeutics, 2017; 23: 885–893.
8. Xu C, Yu L, Hou J, Jackson RJ, Wang H, Huang C, Liu T, Wang Q, Zou X, Morris RGM, Spires-jones TL, Yang Z, Yin Z*, Xu Y* and Chen G*. Conditional deletion of PDK1 in the forebrain causes neuron loss and increased apoptosis during cortical development. Frontiers in Cellular Neuroscience, 2017; DOI: 10.3389/fncel.2017.00330.
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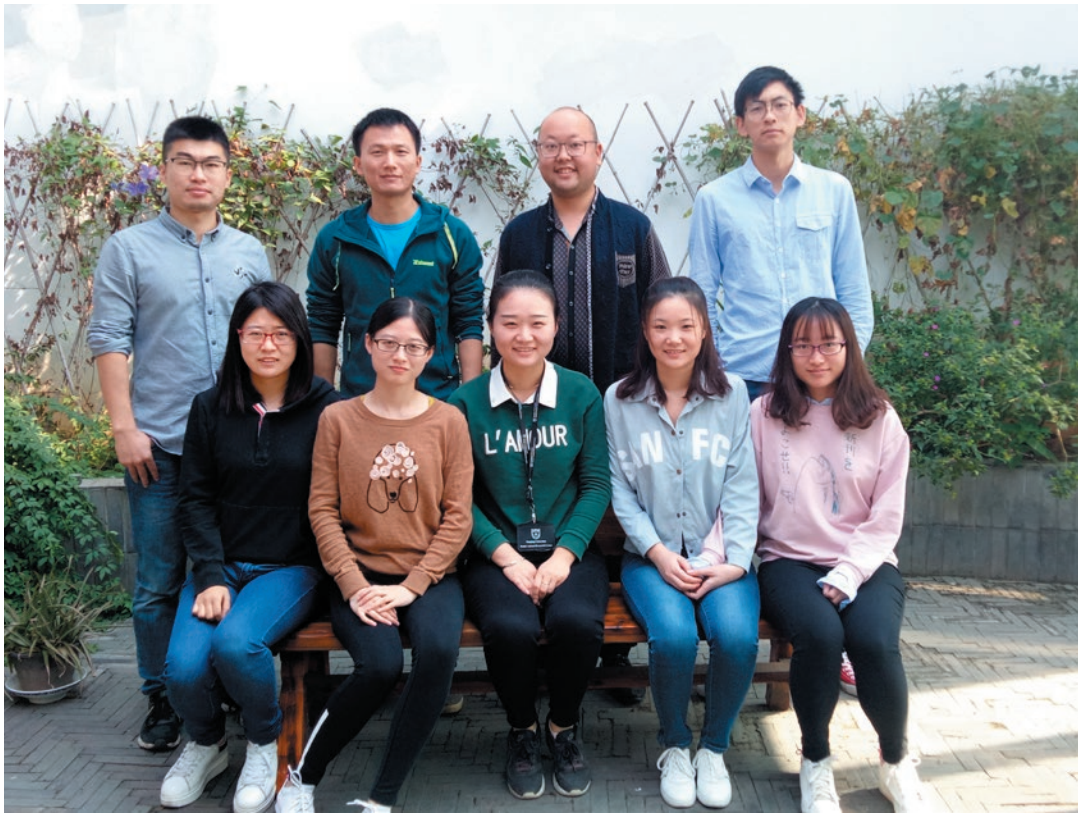

Group members

Group leader

Guiquan Chen

Graduate students

Chaoli Huang
Tingting Liu
He Wang
Jinxing Hou
Huiru Bi
Yingqian Xia
Xiaolian Ye
Mengjia Liu

Former members of the lab

Long Wang
Shanshan Cheng
Congyu Xu
Chen Zhang

Huiming Gao M.D., Ph.D.

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

Contact Information

Tel : +86-25-58641563 (Office) +86-25-58641561 (Lab)
Fax: +86-25-58641500
Email: gaohm@nicemice.cn; gaohm@nju.edu.cn

Neuroinflammation, neurodevelopment, and neurodegeneration

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

Metabolic and inflammatory dysregulation contributes to the pathogenesis of Parkinson's disease (PD). Postmortem studies of PD brains reveal perturbation of the pentose-phosphate pathway (PPP). Paralleling to glycolysis, the PPP converts glucose-6-phosphate into pentoses (5-carbon sugars) and generates NADPH and ribose 5-phosphate playing vital roles in redox homeostasis and anabolic biosynthesis. Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the PPP, displays high activity in the brain and immune cells. It remains unknown whether the PPP affects metabolic-inflammatory interface to participate in PD pathogenesis. Here, we found that inflammation induced PPP disruption and aberrant metabolic-inflammatory axis in brain microglia mediating chronic dopaminergic neurodegeneration. We detected sustained elevation in the expression and activity of G6PD in lipopolysaccharide (LPS)-treated mesencephalic neuron-glia cultures (an in vitro PD model) and in the substantia nigra of mice with an intranigral or intraperitoneal injection of LPS or with daily subcutaneous injection of MPTP for 5 consecutive days (three in vivo PD models). Pharmacological inhibition of G6PD activity by commonly used inhibitors, 6-aminonicotinamide (6-AN) and dehydroepiandrosterone (DHEA), and siRNA-mediated knockdown of microglial G6PD attenuated LPS-elicited chronic dopaminergic neurodegeneration. Microglia with elevated G6PD activity produced excessive NADPH and provided abundant substrate to over-activated NADPH oxidase leading to increased production of reactive oxygen species (ROS). Collectively, we demonstrated that PPP-mediated glucose metabolism disruption and neuroinflammation exacerbated each other mediating chronic neurodegeneration. Insight into metabolic-inflammatory interface suggests that manipulation of activity of the PPP and NADPH oxidase is potential therapeutic interventions in PD.

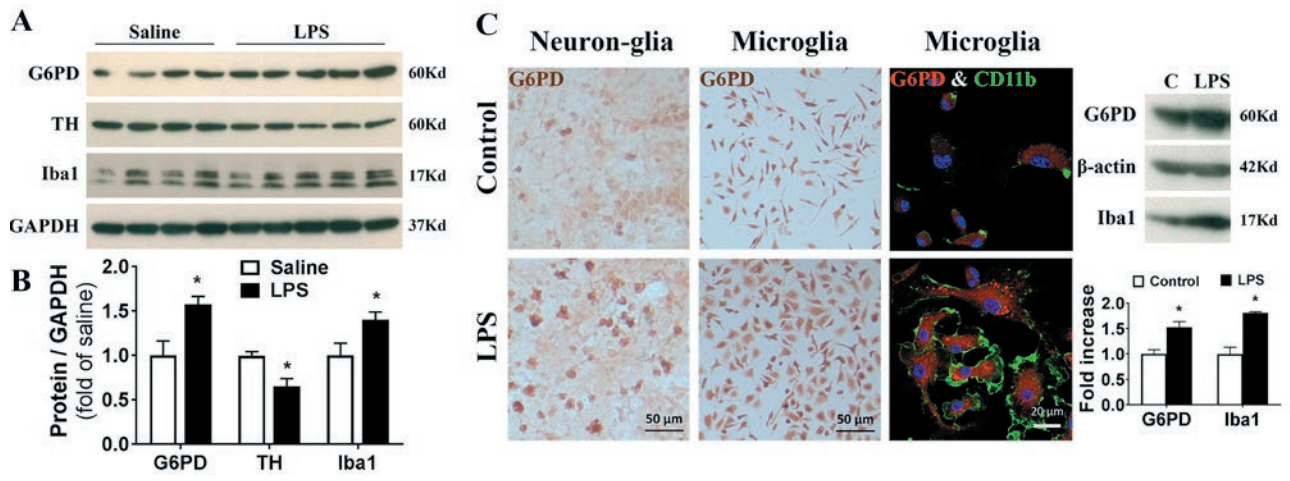

Figure 1. LPS stimulus induced sustained high activity of the pentose phosphate pathway.

(A, B) Sustained high activity of G6PD after LPS treatment in rodent mesencephalic neuron-glia cultures. (C) Immunocytochemical staining for G6PD on mouse neuron-glia cultures and microglia-enriched cultures, and double-labeled immunofluorescence on microglia. (D) Increased expression of G6PD and Iba1 in microglia-enriched cultures upon treatment with 10 ng/ml LPS for 24 hours.

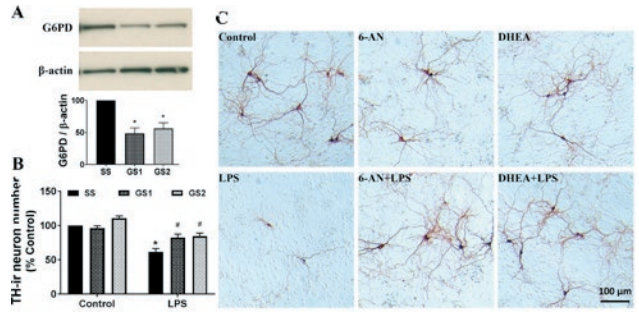

Figure 2. Suppression of G6PD activity by pharmacological inhibition and biological knockdown protected dopamine neurons from LPS-elicited inflammatory insult.

(A) Both siRNAs of G6PD displayed apparent knockdown of G6PD in primary microglia-enriched cultures transfected with scramble RNA (SS) or siRNAs of G6PD (GS1 and GS2) for 30 hours. (B) Both siRNAs of G6PD but not scramble siRNA protected DA neurons against LPS-elicited neurodegeneration in the reconstituted cultures. (C) Representative images indicated that G6PD inhibitor 6-AN (10 μM) and DHEA (30 or 100 μM) displayed significant dopaminergic neuroprotection in rodent mesencephalic neuron-glia cultures pretreated with vehicle or LPS.

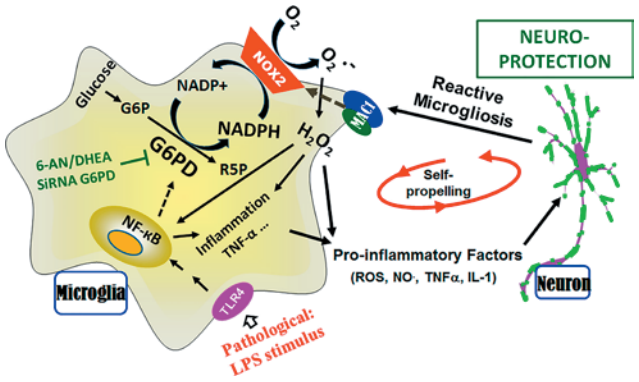

Figure 3. Exacerbation between metabolic disruption in the pentose phosphate pathway and neuroinflammation and consequent neurodegeneration.

Selected publications(* Corresponding author)

1. Gao H-M*, Gao Y, Tu DZ, Liu Y, Zhou H, and Hong JS (2018) Inflammation-mediated Neurodegeneration: Models, Mechanisms, and Therapeutic Interventions for neurodegenerative Diseases. In: Inflammation—From Molecular and Cellular Mechanisms to the Clinic (Jean-Marc Cavaillon & Mervyn Singer, ed). pp1255-1278. Wiley-VCH
2. Gao H-M*, Tu DZ, Gao Y, Liu QY, Yang R, Liu Y, Guan T, and Hong J-S (2017) Roles of microglia in inflammation-mediated neurodegeneration: Models, mechanisms, and therapeutic interventions for Parkinson's disease In: Advances in Neurotoxicology (Michael Aschner and Lucio Costa, ed), pp185-209. Elsevier
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8. 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
9. 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)
10. 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
11. 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
12. 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)
13. 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

Group members

Principal investigator

Huiming Gao

Graduate students

Yun Gao

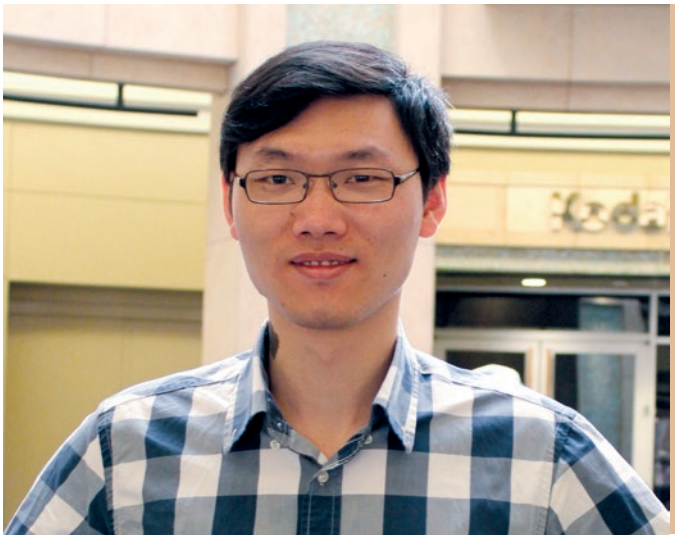
De-Zhen Tu

Tian Guan

Ru Yang

Hui Li

Wei Huang



Guoqiang Wan, Ph.D.

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

Contact Information

Tel (Office): 025-58641594
Tel (Lab): 025-58641544
Email: wangq@nicemice.cn

Development and Regeneration of Auditory Sensory Cells and Synapses

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 (Figure 1A). Our lab aims to identify novel molecular targets and pathways for the development and regeneration of cochlear sensory cells and synapses and to explore therapeutic potentials of these targets for treatment of sensorineural hearing loss.

1) Novel regulators of cochlear hair cell development and differentiation

Loss of hair cells is the primary cause of sensorineural hearing loss. Unlike fish, birds and amphibians, mammalian hair cells do not regenerate, posing great challenge in restoration of auditory function in deaf humans. Many regulators are known to be critical for hair cell development and fate determination, including Atoh1, Pou4f3 transcription factors and Notch signaling pathway. An ongoing effort in our lab is to understand the mechanisms of Atoh1 and Pou4f3 in regulating hair cell fate. However, genetic transfer or pharmacological manipulations of these regulators fail to produce mature and functional hair cells in vivo, suggesting the presence of yet unknown factors essential for hair cell development and maturation. Due to the scarcity of cochlear sensory cells and lack of appropriate cell culture models, high throughput screening (HTS) for regulators of hair cells is severely limited. To circumvent this problem, we have developed a HTS platform based on cochlear progenitor-derived organoids which yields more than 5,000 hair cell-containing organoids from each mouse (Figure 1B). We are now in the process of discovering novel small molecules and transcription factors required for hair cell development and maturation.

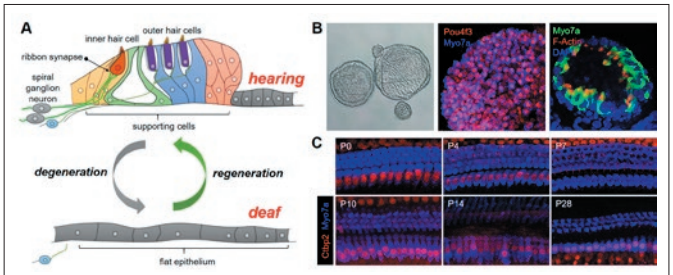
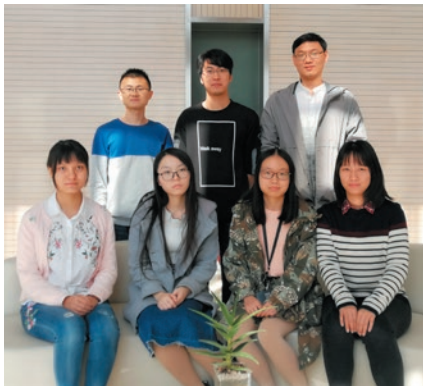


Figure 1. Research overview. (A) In mammalian cochlea, sensory hair cells (IHC and OHC), neurons (SGN), supporting cells and the auditory ribbon synapses are required for normal hearing, damage of these cells and synapses results in deafness. (B) Cochlear organoids after guided differentiation show hair cell markers including Myo7a, Pou4f3 and presence of stereocilia structures. (C) Dynamic pruning of ribbon synapses after birth in both IHCs and OHCs.

2) Mechanisms of synaptic pruning, degeneration and regeneration

Cochlear ribbon synapses are required for synchronized transmission of electric acoustic signals from hair cells to the SGNs. Ribbon synapses, similar to synapses of central nervous system, undergo extension pruning after birth (Figure 1C). However, the mechanisms regulating ribbon synaptic pruning remain elusive. We aim to identify the cellular and molecular mechanisms of synaptic pruning and explore whether the similar signals are involved in degeneration and regeneration of ribbon synapses after acoustic trauma and aging. Additionally, we are also exploring how SGNs can be regenerated after injury.



Graduate students

Zhen Chen
Sihao Gong
Yuhang Huang
Qing Liu
Chang Ye (co-supervision)
Chaorong Yu
Linqing Zhang

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

1. Schaefer, S.A., Higashi A.Y., Loomis, B., Schrepfer, T., Wan, G., Corfas, G., Dressler, G.R. and Duncan R.K. (2018). From Otic Induction to Hair Cell Production: Pax2EGFP Cell Line Illuminates Key Stages of Development in Mouse Inner Ear Organoid Model. Stem Cells and Development, 27(4), 237-251.
2. Long P.*, Wan, G.*, Roberts, M.T., Corfas, G. (2018). Myelin development, plasticity, and pathology in the auditory system. Developmental Neurobiology, 78(2), 80-92.
3. Wan, G.# and Corfas, G.# (2017). Transient auditory nerve demyelination as a new mechanism for hidden hearing loss. Nature Communications, 8, 14487.
4. Wan, G. & Corfas, G. (2015). No longer falling on deaf ears: mechanisms of degeneration and regeneration of cochlear ribbon synapses. Hearing Research, 329, 1-10.
5. 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.
6. 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.
7. 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.

Organogenesis

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.

Contact Information

Tel : +86-25-58641507
Fax: +86-25-58641507
Email: chenjiong@nju.edu.cn

Understanding the driving forces underlying collective cell Migration

Cells do not always migrate individually; they often migrate collectively as a cluster, a sheet, or a strand under physiological, developmental and cancer metastatic conditions. Collective cell migration has recently received much attention from cell and developmental biologists, and it has emerged as an important field of study with many characteristics distinct from those of single cell migration. As a new field, collective migration still has many fundamental questions unresolved. For example, what intrinsic factors or signals pre-determine the migratory fate of a group of cells that will later collectively detach and migrate away from the host tissue (likened to a group of runners pre-selected from a larger group of candidate runners)? How can the group of cells communicate with each other and collectively know the front vs. back, top vs. bottom and inside vs. outside during migration? Finally, what powers the group to migrate collectively?

A recent and primary focus of my lab has been to address these key questions. We utilize the border cells in Drosophila ovary to study collective migration during development, and they are genetically tractable and amenable to live imaging and optogenetic manipulation.

Cell growth regulates fate determination of border cells. Recently, my lab found that the fate determination of border cells was negatively regulated by the growth-promoting InR/Akt/TORC1 signaling pathway (Fig 1; Kang et al., Dev Cell, 2018). During development, cell growth and cell differentiation are two distinct yet coupled fundamental processes to give rise to tissues or organs. However, the mechanisms underlying the coordination or coupling between cell growth and cell differentiation are largely unknown. Our novel finding suggests that specification and differentiation of migratory cells is negatively coupled to cell growth during development.

Control of front-back polarity. It is known that the chemotactic migration of border cells is guided by the guidance receptor PVR, in response to extracellular signals secreted from oocyte. But, how guidance signaling sets up the front-back polarity of the entire border cell cluster is not well understood. We've made an interesting discovery that the guidance receptor PVR mediates the asymmetric distribution of exocyst and recycling endosome to set up the front-back polarity. (Wan et al., Development, 2013). Furthermore, we find that molecules crucial in apical-basal polarity, including aPKC and Crumbs complex, are required for the establishment of front-back polarity (Fig 2; Wang et al., Development, 2018). In addition, we find interesting coordination among the front-back, apical-basal and inside-outside polarities within the border cell cluster.

Power control of collective migration. We found that the actin depolymerizing factor Cofilin is required for the formation of actin-based lamellipodia, whose protrusion and adhesion provide force for migration of border cells (Zhang et al., Development, 2011). Moreover, Cofilin localization and phosphorylation are regulated by guidance receptor (PVR) signaling in such a way that active and unphosphorylated Cofilin are enriched in the leading border cell, resulting in the predominant protrusion forming only at the front of border cell cluster.

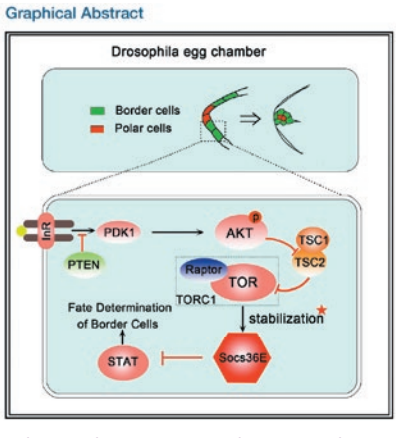


Figure 1 The growth promoting InR/Akt/TORC1 pathway attenuates migratory cell fate of border cells by down-regulation of JAK/STAT and destabilization of Socs36E. (from Kang et al., 2018)

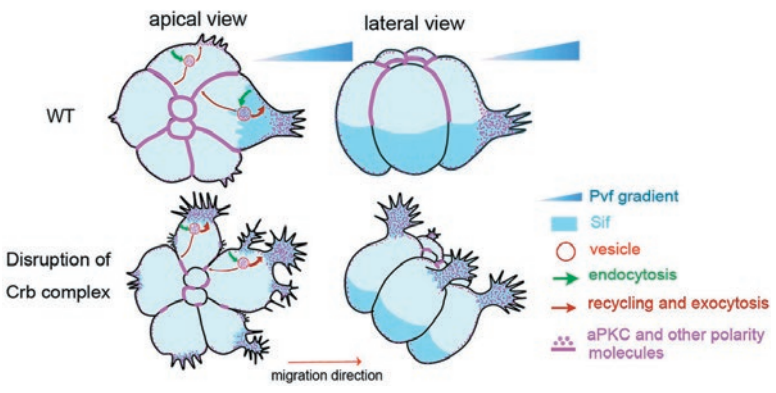


Figure 2. Diagram showing WT and Crb complex-deficient border cells in apical and lateral views. The model proposes how the two pools of aPKC and three distinct cell polarities are established by the Crb and Par complexes and endocytic recycling machinery. (from Wang et al., 2018)

Publications and Manuscripts in 2018 (*Selected Publications)

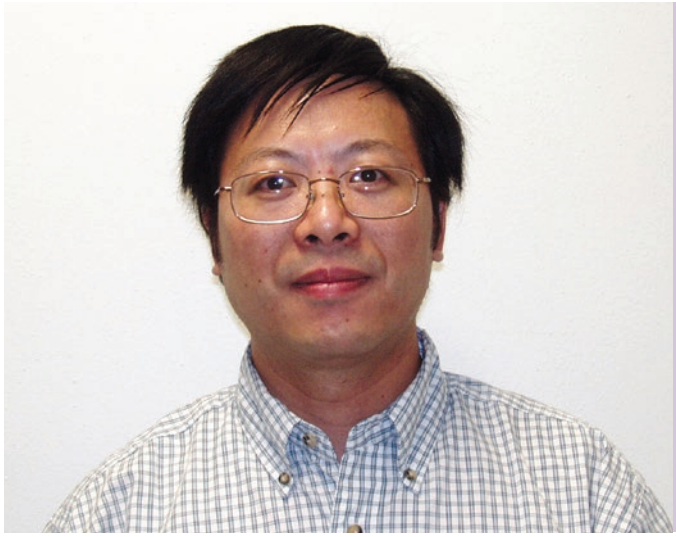
1. Wang, H., Qiu, Z., Xu, Z., Chen, S., Luo, J., Wang, X.*and Jiong Chen*. aPKC is a key polarity molecule coordinating the function of three distinct cell polarities during collective migration, Development (2018)
2. Kang, D., Wang, D., Xu, J., Quan, C., Guo1, X., Wang, H., Luo, J., Yang, Z., Chen, S.* and Jiong Chen*. The InR/Akt/TORC1 growth-promoting signaling negatively regulates JAK/STAT activity and migratory cell fate during morphogenesis, Developmental Cell (2018)
3. Wu, J., Wang, H., Xuan, G., Chen, J.* Cofilin-mediated actin dynamics promotes actin bundle formation during Drosophila bristle development Molecular Biology of the Cell (2016)
4. Luo, J., Wang, H., Kang, D., Guo, X., Wan, P., Wang, D., Chen, J.* Dlg5 maintains apical polarity by promoting membrane localization of Crumbs during Drosophila oogenesis. Scientific Reports (2016).
5. 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).
6. 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)
7. Zhang, L., Luo, J., Wan, P., Wu, J., Laski, F. and Chen, J.* Regulation of cofilin phosphorylation and asymmetry in collective cell migration during morphogenesis. Development 138, 455-64. (2011)

Group members

Technical Staff:
Zhou Ping

Graduate Students:
Guo Xuan
Wang Dou
Wang Xianping
Wu Mengqi
Qu Chen

Former Graduate Students:
Zhang Lijun (Ph.D)
Chu Dandan (Ph.D)
Wan Ping (Ph.D)
Wu Jing (Ph.D)
Luo Jun (Ph.D)
Kang Di (Ph.D)
Wang Heng (Ph.D)
Xu Jianbing (MS)
Qiu Zhiqian (MS)
Zuo Juntao (MS)



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.

Contact Information

Tel : +86-25-58641597
Fax: +86-25-58641500
Email: zhangqing@nicemice.cn, zhangqing@nju.edu.cn

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.

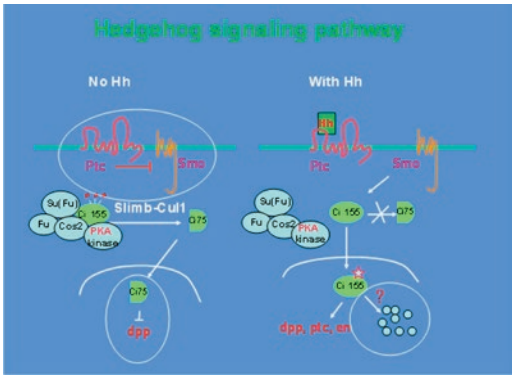


Fig1. Hedgehog signaling pathway in Drosophila.

The deubiquitinase UCHL5/UCH37 positively regulates Hedgehog signaling by deubiquitinating Smoothened.

Hedgehog (Hh) signaling pathway plays important roles in developmental processes including pattern formation and tissue homeostasis. The seven-pass transmembrane receptor Smoothened (Smo) is the pivotal transducer in the pathway; it, and thus the pathway overall, is regulated by ubiquitin-mediated degradation, which occurs in the absence of Hh. In the presence of Hh, the ubiquitination levels of Smo are decreased, but the molecular basis for this outcome is not well understood. Here, we identify the deubiquitinase UCHL5 as a positive regulator of the Hh pathway. We provide both genetic and biochemical evidence that UCHL5 interacts with and deubiquitinates Smo, increasing stability and promoting accumulation at the cell membrane. Strikingly, we find that Hh enhances the interaction between UCHL5 and Smo, thereby stabilizing Smo. We also find that proteasome subunit RPN13, an activator of UCHL5, could enhance the effect of UCHL5 on Smo protein level. More importantly, we find that the mammalian counterpart of UCHL5, UCH37, plays the same role in the regulation of Hh signaling by modulating hSmo ubiquitination and stability. Our findings thus identify UCHL5/UCH37 as a critical regulator of Hh signaling and potential therapeutic target for cancers.

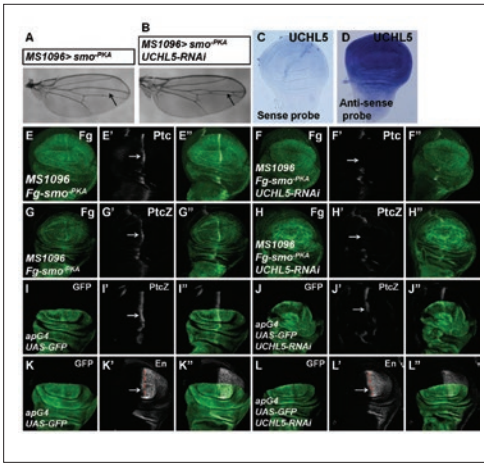


Fig. 2 Knockdown of UCHL5 represses Hh signaling.

(A-B) Comparison of adult wing phenotypes from control (A), and UCHL5 knockdown flies (B). Arrows indicate the space between vein 3 and vein 4. (C-D) The expression pattern of UCHL5 in wing discs was determined by in situ hybridization with DIG-labeled mRNA probe against UCHL5. The sense probe acts as negative control (C). Of note, UCHL5 ubiquitously expresses in wing discs. (E-H") Knockdown of UCHL5 with MS1096-Gal4 under the background of Smo-PKA attenuated the expression of Ptc (compare F-F" with E-E") and ptc-lacZ. Arrows indicate the decrease of Ptc and ptc-lacZ. (I-I") UAS-GFP (green) marks the apG4-mediated gene expression pattern. apG4 drives UAS transgenes to be specifically expressed in the dorsal region of wing discs. (J-J") Knockdown of UCHL5 by apG4 attenuated the expression of ptc-lacZ (arrow). (K-L") Knockdown of UCHL5 with apG4 attenuated the expression of En (compare L–L" with K-K").

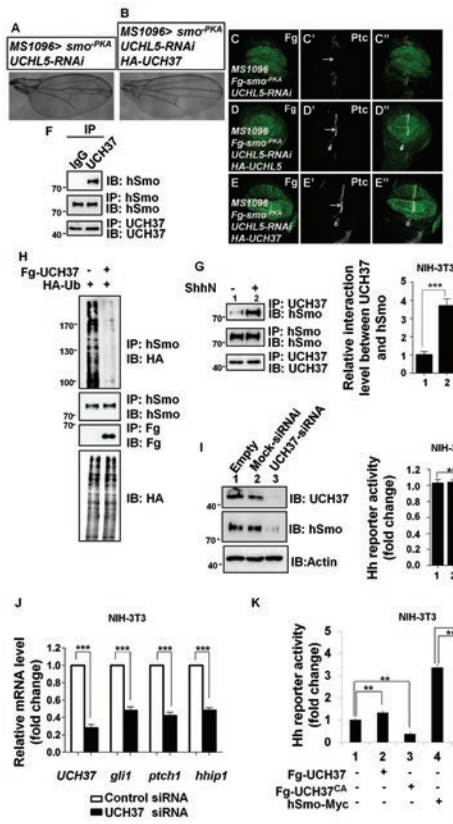


Fig.3 UCH37 deubiquitinates hSmo and promotes Hh Signaling activity in mammalian cells.

(A-B) Adult wings express UCHL5-RNAi alone, or UCHL5-RNAi plus HA-tagged UCH37 under the background of Smo-PKA through MS1096. (C-E″) Wing discs expressing UCHL5-RNAi alone, UCHL5-RNAi plus HA-UCHL5, or UCHL5-RNAi plus HA-UCH37 with MS1096 under the background of Smo-PKA were stained for Ptc (white). The decrease of Ptc protein caused by UCHL5 knockdown (C-C″) was restored via overexpressing HA-UCHL5 (D-D″) or HA-UCH37 (E-E″) (arrows). (F) UCH37could bind hSmo in NIH-3T3 cells. (G) ShhN treatment promoted the binding of UCH37 and hSmo in NIH-3T3 cells. (H) UCH37 inhibited the ubiquitination of hSmo protein in NIH-3T3 cells. (I) Knockdown of UCH37 decreased hSmo levels and Hh pathway activity in NIH-3T3 cells. (J) Relative mRNA levels of gli1, ptc1 and hhip were revealed by real-time PCR in UCH37 knockdown NIH-3T3 cells. (K) Gli-luciferase (Gli-luc) reporter assay in NIH-3T3 cells transfected with indicated constructs. Gli luciferase activities were normalized to Renilla luciferase activities. UCH37 promoted, but UCH37CA attenuated Shh pathway activity.

Selected Publications

1. Zhou Z#, Yao X#, Pang S, Chen P, Jiang W, Shan Z, Zhang Q*. (2018) The deubiquitinase UCHL5/UCH37 positively regulates Hedgehog signaling by deubiquitinating Smoothened. J Mol Cell Biol. 10(3):243-257.
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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.

Group members

Group leader

Qing Zhang

Technical Assistants

Yan Li

Xiatao Zhao

Graduate Students

Zhaoliang Shan
Wenting Li
Chunhua Jian
Xiuxiu Ti
Yuxue Gao
Hui Zuo

Former students

Chen Liu
Zizhang Zhou
Ping Chen
Siya Su
Xia Yao
Wan Gao
Shu Pang
Weirong Jiang

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 embryonic cell differentiation. From October 2008, he was offered the professor at MARC and set up the laboratory for developmental biology and cancer biology.

Contact Information

Tel : +86-25-58641537 (Office) +86-25-58641539 (Lab)
Fax: +86-25-58641500
Email: caoying@nju.edu.cn, caoying@nicemice.cn

Mechanisms of embryonic and cancer cell differentiation

Protein lysine methyltransferase EZH2 as a hub for regulating different types of chromatin modifications in cancer cells.

Our previous studies demonstrated that inhibition of epigenetic modification factors EZH2, LSD1, DNMT1 and HDAC1 caused post-mitotic neuron-like differentiation in cell lines of different cancer types, and cancer cells share regulatory networks with embryonic neural cells. This led us to the proposal that cancer cells have the properties of neural progenitor/stem cells. However, how these epigenetic factors regulate neuronal differentiation in cancer cells and whether inhibition of these factors also causes neuronal differentiation in neural progenitor/stem cells are still unknown. We show that EZH2, LSD1, DNMT1 and HDAC1 forms interactions themselves, and meanwhile, they also interact with SMAD proteins and beta-CATENIN in cancer cells (Figure 1). Inhibition of these epigenetic factors leads to reduced expression of these proteins, changed binding to neuronal gene promoters, changed chromatin modifications on neuronal gene promoters, and consequently, stimulation of transcription of neuronal genes. This effect is also reflected by neuronal differentiation of mouse embryonic stem cell-derived neural progenitor/stem cells after inhibition of these epigenetic factors (Figure 2). Importantly, interaction of EZH2 to LSD1, HDAC1, DNMT1, beta-CATENIN or SMAD2/4 is required for the stability of these proteins. Reduced EZH2 leads to enhanced ubiquitination and degradation of these proteins, and consequently, causes neuronal differentiation in cancer cells. Vice versa, increased EZH2 promotes their expression (Figure 3). Considering together with other studies, we propose that these proteins form a regulatory network conferring neural stemness in both cancer cells and neural progenitor/stem cells, reinforcing the similarity between cancer cells and neural progenitor/stem cells.

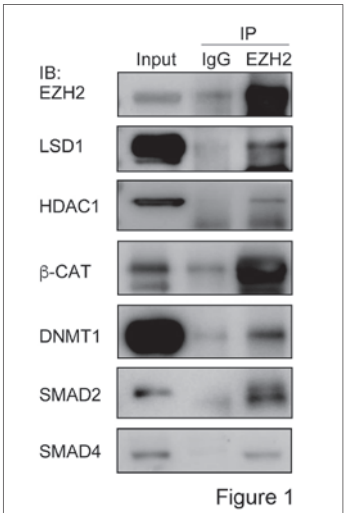


Figure 1. Co-IP shows that EZH2 forms complexes with other chromatin modification enzymes and signal transducers in SW480 cells.

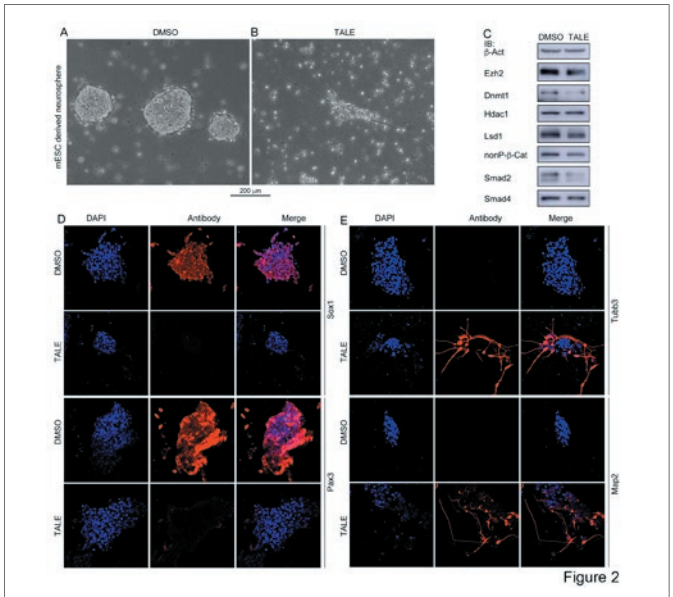


Figure 2. Combined inhibition of EZH2, LSD1, HDAC1 and DNMT1 leads to neuronal differentiation in mouse embryonic stem cell-derived neural progenitor cells, as in cancer cells.

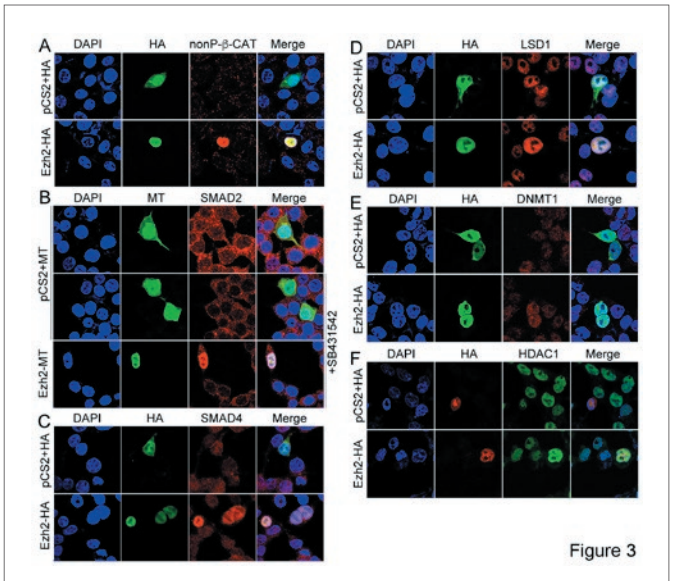


Figure 3. EZH2 overexpression leads to upregulation of other chromatin modification enzymes as well as signal transducers.

Selected publications (*Correspondence author)

1. Anhua Lei, Lu Chen, Min Zhang, Xiaoli Yang, Liyang Xu, Zan Zhang, Yan Gao, Ning Cao, and Ying Cao. 2018. EZH2 as a hub to regulate different types of chromatin modifications in cancer cells (to be submitted).
2. Cao Y*. Tumorigenesis as a process of gradual loss of original cell identity and gain of properties of neural precursor/progenitor cells. Cell Biosci. 2017 Nov 7;7:61. (Review)
3. Zhang Z, Lei A, Xu L, Chen L, Chen Y, Zhang X, Gao Y, Yang X, Zhang M, Cao Y*. 2017. Similarity in gene-regulatory networks suggests that cancer cells share characteristics of embryonic neural cells. J Biol Chem. 292(31):12842-12859.
4. 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
5. 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.
6. 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.
7. Cao Y*. 2015. Germ layer formation during Xenopus embryogenesis: the balance between pluripotency and differentiation. Sci China Life Sci. 58(4):336-42.
8. Cao Y*. 2013. Regulation of germ layer formation by pluripotency factors during embryogenesis. Cell Biosci. 3(1):15.
9. Cao Q, Zhang X, Lu L, Yang L, Gao J, Gao Y, Ma H, Cao Y*. (2012) Klf4 is required for germ-layer differentiation and body axis patterning during Xenopus embryogenesis. Development 139:3950-3961.

Group members

Principal investigator:
Cao Ying

Graduated students:
Cao Qing, PhD
Yang Linan, MSc
Lu Lei, PhD
Zhang Xuena, PhD
Gao Yan, PhD
Zhang Zan, PhD

Graduate students:
Lei Anhua
Xu Liyang
Chen Lu
Yang Xiaoli
Zhang Min
Shi Lihua
Cao Ning

Technicians:
Ma Haihua

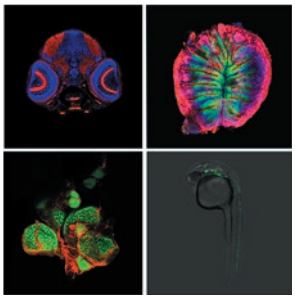
Xin Lou Ph.D.

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.

Contact Information

Tel : +86-25-58641547 (Office) +86-25-58641563 (Lab)
Fax: +86-25-58641500
Email: xin.lou@nju.edu.cn

Vertebrate Organogenesis and regeneration



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

Currently, our research focuses on the following aspects

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

The mammalian heart is incapable of significant regeneration following injury such as an acute myocardial infarction. Unlike the mammalian heart, the injured zebrafish heart normally undergoes minimal scarring and in 30 days the transient fibrin clot is replaced with new contractile muscle. Epigenetic regulation involves all stages of cellular processes in cardiac regeneration: stress-response, re-entry into mitotic cell cycles, "de-differentiation" and re-establishment of mature cell types. We applied transcription array and proteomics approaches on regenerating adult zebrafish heart, characterized the dynamic expression change of epigenetic regulators during heart regeneration. Now we are focusing on a set of chromatin modulators (including components of PRC2 complex and NuRD complex). By using a battery of strategy ranging from experimental molecular genetics to bioinformatics, we are studying the detail function and mechanism of these genes in heart regeneration.

2)IDENTIFICATION OF NOVEL REGULATORS OF ORGANOGENESIS.

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

Selected Publications

1.Zhang L, Yang Y, Li B, Scott IC, Lou X*. The DEAD-box RNA helicase Ddx39ab is essential for myocyte and lens development in zebrafish. Development. 2018 Apr 23;145(8). doi: 10.1242/dev.161018.

2.Ma D, Tu C, Sheng Q, Yang Y, Kan Z, Guo Y, Shyr Y, Scott IC, Lou X*. Dynamics of zebrafish heart regeneration using an HPLC-ESI-MS/MS approach. Journal of Proteome Research. 2018 Jan 25. doi: 10.1021/acs.jproteome.7b00915.

3. Hou N, Yang Y, Scott IC, Lou X*. The Sec domain protein Scfd1 facilitates trafficking of ECM components during chondrogenesis. Developmental Biology. 2017 Jan 1;421(1):8-15.

4. Zhang L, Zhou J, Han J, Hu B, Hou N, Shi Y, Huang X, Lou X*. Generation of an oocyte-specific Cas9 transgenic mouse for genome editing. PLoS ONE 2016 11(4): e0154364.

5. Lou X*, Burrows JT, Scott IC. Med14 cooperates with brg1 in the differentiation of skeletogenic neural crest. BMC Developmental Biology 2015 Nov 15:41.

Group members

Lab Head	Graduate Students
Xin Lou	Linlin Zhang
	Yuxi Yang
	Beibei Li
	Xue Zhang

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.

Contact Information

Tel : +86-25-58641527
Fax: +86-25-58641500
Email: qingshun@nju.edu.cn

Zebrafish development

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

RA (retinoic acid) plays essential roles in vertebrate embryogenesis. Its homeostasis in embryos is determined by the presence of Aldh1A that produces RA and Cyp26 that metabolizes RA into bio-inactive metabolites. Unlike mammals, zebrafish only have aldh1a2, aldh1a3 and aldh8a1 but not aldh1a1. Because both aldh1a3 and aldh8a1 are expressed in late organogenesis, aldh1a2 is the major gene that is responsible for RA synthesis in zebrafish early development (Liang et al, 2008). Like mammals, zebrafish possesses a third cyp26 gene (cyp26c1) (Gu et al., 2005) in addition to cyp26a1 and cyp26b1. The Cyp26c1 metabolizes RA but not retinol or retinal in a similar way to Cyp26a1, the major gene that is responsible for RA metabolism in zebrafish early development (Gu et al., 2006). Like cyp26a1, proper expression of cyp26c1 at early developmental stage is essential for the development of anterior–posterior axis and left–right symmetry in zebrafish embryos (Gu et al., 2005; Gu et al., 2006). Analyzing the promoter of cyp26a1, we reveal that zebrafish cyp26a1 possesses three conserved RAREs in response to RA signal and therefore maintains RA homeostasis in a feedback way (Hu et al., 2008; Li et al., 2012). Other than Cyp26s that can limit RA signaling, Ncor1 (nuclear receptor co-repressor) is essential for patterning the anterior–posterior axis of zebrafish hindbrain by actively repressing RA signaling (Xu et al., 2009). Consistent with these results, znfl1 whose expressions are in response to RA signaling, mediate the roles of RA in patterning zebrafish posterior neuroectoderm by acting upstream of pou5f3 and sall4 (Dong et al., 2017). Additonally, Znfl1s regulates left-right asymmetry patterning through controlling the expression of fgfr1a (Li et al., 2018).

RA signaling is also essential to vertebrate mesoderm differentiation. It plays a restrictive role in primitive myelopoiesis by inhibiting the specification of ventral mesoderm cells into anterior hemangioblasts through acting downstream of gata4/5/6, upstream of or parallel to cloche, and upstream to scl in a dose dependent manner (Liang et al., 2012). On the other hand, it is also essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos (Junbo Li et al., 2016). Moreover, Ncor1 and Ncor2 play essential but distinct roles in zebrafish primitive myelopoiesis (Jingyun Li et al., 2014). Other than RA signaling, the differentiation of ventral mesoderm is affected by environmental factors, excessive sodium nitrite affects zebrafish valve leaflet formation by producing too much NO signaling (Junbo Li et al., 2014).

RA signaling is genetically controlled by upstream genes. Foxc1a is a member of the forkhead transcription factors. By generating foxc1a knockout zebrafish using TALEN (transcription activator-like effector nuclease) technology, we demonstrated that knocking out foxc1a caused defective somites by controlling Fgf and Notch signaling through restricting the expression of aldh1a2 in zebrafish paraxial mesoderm directly (Jingyun Li et al., 2015). Additionally, we revealed that Foxc1a plays essential roles in zebrafish cardiogenesis by directly regulates expression of nkx2.5, encoding a transcriptional regulator of cardiac progenitor cells (Figure 1. Yue et al., 2018).

Engineered endonuclease (EENs) including ZFN, TALEN and CRISPR/Cas9 are powerful tools to create genome edited animals without species limitation. Using the knock out tools of ZFN and TALEN, we produced heritable targeted inactivation of myostatin genes in yellow catfish, the first endogenous gene knockout in aquaculture fish (Dong et al., 2011, Dong et al., 2014). To increase the efficiency of germline transmission of induced mutations and particularly knockin alleles created by CRISPR/Cas9, we co-microinjected yfp-nanos3 mRNA with Cas9 mRNA, sgRNA and ssDNA donor. In comparison with the common practice of selecting founders by genotyping fin clips, our new strategy of selecting founders with tentatively fluorescent-labeled PGCs significantly increases the ease and speed of generating heritable knocking and knockout animals with CRISPR/Cas9 (Dong et al., 2014). Using this strategy, we develop "two-step strategy" to generate an aldh1a2 flox zebrafish line (aldh1a2flox/flox) by first inserting mloxP sites into the 3rd intron and then into the 4th introns of aldh1a2. With the systemic expression of Cre in the eggs of aldh1a2flox/flox zebrafish, we obtained an aldh1a2 conventional knockout zebrafish line (aldh1a2+/-)(Figure 2, Gu et al., Unpublished data). Interstingly, the embryos whose primordial germ cells are eliminated at early development grow up as all-male-like sterile zebrafsh (Zhou et al., 2018). Collaborating with the groups of Professors Zhou and Zhu, we developed an alternative novel tool for DNA editing (SGN: structure-guided nuclease) without target sequence limitation (Xu et al., 2016). Unfortunately, our furthur efforts do not support that the system works in human colorectal carcinoma cell line (HCT116), nor in producing any germline transmssion zebrafish mutans (Zhang et al., Unpublished data).

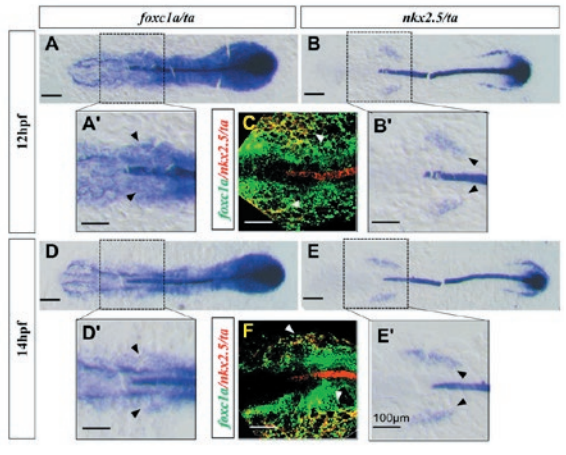


Figure 1. Zebrafish foxc1a is co-expressed with nkx2.5 in the caudal portion of ALPM at somite stage.

A - D, The expressions of foxc1a at 12 hpf (A) and 14 hpf (D), and nkx2.5 at12 hpf (B) and 14 hpf (E). A'-D', The magnification of the expression in LPM. C and F, Double florescence ISH of foxc1a and nkx2.5 in wild type zebrafish embryos at 12 hpf (C) and 14 hpf (F). Embryos were co-stained with ta to indicate the midline (A-F). The black arrow head indicates the expressions of foxc1a (A' and D') and nkx2.5 (B' and E'). The white arrow head indicates the merged yellow signal with green foxc1a expression and red nkx2.5 expression (C and F).

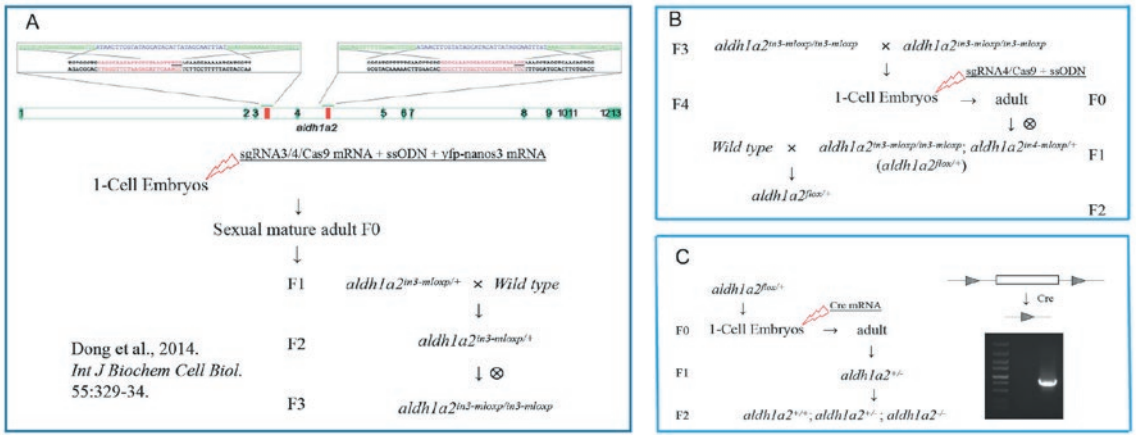


Figure 2. Generation of aldh1a2 conditional knockout zebrafish using "two-step" strategy.

(A) Shematic showing "the first step" to insert a mloxp site into the 3rd intron of aldh1a2 using CRISPR/Cas9 technology and generation of aldh1a2in3-mloxp/in3-mloxp. (B) Shematic showing "the second step" to insert a second mloxp site into the 4th intron of aldh1a2 using CRISPR/Cas9 technology and generation of aldh1a2flox/+ zebrafish. (C) Shematic showing the mloxp sites in aldh1a2flox/+ zebrafish are functional and can be recognized by Cre to conditinally remove the 4th exon of aldh1a2 in zebrafish.

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

1) Yunyun Yue, Mingyang Jiang, Luqingqing He, Zhaojunjie Zhang, Qinxin Zhang, Chun Gu, Meijing Liu, Nan Li, Qingshun Zhao*. 2018. The transcription factor Foxc1a in zebrafish directly regulates expression of nkx2.5, encoding a transcriptional regulator of cardiac progenitor cells. The Journal of Biological Chemistry, 293(2):638-650.

2) Li Zhou, Yongyong Feng, Fang Wang, Xiaohua Dong, Lan Jiang, Chun Liu, Qinshun Zhao**, Kaibin Li*. 2018. Generation of all-male-like sterile zebrafsh by eliminating primordial germ cells at early development. Scientific Reports, 8:1834.

3) Xiaohua Dong, Jingyun Li, Luqingqing He, Chun Gu, Wenshuang Jia, Yunyun Yue, Jun Li, Qinxin Zhang, Lele Chu, Qingshun Zhao*. 2017. Zebrafish Znfl1s control the expression of hoxb1b in the posterior neuroectoderm by acting upstream of pou5f3 and sall4. The Journal of Biological Chemistry, 292(31):13045-13055.

4) Shu Xu, Shasha Cao, Bingjie Zou, Yunyun Yue, Chun Gu, Xin Chen, Pei Wang, Xiaohua Dong, Zheng Xiang, Kai Li, Minsheng Zhu**, Qingshun Zhao**, Guohua Zhou*. 2016. An alternative novel tool for DNA editing without target sequence limitation: the structure-guided nuclease. Genome Biology. 17(1):186.

5) Junbo Li, Yunyun Yue, Qingshun Zhao*. 2016. Retinoic acid signaling is essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos. Zebrafish, 13(1):9-18. (Cover)

6) 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.

7) 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.

8) 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.

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.

Group members

Graduate Students

Chun Gu
Meijing Liu
Qinxin Zhang
Luqingqing He
Nan Li
Zhaojunjie Zhang
Dongya Jiang

Technician Staff

Xiaochun Yu

Old Lab Members

Fang Xu, PhD (2007)
Ping Hu, PhD (2009)
Jie Bao, PhD (2009)
Dong Liang, PhD (2011)
Kui Li, PhD (2011)
Jingyun Li, PhD (2013)
Junbo Li, PhD (2013)
Zhangji Dong, PhD (2014)
Wenshuang Jia, PhD (2015)
Xiaohua Dong, PhD (2015)
Shasha Cao, PhD (2016)
Yunyun Yue, PhD (2017)
Zhiying Zou, MS (2005)
Lu Sun, MS (2005)
Wei Song, MS (2006)
Xiaolin Wang, MS (2006)
Mei Zhang, MS (2008)

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.

Contact Information

Tel: +86-25-58641503(office) +86-25-58641521(lab)
Email: zhongzhouyang@nju.edu.cn, yangzz@nicemice.cn

Heart Development and Regeneration

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 utilized to study heart developmental regulation.

The right-sided heart development

During murine early heart development, the linear heart tube loops towards the right (~E9.0) resulting in a heart structure with two sides, the left side and the right side. The left side of the heart comprises of the left ventricle and the right side of the heart consists of the right ventricle (RV) and the outflow tract (OFT). The OFT further develops to the great aorta and the pulmonary artery (PA) through septation and rotation. The right-sided heart is prone to malformations, such as Tetralogy of Fallot (TOF), transposition of the great arteries (TGA) and the hypoplastic right ventricle syndrome (HRHS). Because majority of the heart defects occur in the right-sided heart, we aim to investigate the regulatory mechanisms of the right-sided heart at cellular and molecular levels, in order to understand human congenital heart defects.

The Mef2c-anterior heart field (Mef2c-AHF) enhancer cloned by Dr. Brian Black's group is active in a subset of the second heart field (SHF) progenitors located in the cardiac crescent at E7.5 and in the pharyngeal mesoderm (PM) and splanchnic mesoderm (SM) and heart tube during E8.0-9.5, and continues to be active in the RV and OFT as well as it derivatives till E14.5. Therefore, the Mef2c-AHF enhancer is a unique and informative tool to study the right-sided heart development (RV and OFT development) (Fig.1).

Our current understanding of the critical regulatory molecules involved in heart development fall into the three categories: the transcription factors (Nkx2.5, Hand2, GATA5, TBX5 etc), the signaling ligands and receptors, and the structural proteins. Using the Mef2c-AHF-Cre tool, we investigate the functions of the gene expression regulators (transcription factor, epigenetic regulator and RNA binding protein) and signaling regulator in the right-sided heart development.

PDK1 is a pivotal kinase and a critical component of the PI3K signaling pathway. Deletion of PDK1-encoding gene (Pdk1) gives rise to a hypoplastic right ventricle syndrome (HRHS) mouse model including a small RV, severe interventricular septum (IVS) and hypoplastic pulmonary artery (PA) or PA stenosis (Fig. 2).

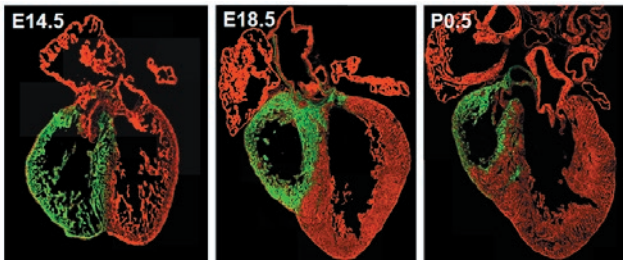


Fig. 1. Mef2c-AHF-Cre; Rosa26-mTmG lineage tracing of mouse heart. The green cells are Mef2c-AHF $^{+}$.

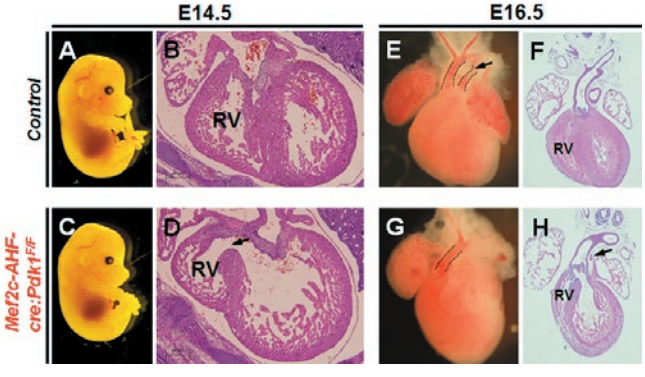


Fig.2. Deletion of PDK1-encoding gene (Pdk1) gives rise to a hypoplastic right ventricle syndrome (HRHS) mouse model including a small RV, severe interventricular septum (IVS) and hypoplastic pulmonary artery (PA) or PA stenosis

Heart regeneration and repair

In collaboration with Dr. Geng Liu's group, we established a p53 based genetic tracing system to investigate postnatal cardiomyocyte proliferation and heart regeneration. By selectively tracing the proliferative cardiomyocytes, a differential pattern of clonal expansion in p53+ cardiac myocytes was revealed in neonatal, adolescent and adult stages. In addition, the percentage of p53+ lineage cardiomyocytes displayed continuous increase in the first month. Furthermore, these cells rapidly responded to heart injury and greatly contributed to replenished myocardium. Therefore, this study revealed complex proliferating dynamics in postnatal cardiomyocytes and heart repair, and provided a novel genetic tracing strategy to study postnatal cardiac turnover and regeneration (Fig. 3).

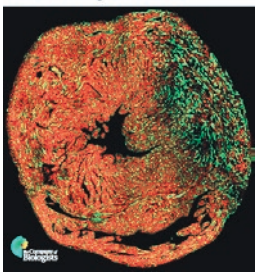


Fig.3. p53 based genetic tracing system to investigate postnatal cardiomyocyte proliferation and heart regeneration.

Selected Publications

1. Qi Xiao, Guoxin Zhang, Huijuan Wang, Lai Chen, Shuangshuang Lu, Dejing Pan, Geng Liu* and Zhongzhou Yang*. (2017) A p53 based genetic tracing system to follow postnatal cardiomyocyte expansion in heart regeneration. Development 144: 580-589. (Cover story/featured article, *Co-corresponding author)
2. 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)
3. 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.
4. 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
5. 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.
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8. 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).
9. Ruomin Di, Xiangqi Wu, Zai Chang, Xia Zhao, Qiuting Feng, Shuangshuang Lu, Qing Luan, Brian A. Hemmings, Xinli Li and Zhongzhou Yang. (2012) S6K inhibition renders cardiac protection against myocardial infarction through PDK1 phosphorylation of Akt. Biochem. J. 441:199-207.
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11. 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)

Group members

Group leader

Zhongzhou Yang

Secretary

Xiaoli Zhou

Technical staff

Congjia Shan

Graduate students

Hengwei Jin
Meng Xia
Yingchao Shi
Xinyi Huang
Huijuan Wang
Mingjie Xu
Ke Zhao
Shan Yu
Jie Li
Jie Yao
Wenli Fan

Yohei Niikura, Ph.D.

Yohei obtained his PhD in Chemistry at the University of Florence, Italy in 2000 and then conducted his postdoctoral research at Switzerland (Friedrich Miescher Institute), Japan (National Center for Geriatrics and Gerontology), and US (St. Jude Children's Research Hospital; Nationwide Children's Hospital; Greehey Children's Cancer Research Institute). He joined the MARC of Nanjing University as a Principle Investigator in September of 2018. His current research interest is molecular mechanism of cell division and fusion in human diseases and regeneration.

Contact Information

Tel: +86-25-585-00973
Fax: +86-25-58641500
Email: niikura@nicemice.cn

Mitotic regulators in diseases

Our lab is interested in the molecular mechanism involved in both cell division and human diseases, currently focusing on cancer and nervous system disorder.

During cell division, proper chromosomes segregation must be achieved otherwise it can result in unequal distribution of chromosomes to daughter cells. Spindle microtubules must attach to a single region of each chromosome, termed the "centromere," in most eukaryotes. The kinetochore is a complex of proteins that is located at the centromere (Figure 1). Defects in the centromere-kinetochore function leads to chromosome instability (CIN). CIN, unequal distribution of chromosomes to daughter cells results in aneuploidy (i.e., an incorrect number of chromosomes) – the consequences of aneuploidy are usually profound and may include cancer, birth defects, and developmental disorders such as Down syndrome. CIN is a hallmark of cancers, and is often associated with a poor prognosis. Therefore, it is highly important to study the temporal-special regulation and the structure of centromere and kinetochore protein(s) to understand CIN and cancer progression.

However, the relationship between centromere-kinetochore components and tumor regulating components and its precise mechanism during mitosis remain unclear, and many mutational studies of tumor suppressors and oncoproteins in past were limited in non-mitotic stage. Mitotic regulators of our interests include but not limited to centromere-kinetochore proteins, microtubule binding proteins, mitotic enzymes (e.g., kinases, phosphatases, ubiquitin ligases, deubiquitinases etc.), and chaperons and co-chaperons that facilitate proper chromosome segregation. Importantly, the functions of mitotic regulators are neither limited to the roles of cell division, and often extended to the roles of other cell cycle stages such as G1(G0)/S phases. Moreover, numerous mitotic regulators are often widely expressed in different tissues, it is very interesting to investigate how these regulators change their functions in different tissues and organs. In tissues such as the brains, hearts, and muscles where mitosis is not frequent and have lost the ability of spontaneous regeneration, error of the G1(G0)/S functions (e.g., protein quality control such as ubiquitin-proteasome machinery) would be a serious problem. While such as the blood, skin, bone, and gut where mitosis is frequent and have high turnover rates of the regeneration, error of (G2)/M functions (e.g., chromosome segregation) would be a serious problem. We note that the mechanism of the chromosome segregation is also highly regulated by the ubiquitin-proteasome machinery. Therefore, studying the cell cycle-specific functions of mitotic regulators involved in different type of cancer and other diseases in different organs would be expected for future research. Our lab is currently focusing on the function of the mitotic regulators in cancer and nervous system disorder.

TSG101-DAXX in cancer

Mitotic arrest deficiency 2 (Mad2), a critical component of the spindle checkpoint, is overexpressed in many cancer cells. Thus, we hypothesized that Mad2 overexpression could specifically make cancer cells susceptible to death by inducing a synthetic dosage lethality defect. We performed a synthetic genetic array analysis in yeast and revealed that Mad2 overexpression induced lethality in 13 gene deletions. Yeast STP22 (homolog of mammalian TSG101) is among 13 genes whose deletion caused synthetic dosage lethality in Mad2- overexpressing yeast cells. TSG101 is commonly known as a component of the ESCRT-I complex, a regulator of vesicular trafficking process, and is required for the sorting of endocytic ubiquitylated cargos into multivesicular bodies (MVBs). While initially discovered as negative regulator for tumorigenesis, accumulating evidence now describes TSG101 as a positive modulator of cancer progression. Consistent with this notion, overexpression of TSG101 has been reported in various cancer types. The challenge will be to define precisely how TSG101 exerts its oncogenic properties in cancer development.

TSG101 physically interacts with the H3.3 chaperone DAXX, and they cooperatively repress glucocorticoid receptor (GR)-mediated transcriptional activity. DAXX also protects protein degradation of DNA methyltransferase 1 (DNMT1)-associated protein (DMAP1) in vivo. Ectopic localization of the histone variant CENP-A in human cells depends on DAXX, and this aberrant nucleosome occludes CTCF binding, forming a heterotypic particle with H3.3., and has a minor effect on gene expression. Cells overexpressing CENP-A are more tolerant of DNA damage, and both the survival advantage and CTCF occlusion in these cells are dependent on DAXX. DAXX is a death domain-binding protein implicated in Fas-mediated cell death and physically interacts with CENP-C mediated by the amino-terminal 315 amino acids of CENP-C and the carboxyl-terminal 104 amino acids of DAXX. In normal conditions DAXX is mainly accumulated at Promyelocytic Leukemia Nuclear Bodies (PML NBs), and has a minor association with centromeres and pericentromeres (CEN/ periCEN). Application of physiological Heat Shock (HS) changes this balance forcing very robust and reversible accumulation of DAXX on CEN/periCEN heterochromatin. Depletion of DAXX leads to HS-induced changes in the balance of epigenetic modifications at heterochromatin, most dramatically elevating levels of active H3K4Me2 modification at periCEN, suggesting dualistic function of DAXX-containing complexes at CEN/periCEN: (1) regulation of H3.3 loading in normal conditions and (2) protection of epigenetic status upon stress-induced accumulation, thus collectively guarding epigenetic identity of CEN/periCEN heterochromatin. DAXX-USP7 (ubiquitin-specific-processing protease 7) regulates Aurora A stability, and DAXX-RSSF1 (RAS-association domain family protein 1; a mitotic checkpoint protein) regulates taxane response during mitosis. Interestingly, PTEN regulates glioblastoma oncogenesis through chromatin-associated complexes of DAXX and histone H3.3. Many PTM sites of DAXX are reported (data not shown), but their functional roles and their mechanisms to cause/avoid the genomic instabilities and cancer are poorly understood.

TSG101-DAXX in cancer (preliminary Investigation)

In our preliminary study, we detected aberrant mitotic progression in HeLa cells depleted of TSG101, which is reminiscent of kinetochore-defective cellular phenotype (Figure 2) which is consistent with the previous report. These cells also demonstrated significant increase of number of metaphase cells with misaligned chromosome and abnormal anapahase B or telophase cells with lagging chromosome. These morphological abnormalities were similar to those observed in cells which are defective of other centromere/kinetochore components or spindle checkpoint proteins. Interestingly, we detected that outer kinetochore protein HEC1 delocalizes from kinetochore in TSG101-depleted cells, although HEC1 protein level is increased in total HeLa cell lysates (data not shown). This phenotype is mimic of cells overexpressing CENP-H and we note that CENP-H is suggested to be a good prognostic marker and therapeutic target of cancer. In addition, we also found multiple consensus sites for mitotic kinases (e.g., PLK1, CDK1/Polo kinases, Mps1, Aurora-A, etc.) in TSG101 (Table 1). We are going to construct site-specific mutants and apply them for further analyses. We are also interested in identifying what protein depletion synthetically suppresses the cell growth of TSG101-overexpressing cancer cells by conducting high throughput RNAi and/or Crsipr KO library screens. Further study will be promised to confirm these interactions and to address the signaling pathway involving PTM of TSG101.

Table 1: Consensus sites found in TSG101.

consensus seq.	consensus for...	TSG101 peptide seuquence
S(S/T)(P/X)	CDK1/some Polo	SSR, SSM, SSQ, SSA
Similar to PLK1 consensus.	Mps1	DLTVRET, DGSSRE, DGTISE
KI(D/N)XXXF(L/I)XXLK	Bubs binding	(**KI**YLPYLHEWK)
RX(S/T)(L/V)	Aur-A	RETV, RASL,
(S/T)XX(S/T)	CK-I	SSMT, SEDT, SLIS, SAVS, TIKT, TTSS
SXX(E/D/Yp/Sp or any acidic)	CK-II	SSRE
Psi KX(E/D)	SUMOylation	MKEE, QKLE
P(S/T)AP	Late budding motif	PTAP

Figure Legends

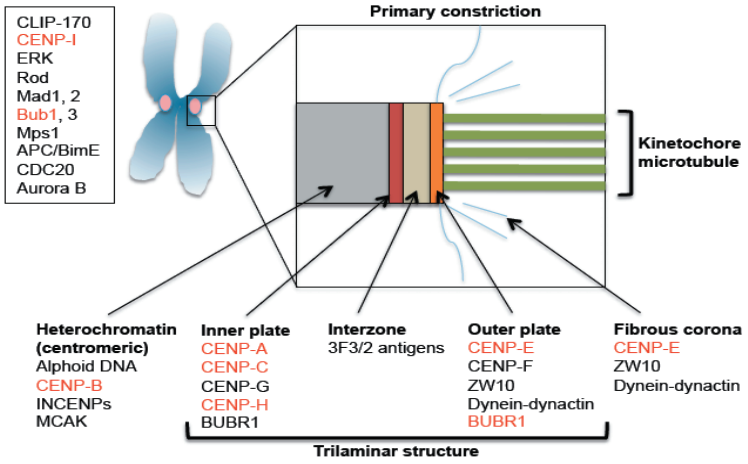

Figure 1. Human kinetochore structure.

Electron microscopic analyses revealed that the human kinetochore consists of a heterochromatin region, a trilaminar structure, and a fibrous corona. The box to the left of the diagram lists proteins whose locations in the structure have not been determined by immunoelectron microscopy. The kinetochore localizations of proteins in red were examined in 17-AAG treated cells.

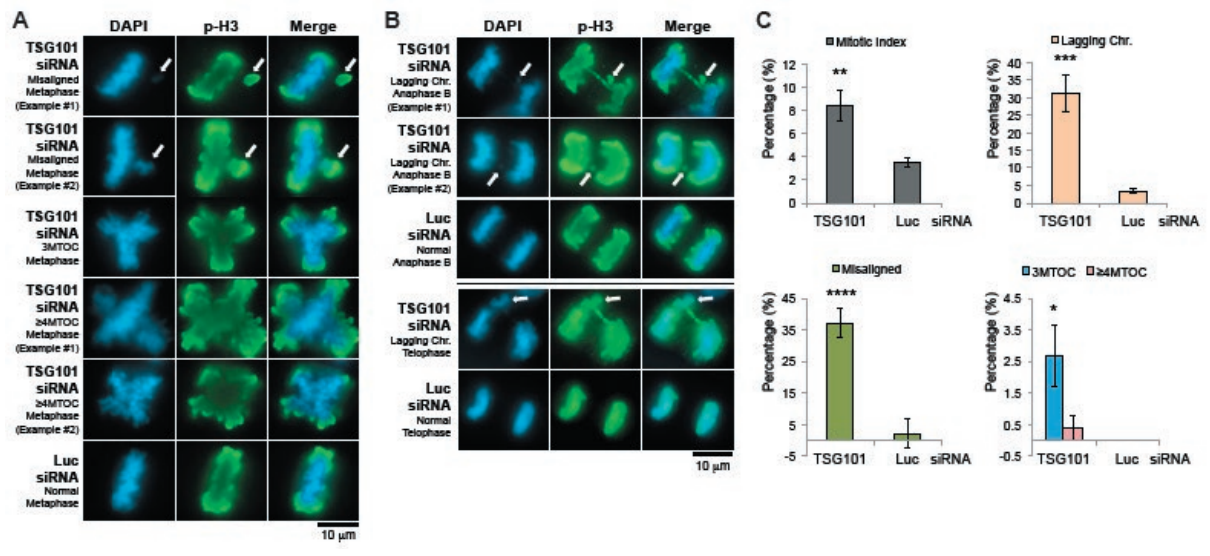

Figure 2. Abnormal mitotic progression in TSG101-depleted HeLa cells.

(A) Abnormal metaphase was observed by DAPI stain in TSG101 siRNA-treated Hela cells. Arrows indicate misaligned metaphase chromosomes. (B) Abnormal anaphase B or telophase was observed by DAPI stain in TSG101 siRNA-treated Hela cells. Arrows indicate lagging chromsomes. (C) Histogram summarizing abnormal metaphase, and anaphase B or telophase cells shown in (A) and (B). Mitotic index was shown. Misaligned, misaligned metaphase cell [arrows in (A)]; Lagging Chr., anaphase B or telophase cell with lagging chromosome [arrows in (B)]; 3 MTOC, cell with 3 microtubule-organizing center (MTOC); ≥4 MTOC, cell with four or more microtubule-organizing center (MTOC).

Selected publications (*corresponding author)

1. Niikura, Y*., and Kitagawa, K*. (2019a). The function of SUGT1 (the human homolog of SGT1), a co-chaperon. Heat Shock Protein 90 in Human Diseases and Disorders (Dordrecht, Netherlands: Springer Nature Publishers). In Press.
2. Niikura, Y*., and Kitagawa, K*. (2019b). CENP-A ubiquitylation contributes to maintain the chromosomal location of the centromere. Molecules. In Press
3. Niikura, Y., Kitagawa, R., and Kitagawa, K. (2017a). CENP-A Ubiquitylation Is Required for CENP-A Deposition at the Centromere. Developmental cell 40, 7-8.
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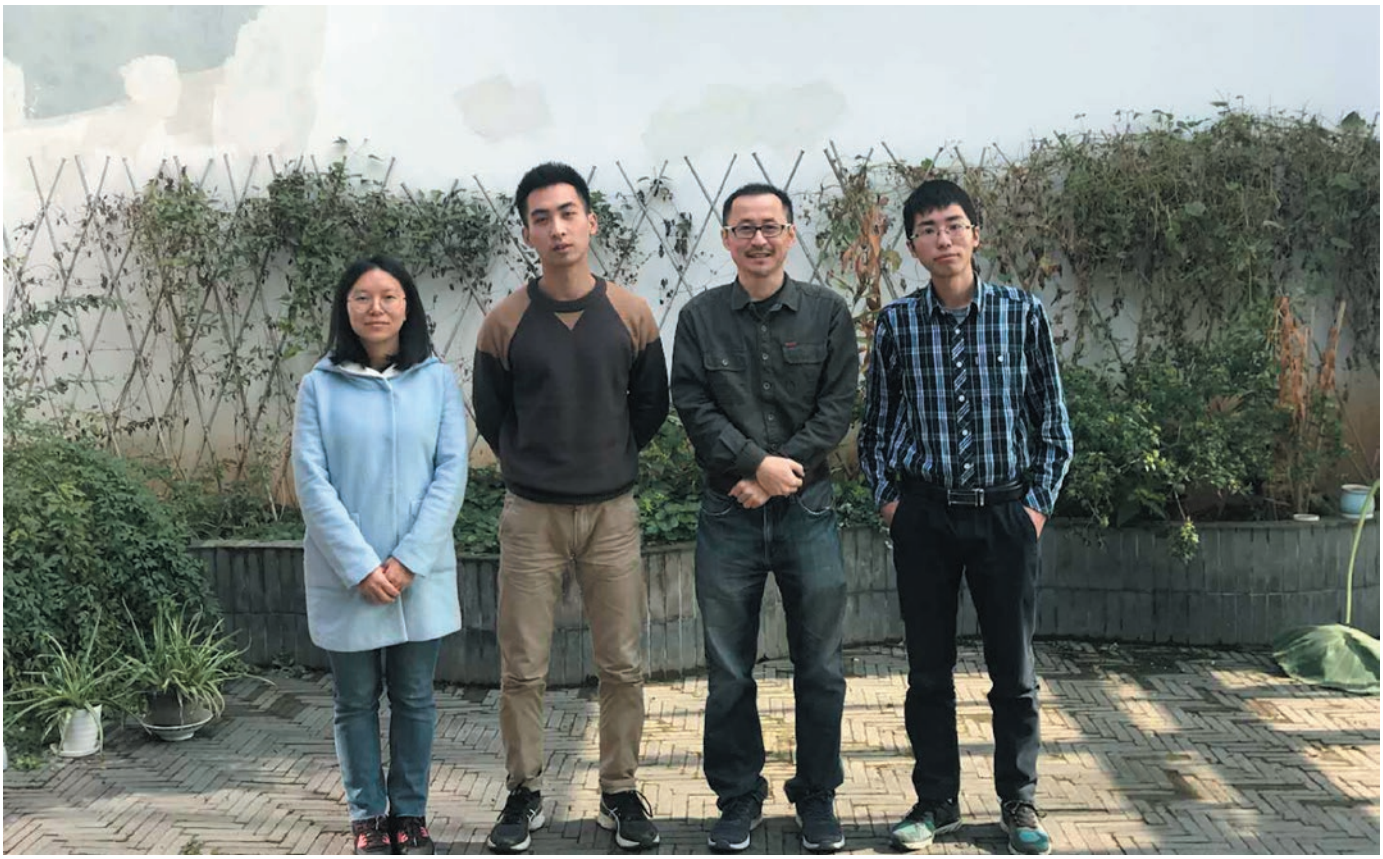

Group members

Graduate students

Yao Xi

Peizhao Li

Technician Staff

Ju You

Metabolism and Immunity

Xiang Gao, Ph.D.

Xiang was an alumina of Nanjing University. He received his Ph.D. degree from Thomas Jefferson University in 1994, then did his postdoctoral training at the Jackson Laboratory and University of North Carolina at Chapel Hill. In 2000, Xiang was recruited back to Nanjing University. He later founded both MARC and National Resource Center of Mutant Mice of China. He is also the current director for the State Key Laboratory of Pharmaceutical Biotechnology. Xiang is the recipient for Cheung Kong Scholar from Ministry of Education and Distinguished Young Scholar from National Science Foundation.

Contact Information

Tel : +86-25-58641598 (Office) +86-25-58641511 (Lab)
Fax: +86-25-58641500
Email: gaoxiang@nju.edu.cn

Physiological regulation and metabolic homeostasis

The advance of modern technologies, especially the NGS and gene editing, transform the biomedical fields. The complicated metabolic regulatory networks crossing the variety of tissues and organs are becoming tangible with these new tools. We are excited to embrace these promising progresses for identifying the previous unsolvable biological questions. In my laboratory, we are more interested in defining the global regulators for crucial physiological processes. Following are some of our publications:

1. Defining the mechanisms behind the "obesity memory" (Figure 1)

Body weight regain often causes failure of obesity therapies while the underlying mechanism remains largely unknown. In this study, we report that immune cells, especially CD4+ T cells, mediate the 'memory' of previous obese status. In a weight gain-loss-regain model, we found that C57BL/6J mice with an obesity history showed a much faster rate of body weight regain. This obesity memory could last for at least 2 months after previously obese mice were kept at the same body weight as non-obese mice. Surprisingly, such obesity memory was abrogated by dexamethasone treatment, whereas immunodeficient Rag1− / − and H2A− / − mice failed to establish such memory. Rag1− / − mice repossessed the obesity memory when immune cells or CD4+ T cells isolated from previously obese mice were transferred. Furthermore, depletion of CD4+ T cells led to obesity memory ablation. Taken together, we conclude that CD4+ T cells mediate obesity memory and promote weight regain (Zou et al, Cell Mol Immun).

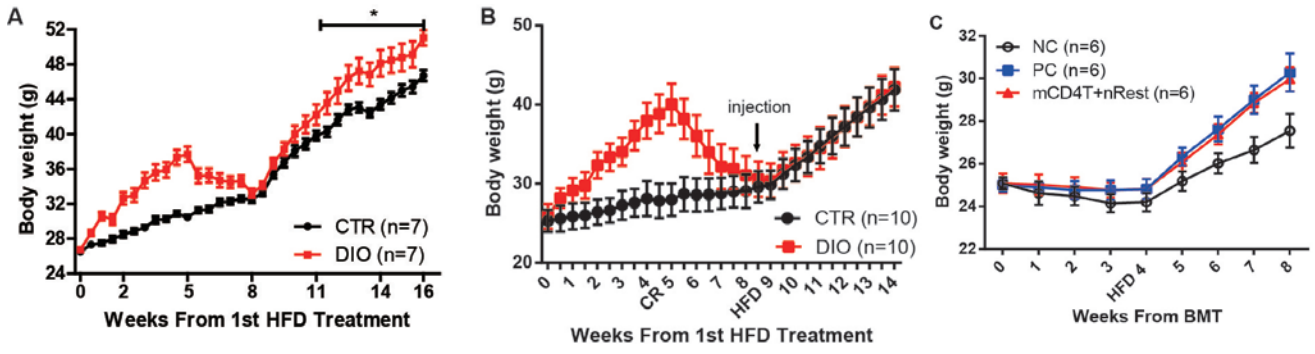

Figure 1. Obesity memory is stored and carried by CD4+ T lymphocytes.

(A) Body weight curve showing HFD-induced obesity memory in C57BL/6J mice. (B) Obesity memory vanished when C57BL/6J mice received anti-CD4 antibody injection. (C) Body weight curve of C57BL/6J mice that subjected to bone marrow transplantation and received memory CD4+ T and normal other cells.

2. Dissecting lipid metabolic change in the reprogramming process of induced pluripotent stem cells (Figure 2)

Changes of metabolic pathway preference are key events in the reprogramming process of somatic cells to induced pluripotent stem cells (iPSCs). The optimization of metabolic conditions can enhance reprogramming; however, the detailed underlying mechanisms are largely unclear. By comparing the gene expression profiles of somatic cells, intermediate-phase cells and iPSCs, we found that Cpt1b, a rate-limiting enzyme in fatty acid oxidation, was significantly upregulated at the early stage of the reprogramming process. Palmitoylcarnitine or acetyl-CoA, the primary and final products of Cpt1-mediated fatty acid oxidation, enhanced the reprogramming efficiency. Mechanistically, we demonstrated that these metabolites upregulated oxidative phosphorylation (OXPHOS) and downregulated protein kinase C activity at the early stage of the reprogramming process. This study reveals that fatty acid oxidation is crucial for reprogramming (Lin et al, Stem cell research & therapy).

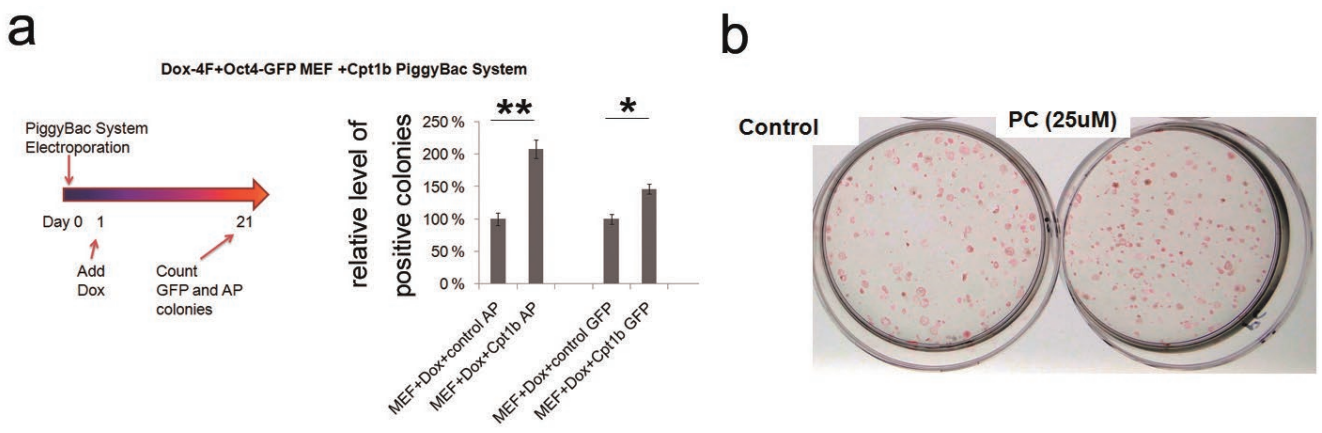

Figure 2. The role of lipid oxidation in reprogramming.

(A) Strategy of the reprogramming and the relative levels of AP- and GFP-positive colonies with or without cpt1b overexpression.(B) Images of AP-stained colonies in control and palmitoylcarnitine (PC) treated plates.

3. Uncovering the role of GSDMB in non-canonical pyroptosis (Figure 3)

Gasdermin B (GSDMB) has been reported to be associated with immune diseases in humans, but the detailed molecular mechanisms remain unsolved. The N-terminus of GSDMB by itself, unlike other gasdermin family proteins, does not induce cell death. Here, we show that GSDMB is highly expressed in the leukocytes of septic shock patients, which is associated with increased release of the GSDMD N-terminus. GSDMB expression and the accumulation of the N-terminal fragment of GSDMD are induced by the activation of the non-canonical pyroptosis pathway in a human monocyte cell line. The downregulation of GSDMB alleviates the cleavage of GSDMD and cell death. Consistently, the overexpression of GSDMB promotes GSDMD cleavage, accompanied by increased LDH release. We further found that GSDMB promotes caspase-4 activity, which is required for the cleavage of GSDMD in non-canonical pyroptosis, by directly binding to the CARD domain of caspase-4. Our study reveals a GSDMB-mediated novel regulatory mechanism for non-canonical pyroptosis and suggests a potential new strategy for the treatment of inflammatory diseases (Chen et al, J Mol Cell Biol).

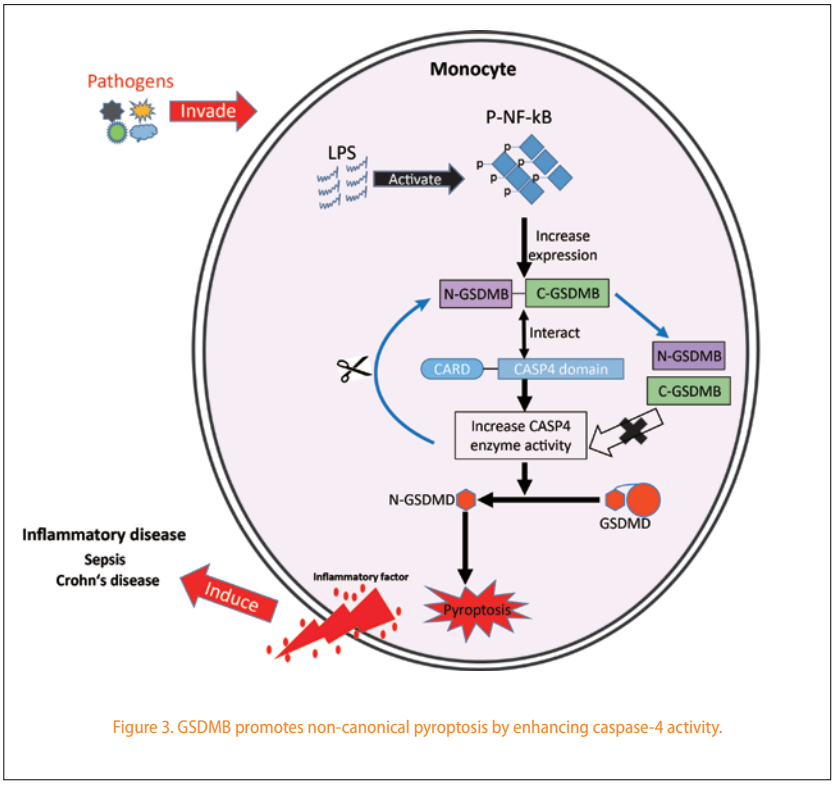

Figure 3. GSDMB promotes non-canonical pyroptosis by enhancing caspase-4 activity.

4. Analyzing the function of all coding genes by International Mouse Phenotype Consortium (Figure 4)

Although next-generation sequencing has revolutionized the ability to associate variants with human diseases, diagnostic rates and development of new therapies are still limited by a lack of knowledge of the functions and pathobiological mechanisms of most genes. To address this challenge, the International Mouse Phenotyping Consortium is creating a genome- and phenome-wide catalog of genefunction by characterizing new knockout-mouse strains across diverse biological systems through a broad set of standardized phenotypingtests. All mice will be readily available to the biomedical community. Analyzing the first 3,328 genes identified models for 360 diseases, including the first models, to our knowledge, for type C Bernard-Soulier, Bardet-Biedl-5 and Gordon Holmes syndromes. 90% of our phenotype annotations were novel, providing functional evidence for 1,092 genes and candidates in genetically uncharacterized diseases including arrhythmogenic right ventricular dysplasia 3. Finally, we describe our role in variant functional validation with The 100,000 Genomes Project and others (Meehan et al, Nature Genetics; Karp et al, Nature Comm; Rozman et al, Nature Comm).

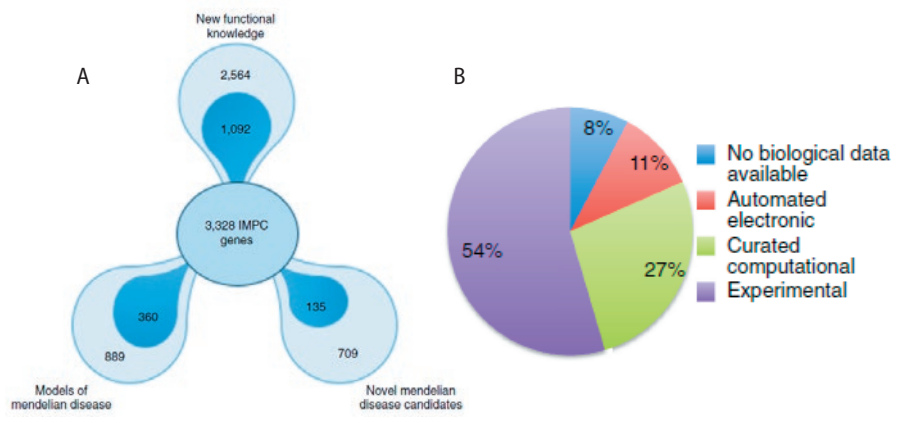

Figure 4. Novel mouse models of disease.

(A) IMPC mutant models of human disease and gene function. Human disease models were identified by measuring the degree of phenotypic similarity between IMPC null mutant mouse strains and their orthologous genetic loci associated with human diseases.

(B) Over 40% of IMPC strains are based on genes that lack experimental evidence for function according to the Gene Ontology Consortium (blue, red and green).

Selected publications

1. Zou J, Lai B, Zheng M et al. CD4+ T cells memorize obesity and promote weight regain. Cell Mol Immunol. 2018;15:630-639.
2. Wang Q, Tang J, Jiang S et al. Inhibition of PPARgamma, adipogenesis and insulin sensitivity by MAGED1. J Endocrinol. 2018. In press
3. Rozman J, Rathkolb B, Oestereicher MA et al. Identification of genetic elements in metabolism by high-throughput mouse phenotyping. Nature Comm. 2018;9:288.
4. Luan XR, Chen XL, Tang YX et al. CRISPR/Cas9-Mediated Treatment Ameliorates the Phenotype of the Epidermolytic Palmoplantar Keratoderma-like Mouse. Mol Therapy Nucleic Acids. 2018;12:220-228.
5. Lin Z, Liu F, Shi P et al. Fatty acid oxidation promotes reprogramming by enhancing oxidative phosphorylation and inhibiting protein kinase C. Stem Cell Res Therapy. 2018;9:47.
6. Lai B, Zou J, Lin Z et al. Haploinsufficiency of hnRNP U Changes Activity Pattern and Metabolic Rhythms. Am J Pathol. 2018;188:173-183.
7. Chen Q, Shi P, Wang Y et al. GSDMB promotes non-canonical pyroptosis by enhancing caspase-4 activity. J Mol Cell Biology. 2018.

Group members

Group Leader

Xiang Gao

Co-PI

Zhaoyu Lin

Graduate Students

Dayuan Zou
Qin Chen
Yufang Wang
Dingyu Wang
Yujie Zou
Xu Li
Jingwen Pei
Yue Liu
Qiyao Liu
Qianyue Chen
Shang Zheng

Shuai Chen, Ph.D.

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

Contact Information

Tel : +86-25-58641552 (Office) +86-25-58641557 (Lab)
Fax: +86-25-58641500
Email: chenshuai@nicemice.cn, chenshuai@nju.edu.cn

Cell Signaling and Type II Diabetes

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

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

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

The recent progresses of my lab is as follows:

1.Rab8a deficiency in skeletal muscle causes hyperlipidemia and hepatosteatosis by impairing muscle lipid uptake and storage

Nonalcoholic fatty liver disease (NAFLD) has become prevalent in the last few decades, which heightens the needs to elucidate the mechanisms underlying the pathogenesis of this disease. In this study, we show that skeletal muscle plays a critical role in the pathogenesis of hyperlipidemia and hepatosteatosis. We found that a small GTPase Rab8a controlled muscle lipid uptake through regulating translocation and expression of a fatty acid translocase CD36 and regulated muscle lipid storage via controlling fusion of lipid droplets. Muscle lipid uptake and storage were impaired when Rab8a was knocked-out in skeletal muscle, which consequently caused hyperlipidemia and exacerbated HFD-induced hepatosteatosis through promoting lipogenesis and cholesterol biosynthesis in the liver (Fig. 2). We therefore propose the existence of myogenic NAFLD, a possible subgroup of this disease, which may help for development of precision medicine to treat such disease in the future. (Chen Q.L., Rong P., …, Wang H.Y.*, Chen S.* 2017 Diabetes).

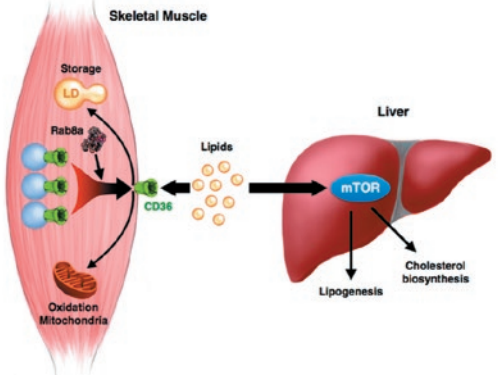

Figure 2. Lipid uptke and storage in skeletal muscle plays a critical role in the development of NAFLD.

2. A Tbc1d1^{Ser231Ala}-knockin mutation partially impairs AICAR- but not exercise-induced muscle glucose uptake in mice

Regulation of GLUT4 trafficking and glucose transport by insulin-dependent and insulin-independent/AMPK-mediated mechanisms is a major research focus, largely because it has immense implications in treating type 2 diebetes. We previously identified a RabGAP protein TBC1D1 as an AMPK substrate, and found that TBC1D1 can bind to 14-3-3 proteins in muscle cells mainly through its phosphorylated Ser^{231} when AMPK pathway is activated. In the current study, we employ two genetically-modified mouse models, namely a skeletal muscle specific AMPKα double KO model and a recently generated

TBC1D1^{Ser231Ala} knockin model (Chen et al 2016 PNAS, 113(26): 7219-24), to investigate potential roles of the AMPK-TBC1D1 signaling nexus in regulating glucose homeostasis. We provide evidence that TBC1D1 Ser^{231} phosphorylation and/or its binding to 14-3-3s play an important role in regulating glucose homeostasis at both peripheral and whole-body levels in a context-dependent manner. The TBC1D1^{Ser231Ala} knockin mutation impaired the hypoglycaemic effect of a pharmacological AMPK activator AICAR at least partially through inhibiting GLUT4 trafficking and glucose transport in skeletal muscle. However, this TBC1D1^{Ser231Ala} knockin mutation neither impaired exercise-induced muscle glucose uptake nor affected exercise performance in mice (Fig. 3). This study provides mechanistic insights into the AMPK-dependent GLUT4 trafficking process in response to AICAR. (Chen Q.L., Xie B.X., …, Chen S.*, Wang H.Y.* 2017 Diabetologia).

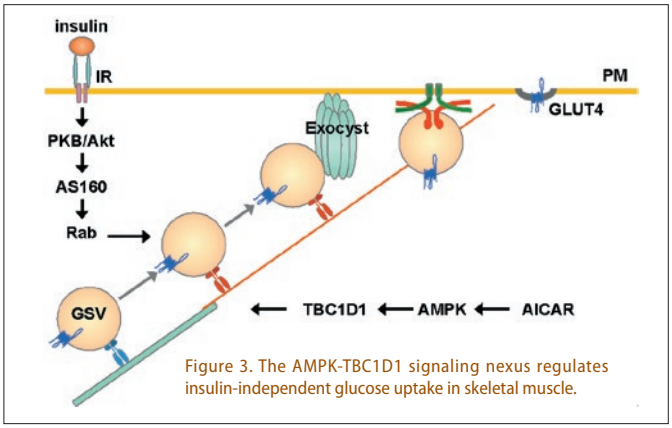

Figure 3. The AMPK-TBC1D1 signaling nexus regulates insulin-independent glucose uptake in skeletal muscle.

Selected Publications(* corresponding author)

1. Kang D, Wang D, Xu J, Quan C, Guo X, Wang H, Luo J, Yang Z, Chen S*, Chen J*. (2018) The InR/Akt/TORC1 Growth-Promoting Signaling Negatively Regulates JAK/STAT Activity and Migratory Cell Fate during Morphogenesis. Dev Cell 44:524-531 e525 (* corresponding author)
2. Chen Q.L., Rong P., Xu D.J., Zhu S.S., Chen L, Xie B.X., Du Q., Quan C., Sheng Y., Zhao T.J., Li P., Wang H.Y.*, Chen S.* (2017) Rab8a deficiency in skeletal muscle causes hyperlipidemia and hepatosteatosis via impairment of muscle lipid uptake and storage. Diabetes 66(9): 2387-2399 (* corresponding author)
3. Chen L., Chen Q.L., Rong P. Wang H.Y.* and Chen S.* (2017) The energy sensing LKB–AMPKα1 pathway regulates IGF1 secretion and consequent activation of the IGF1R–PKB pathway in primary hepatocytes. FEBS J 284(13): 2096-2109 (* corresponding author)
4. Chen QL, Xie BX, Zhu SS, Rong P, Sheng Y, Ducommun S, Chen L, Quan C, Li M, Sakamoto K, MacKintosh C, Chen S* and Wang HY* (2017) A Tbc1d1Ser231Ala-knockin mutation partially impairs AICAR- but not exercise-induced muscle glucose uptake in mice. Diabetologia 60(2): 336-345 (* corresponding author)
5. Xie B.X., Chen Q.L., Chen L., Sheng Y., Wang H.Y.* and Chen S.* (2016) The inactivation of RabGAP function of AS160 promotes lysosomal degradation of GLUT4 and causes postprandial hyperglycemia and hyperinsulinemia. Diabetes 65(11): 3327-3340 (* corresponding author)
6. Chen L., Chen Q.L., Xie B.X., Quan C., Sheng Y., Zhu S.S., Rong P., Zhou S.L., Sakamoto K., MacKintosh C., Wang H.Y. * and Chen S.* (2016) Disruption of the AMPK–TBC1D1 nexus increases lipogenic gene expression and causes obesity in mice via promoting IGF1 secretion. PNAS 113(26): 7219-24 (* corresponding author)
7. Wang HY*, Quan C, Hu CX, Xie BX, Du YN, Chen L, Yang W, Yang L, Chen QL, Shen B, Hu B, Zheng ZH, Zhu HB, Huang XX, Xu GW and Chen S (2016) A lipidomics study reveals hepatic lipid signatures associating with deficiency of the LDL receptor in a rat model. Biology Open 5, 979-986
8. Jiang T.T., Gao X.J., Wu C, Tian F, Lei Q.C., Bi J.C., Xie B.X., Wang H.Y., Chen S.*, Wang X.Y.* (2016) Apple-derived pectin modulates gut microbiota, improves gut barrier function, and attenuates metabolic endotoxemia in rats with diet-induced obesity. Nutrients 8(3). pii: E126. doi: 10.3390/nu8030126. (* corresponding author)
9. 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)
10. 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)
11. Chen Q.L., Quan C., Xie B.X., Chen L., Zhou S.L., Toth R., Campbell D.G., Lu S.S., Shirakawa R., Horiuchi H., Li C.J., Yang Z.Z., MacKintosh C., Wang H.Y.* and Chen S.*. (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)
12. 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)
13. 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)
14. 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)
15. 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)

Group members

PI
Shuai Chen
Postdoc
Dr. Qiaoli Chen
Graduate Students
Chao Quan
Min Li
Yang Sheng
Ping Rong
Qian Du
Sangsang Zhu
Wen Wei
Minjun Liu
Xinyu Yang
Weikuan Feng
Kun Zhou
Former Lab Members
Dr. Bingxian Xie
Dr. Liang Chen

Di Chen, Ph.D.

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

Contact Information

Tel : +86-25-58641512 (Office) +86-25-58641567 (Lab)
Fax: +86-25-58641500
Email: chendi@nju.edu.cn, chendi@nicemice.cn

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

Aging is a process of gradual function decline accompanied with increased mortality rate. The evolutionary theory of aging proposed that aging takes place because natural selection favors genes that confer benefit early on life at the cost of deterioration later in life (antagonistic pleiotropy). Using model organisms, researchers have demonstrated that aging can be modulated by highly conserved signaling pathways. Appropriate genetic or environmental modulations not only extend lifespan but also delay age-related pathologies. Many exciting discoveries on the molecular basis of aging were initially made in C. elegans, which is a great system for biology research because of the ease of genetics analysis and the conservation with higher species.

The highly conserved Insulin/IGF-1 signaling (IIS) and Target of Rapamycin (TOR) pathway play an important role in aging in many species. Our recently published work showed that simultaneous inhibition of DAF-2 (IGF-1 receptor) and TOR target RSKS-1 (ribosomal S6 kinase) leads to a nearly 5-fold, synergistic lifespan extension in C. elegans. We further demonstrated that the underlying mechanisms involve positive feedback regulation of the DAF-16/FOXO transcription factor via the key energy homeostasis regulator AMPK, and the germ line tissue plays a key regulatory role in this process (Figure 1). Currently, we are using polysomal profiling coupled with RNA-Seq techniques to identify genes that are translationally regulated in the daf-2 rsks-1 mutant and characterize their roles in aging (Figure 1).

Dietary restriction (DR) is one of most robust environmental manipulations that slow down aging in various species. However, the molecular mechanisms of DR remain largely unknown. Previously, we demonstrated that the hypoxia inducible factor-1 (HIF-1) plays an important role in DR-induced lifespan extension by regulating the IRE-1 ER stress pathway. To gain better insights on the relationship between nutrients and aging, we performed an RNAi-based genetic screen and identified a key mediator of DR. Mutations in this gene affect DR-induced lifespan extension and lipid metabolism in a tissue-specific manner (Figure 2).

Currently, our research focuses on the following aspects:

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

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

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

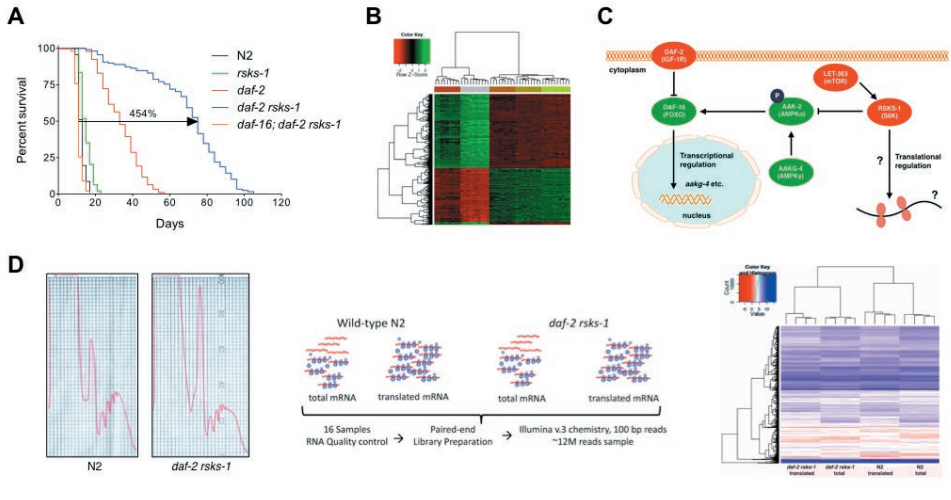

Figure 1. Functional genomics analysis of the super long-lived daf-2 rsks-1 mutant.

(A) Double mutations in DAF-2 (IGF-1 receptor) and RSKS-1 (ribosomal S6 kinase) leads to nearly 5-fold synergistic lifespan extension, which requires the DAF-16 (FOXO) transcription factor. (B) Transcriptome analysis via microarrays helped to identify genes that are differentially expressed in the daf-2 rsks-1 double mutant. (C) A model depicting the positive feedback regulation of DAF-16 via AMPK in the super long-lived daf-2 rsks-1 double mutant. (D) Polysomal profiling and RNA-Seq were performed to identify genes that are regulated at the post-transcriptional levels in the daf-2 rsks-1 mutant.

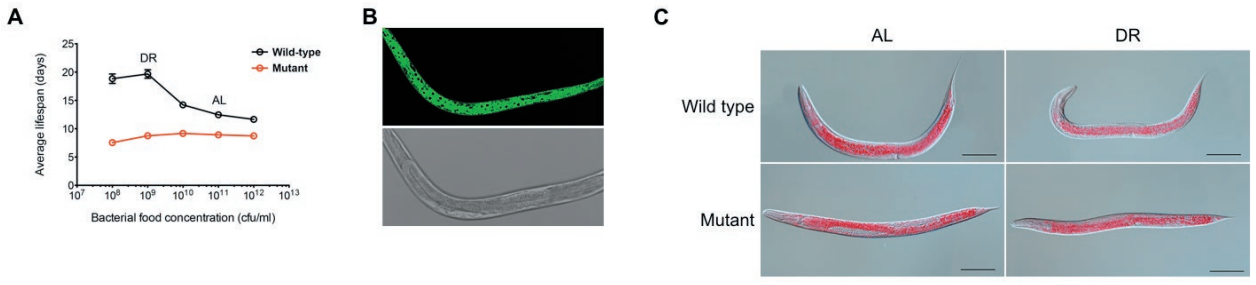

Figure 2. Characterization of lipid metabolism in dietary restriction-induced lifespan extension.

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

Recent publications (*, corresponding authors):

1. Wang D, Hou L, Nakamura S, Su M, Li F, Chen W, Yan Y, Green CD, Chen D, Zhang H, Antebi A and Han JD* (2017). LIN-28 Balances Longevity and Germline Stem Cell Number in Caenorhabditis elegans through let-7/AKT/DAF-16 Axis. Aging Cell 16(1): 113-124.
2. Gao L, Zhang R, Lan J, Ning R, Wu D, Chen D* and Zhao W* (2017). β-Dihydroagarofuran-type Sesquiterpenes from the Seeds of *Celastrus monospermus* and Their Lifespan-extending Effects on the Nematode *Caenorhabditis elegans*. *Journal of Natural Products* 79(12): 3039-3046.
3. Hou L#, Wang D#, Chen D#, Liu Y, Zhang Y, Cheng H, Xu C, Sun N, McDermott J, Mair WB, Han JD*, A Systems Approach to Reverse Engineer Lifespan Extension by Dietary Restriction. Cell Metabolism, 2016, 23(3): 529-540.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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.
10. 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.
11. 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.

Group members

Principle Investigator

Di Chen

Graduate Students

Yanling Bo
Jianfeng Lan
Zi Wang
Di Wu
Xiao Zang
Lina Zou

Lab Manager

Zixing Wu

Former Graduate Student

Xuan Zhang

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.

Contact Information

Tel : 86-25-58641529
Fax: 86-25-58641529
Email: zhums@nju.edu.cn, zhums@nicemice.cn

Smooth muscle and diseases

Smooth muscle is essential for maintaining functional homeostasis of hollow organs 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 pathogenesis of 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 mechanisms of smooth muscle contraction and calcium sensitization are still controversial. To understand the signaling mechanism of smooth muscle contraction and their functional importance in diseases, we developed a series of smooth muscle-specific knockout mice by Cre/LoxP-mediated mutagenesis with deletion of signal module genes, such as MLCK, zip kinase, MYPT1, TMEM16A and Myl-9. Our observations suggest that 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. Our findings reveal that calcium-depending signaling is the basic mechanism for all types of smooth muscle. MYPT1 deletion causes phenotypic transition of phasic and tonic smooth muscles, and the myogenic alteration by MYPT1 deletion is enough for generation of hypertension. We proposed that RhoA/ROCK/MYPT1 axis was not important for calcium-sensitized contraction, while PKC/CPI-17 pathway was critical. We also investigated the mechanism underlying asthmatic hyperresponsiveness of airway smooth muscle, and found that TMEM16A/VDCC/MLCK signaling pathway was adopted by inflammatory constrictors of asthma and hence contributed to synergistic response to nerve activity.

Skeletal muscle is another important tissue of human body and its function and size may be regulated by micro RNA at multiple levels. Our previous studies suggest that the maternally expressed miR-379/miR-544 cluster might regulate skeletal muscle growth through the imprinted Delta-like 1 homolog (Dlk1) gene, thereby underlying the polar overdominance inheritance of callipyge sheep; miRNA23a may regulate muscular fiber property through targeting myosin gene. To understand the mechanistic pathogenesis of skeletal myopathy, we assessed the contribution of intragenic micro RNAs in the pathology of centered nuclear myopathy (CNM). By using transgenic rescue experiments, we demonstrated that intragenic micro RNA involved in the pathogenesis of CNM. This finding will help us develop a therapeutic strategy for this disease.

The Neurite outgrowth requires coordinated cytoskeletal rearrangements in the growth cone and directional delivery of membrane from the neuronal soma. Triple functional domain (Trio) is necessary for cytoskeletal dynamics and regulates neurite outgrowth (Fig.1). We find that Golgi pool of Trio also regulates directional membrane trafficking by controlling the direction maintenance of both Rab8- and Rab10-positive membrane vesicles (Fig.2). GTP-bound Rab8 and Rab10 levels are decreased in Trio deficient cerebellum and constitutive-active Rab8 or Rab10 restores the neurite outgrowth deficit of Trio-deficient cerebellar granule neurons. Trio directly interacts with and activates Rabin8, a common GEF for small GTPases Rab8 and Rab10. Our study delineated a regulatory role of Trio in membrane trafficking during neurite outgrowth and suggested a crosstalk of Rho GEF and Rab GEF in controlling both cytoskeletal dynamics and membrane trafficking during neuronal development.

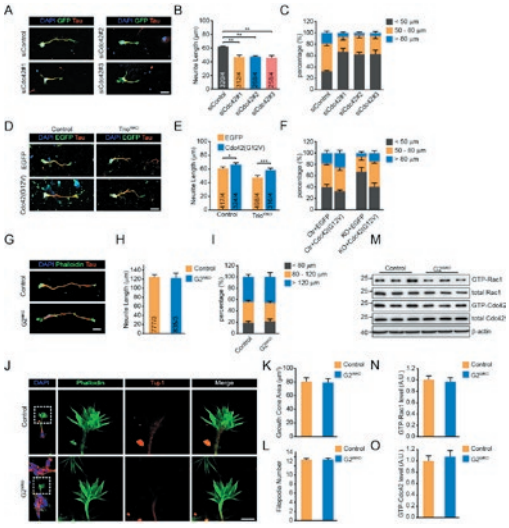

Figure 1. Cdc42 activation by Trio is required for neurite outgrowth of CGNs

(A) CGNs isolated from wild-type mice were nucleofected with either control siRNA or siRNA of Cdc42. pmaxGFP was used as a transfection marker. The transfected CGNs were cultured for 2 DIV, and then stained with DAPI and anti-Tau antibody for neurite outgrowth assay. Scale bar represents 20 μm.
(B and C) Quantification of neurite length (B) and neurite length distribution (C) from images in (A). Each group has 4 mice (n=4) and total numbers of the neurons were indicated.
(D) CGNs isolated from TrioWKO mice and their control littermates were nucleofected with pcDNA-EGFP or pcDNA-Cdc42 (G12V)-P2A-EGFP. After cultured for 2 DIV, the cells were stained with DAPI and the antibodies of EGFP and Tau. Scale bar represents 20 μm.
(E and F) Quantification of neurite length (E) and neurite length distribution (F) from images in (D). Each group has 4 mice (n=4) and total numbers of the neurons were indicated.
(G) CGNs isolated from G2NKO mice and their control littermates were cultured for 2 DIV, and then stained with DAPI, Phalloidin and Tau antibody. Scale bar represents 20 μm.
(H and I) Quantification of neurite length (H) and neurite length distribution (I) from images in (G). Each group has 3 mice (n=3) and total numbers of the neurons were indicated.
(J) CGNs isolated from G2WKO and their control littermates were cultured for 24-48 hours and then stained Phalloidin and DAPI. Tuj-1 was used as a neuronal microtubule marker. Scale bar in left panel represent 20 μm, and that in the right magnified panel represents 5 μm.
(K and L) Quantification of growth cone area (K) and filopodia number (L) from images in (J), n = 3 mice, and total 36 neurons for each group were measured.
(M) Western blot of GST-PAK1 pull-down assay to determine Rac1 and Cdc42 activity in cerebellar tissues of G2NKO and their control littermates.
(N and O) Quantification of GTP-Rac1 level (N) and GTP-Cdc42 level from Western blot in (M), n = 3 mice.

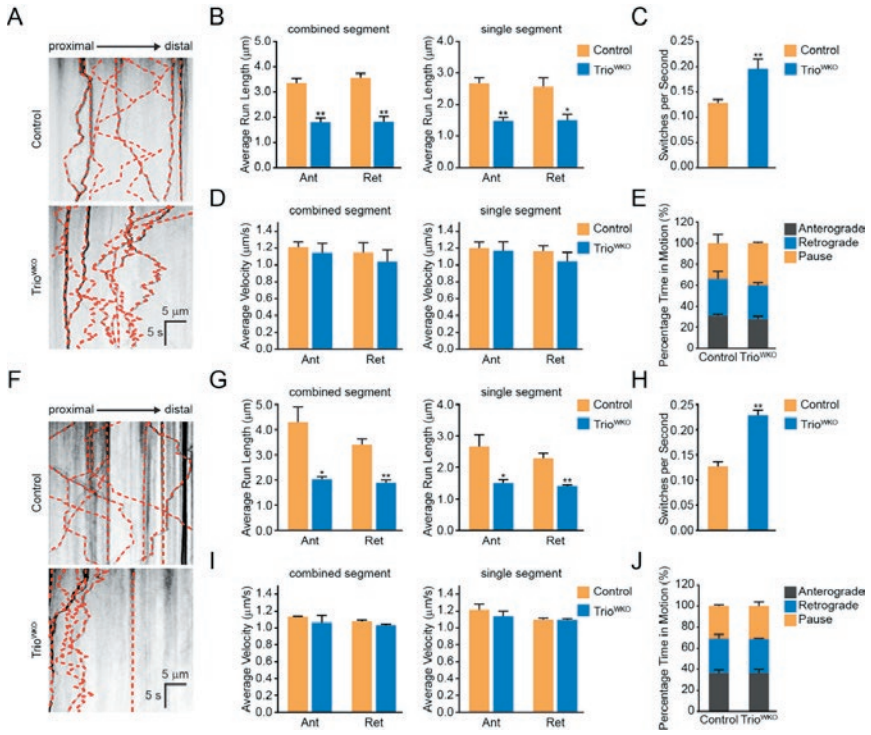

Fig.2 Trio regulates Rab8- and Rab10- positive vesicle trafficking

(A) Representative kymographs of Rab8a vesicles. CGNs isolated from TrioWKO and their control littermates were nucleofected with ptdTomato-Rab8a and cultured for 24-30 hours. Time-lapse images were acquired using GE delta Vision eLite microscope. The kymographs were generated with KymoResliceWide plugin in ImageJ. Scale bars represent 5 µm and 5 seconds, as indicated in the graph.

(B-E) Quantification of Rab8 positive vesicles movements including run length of combined and single segment (B), switching frequencies (C), velocities (D), and percentage time in motion (E). Data was statistically analyzed using KymoAnalyzer macro in ImageJ. n = 3 mice and 30 neurons per group.

(F) Representative kymographs of Rab10 vesicles. CGNs isolated from TrioWKO and their control littermates were nucleofected with ptdTomato-Rab10 and cultured for 24-30 hours. Time-lapse images were acquired using GE delta Vision eLite microscope and kymographs were generated and using KymoResliceWide plugin in ImageJ. Scale bars represent 5 µm and 5 seconds, as indicated in the graph.

(G-J) Quantification of Rab10 positive vesicles moving parameters, including run length of combined and single segment (G), switching frequencies (H), velocities (I), and percentage time in motion (J). Data were statistically analyzed using KymoAnalyzer macro in ImageJ. Data represent mean ± SEM. *p < 0.05, **p < 0.01.

Selected Publications

1. Sun J., Yang GM., Tao T., Wei LS., PanY., Zhu MS. Isometric Contractility Measurement of the Mouse Mesenteric Artery Using Wire Myography. J. Vis. Exp. (138), e58064, doi:10.3791/58064 (2018).
2. Berger SL, Leo-Macias A, Yuen S, Khatri L, Pfennig S, Zhang Y, Agullo-Pascual E, Caillol G, Zhu MS, Rothenberg E, Melendez-Vasquez CV, Delmar M, Leterrier C, Salzer JL. Localized Myosin II Activity Regulates Assembly and Plasticity of the Axon Initial Segment. Neuron. 2018;97(3):555-570
3. Yang GM, Sun J, Pan Y, Zhang JL, Xiao M, Zhu MS. Isolation and identification of a tribenzylisoquinoline alkaloid from Nelumbo nucifera Gaertn, a novel potential smooth muscle relaxant. Fitoterapia. 2018; 124:58-65.
4. Pei Wang, Wei Zhao, Jie Sun, Tao Tao, Xin Chen, Yan-Yan Zheng, Cheng-Hai Zhang, Zhong Chen, Yun-Qian Gao, Fan She, Ye-Qiong Li, Li-Sha Wei, Ping Lu, Cai-Ping Chen, Ji Zhou, Da-Quan Wang, Liang Chen, Xiao-Hao Shi, Linhong Deng, Ronghua ZhuGe, Hua-Qun Chen, Min-Sheng Zhu. Inflammatory Mediators Mediate Airway Smooth Muscle Contraction through a GPCR-TMEM16A-VDCC Axis and Contribute to Bronchial Hyperresponsiveness in Asthma. J Allergy Clin Immunol 2018;141(4):1259-1268

Group members

Group Leader Min-Sheng Zhu

Graduate Students

Tao Tao Jie Sun Yue-Qiong Li Li-Sha Wei

Yan-Yan Zheng Wei Wang Yu-Wei Zhou

Ye Wang

Postdoc Xin Chen Ph.D

Technical Assistant Zhiqing Yu

Graduated Ph.D students and current employment

Wen-Cheng Zhang, Professor of Medical School of Shandong University

Wei-Qi He, Professor of Suzhou University

Cheng-Hai Zhang, Postdoc of Medical School of Harvard University

Yan-Ning Qiao, Associate professor of Shanxi Normal University

Chen Chen, Postdoc of the Cold Spring Harbor Laboratory

Cai-Ping Chen, Instructor of China Pharmaceutical University

Yun-Qian Gao, Postdoc research fellow of Fudan University

Yanjing Peng, Postdoc research fellow of Wiscoxin University

Wei Zhao, Research Fellow of Nanjing General Hospital

Pei Wang, Postdoc research fellow of Stanford University

Chao-Jun Li, Ph.D

Chao-Jun Li received his Ph. D in Physiology from Nanjing University in 1994. He 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 worked as an extinguishing professor in Nanjing Normal University from 1994-2008 before he joined the Faulty of Model Animal Research Center (MARC), Nanjing University in 2008. He is now a professor of Cell Biology and a principal investigator in MARC and the Medical School of Nanjing University. He is elected as the vice-president of Chinese Society for Cell Biology since 2014.

Contact Information

Tel : 86-25-83596289 (Office) 86-25-83596782 (Lab)
Fax: 86-25-83596289
Email: licj@nju.edu.cn, licj@nicemice.cn

Metabolic reprogramming and protein prenylation balance

Protein prenylation is a critical process for the membrane association of plenty of signaling proteins, including small G proteins. Geranylgeranyl diphosphate synthase (GGPPS) catalyzes the formation of geranylgeranyl diphosphate (GGPP) from farnesyl diphosphate (FPP), both of which are used to prenylate proteins with CAAX motif in their carboxyl termini. The prenylated proteins then are able to associate with membrane to initiate their function. We first identified GGPPS as a directly target gene of Egr-1, which can positively feedback to increase Egr-1 accumulation during chronic stress stimulation through enhance Ras prenylation and membrane association (Am J Path, 2011a, 2011b; J Biol Chem 2011; EMBO J, 2011). The prenylation includes two type modifications of protein: farnesylation and geranylgeranylation. Our hypothesis is that the balance of protein farnesylation and geranylgeranylation or FPP and GGPP inside the cell is critical to cell homeostasis by affecting signal transduction and protein functions. Thus, we have constructed GGPPS Floxed mice and conditionally deleted GGPPS gene in different tissues to examine its functions on cell homeostasis and its involvements in human diseases. We found that GGPPS regulated protein prenylation balance is involved in spermatogenesis and infertility (J Exp Med, 2013; Sci Rep, 2016; PLoS Genetics, 2017); hypertrophy and heart failure (J Path, 2015; Cardiovasc Res. 2018); insulin granule docked pool formation (J Path, 2016); lipid-induced muscle insulin resistance (J Biol Chem, 2015; FASEB J); pulmonary development (Am J Path, 2016) and NAFLD/HCC progression (J Path, 2018). We also studied the function of protein dephosphorylation during liver injury and liver regeneration (J Hepal, 2016). Right now, we are exploring protein prenylation balance and the metabolic reprogramming like glucose/lipid shift during pathological and physiological processes.

1. Geranylgeranyl diphosphate synthase reduction accelerates hepatocellular carcinogenesis via rerouting glucose toward pentose phosphate pathway through interaction with glucose-6-phosphate dehydrogenase (Bin Xue; Chao-Jun Li)

Statin has been widely used as anti-cancer agents, however, they do exhibit potentially severe side-effects, most prominently myopathy, polyneuropathy and tumor recurrence. Looking for possible downstream targets of mevalonate pathway, which may regulate tumor development and progression, with less side-effects and more precisely, becomes an urgent priority. Here we found that, a branch point enzyme in mevalonate pathway that catalyzes the synthesis of geranylgeranyl diphosphate from farnesyl diphosphate, GGPPS was highly expressed in liver tumor tissue and regulated by DDIT3 (DNA damage-inducible transcript 3). Tissue IHC arrays showed that GGPPS improved overall survival in HCC patients and consistently, liver-specific Ggpps knockout mice directly demonstrated that GGPPS acted as defender in hepatocellular carcinogenesis. Lacking Ggpps reroutes glucose metabolism via G6PDH/PPP, meeting the cellular demands for anabolic biosynthesis and providing anti-oxidative defense. Proteomics study suggested that GGPPS interacts with G6PDH, which further affect dimerization of G6PDH and subsequent enzyme activity.

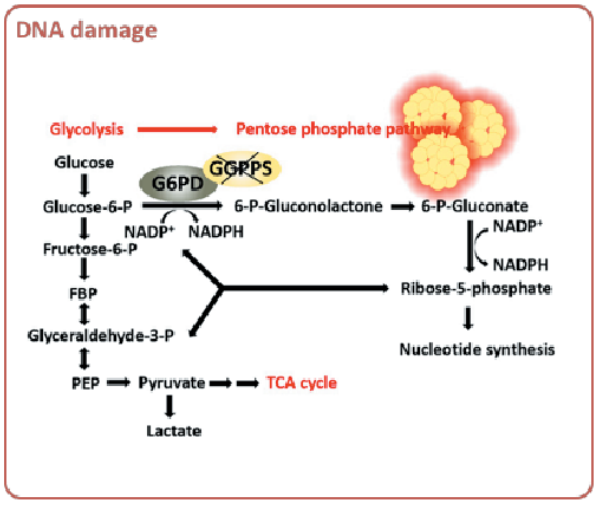

Figure1 Geranylgeranyl diphosphate synthase reduction accelerates hepatocellular carcinogenesis via rerouting glucose toward pentose phosphate pathway through interaction with glucose-6-phosphate dehydrogenase

2. GGPPS-mediated Lipid Droplet Size in Liver Determines the Distinction of metabolically abnormal obese and metabolically-healthy obesity (Yue Zhao; Chao-Jun Li)

Hepatic steatosis, a typical feature of non-alcoholic fatty liver disease (NAFLD), is characterized by the ectopic accumulation of lipid droplets (LDs) in hepatocytes. Hepatic lipotoxicity often leads to systemic insulin resistance (IR). In this study, we find that Ggpps expression is increased in the liver of metabolically abnormal obese (MAO) with large LD size, compared with that of metabolically-healthy obesity (MHO) with small LD size. Additionally, specific inactivation of Ggpps in the liver reduces hepatic LD size and insulin resistance in vivo and in vitro. Mechanistically, Ggpps-mediated Perilipin4 prenylation regulates LD formation in prediabetic state, which determine the LD size and insulin sensitivity. Furthermore, inhibition of Ggpps, either through DGBP in vitro or siRNA in vivo, attenuates hepatic lipid accumulation from high-fat diet feeding. Collectively, our data reveal that lipid droplet morphology of hepatocytes might be a predictive factor for the obesity-induced diabetes, underlying the distinction between MAO and MHO, and provide a new therapeutic strategy to combat hepatic steatosis.

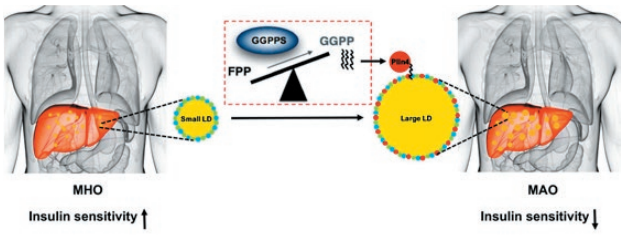

Figure2 Geranylgeranyl diphosphate synthase mediates lipid droplet morphology of hepatocytes, predicting the obesity-induced diabetes and underlying the distinction between MAO and MHO

3. Quantitative map of proteome dynamics during postnatal heart development reveals a particular cluster of proteins potentially accompanied with cardiac regeneration in mammal (Lei Fang; Chao-Jun Li)

Heart failure, the end-stage of almost all of the cardiovascular diseases like myocardial infarction, has been emerging as a global public health concern in recent decades. Current therapeutic approaches, either heart transplantation or stem cell-based therapies, exhibit their inevitable limitations, respectively. To reduce the mortality and limit the necessity for cardiac transplantation, innovative trials based on stimulating endogenous heart regeneration are urgently required. It is commonly accepted that neonatal mammalian heart possesses considerable regeneration capacity with a one-week window after birth, while the mature mammal almost entirely loses this capacity. Numerous reports have shown that the loss of regeneration capacity in heart is tightly regulated by selectively gene expression at different heart developmental stages. In order to systematically study the proteomic dynamics accompanied with regeneration capacity loss, we collected ventricular tissues from mice at different time points during the heart development, and monitored the global protein expression patterns using an iTRAQ (isobaric tags for relative and absolute quantification) based quantitative proteomic approach. In our results, 3,755 proteins were detected, while 3,379 proteins were quantified. By soft clustering analysis, we found the dominating cluster demonstrated concordant expression pattern with cardiomyocyte markers. Intriguingly, a particular cluster of proteins were revealed by showing remarkably high expression in one-week window but low expression in adult hearts. More importantly, among them, several proteins such as HMGB1 and Agrin have been reported to promote cardiac regeneration. Further bioinformatics analysis demonstrated that these proteins were involved in a variety of biological processes including cellular metabolic process, establishment of localization, positive regulation of cell adhesion. Thus, in this study we've established a quantitative map of proteome dynamics during postnatal heart development, which could be a rich resource for further mechanism study of cardiovascular regeneration.

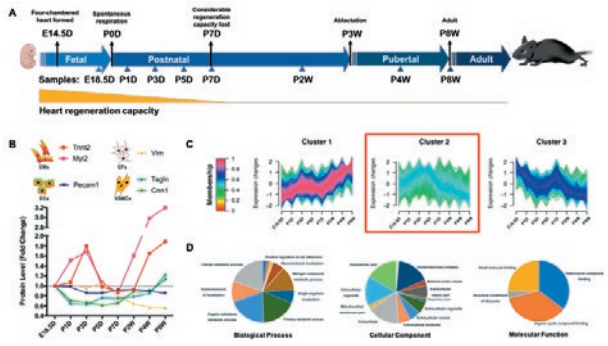

Figure3 Quantitative map of proteome dynamics during postnatal heart development

(A)After four-chambered heart formed, the heart undergoes complicated and labyrinthian transition to adult status during postnatal development. Rapid and characteristic molecular alterations at the indicated timepoints are associated with a range of life events, including spontaneous respiration, rapid development in the early stage, ablactation, pubertas, etc.; (B)Expression pattern of cell type markers in our dataset; (C)Soft clustering of proteome dynamics revealed three clusters with distinct protein expression profiles. (D)Heatmap represents the membership abundance of proteins per cluster;

Gene ontology enrichment analysis of Cluster 2 was performed using a Fisher's exact test (cutoff $p < 0.002$, Benjamini-Hochberg corrected). Representative biological processes (BP), molecular functions (MF), and cellular components (CC) that are overrepresented in the cluster are visualized.

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

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2. Chen Z, Xu N, Chong D, Guan S, Jiang C, Yang Z, Li C.*. Geranylgeranyl pyrophosphate synthase facilitates the organization of cardiomyocytes during mid-gestation through modulating protein geranylgeranylation in mouse heart. Cardiovasc Res. 2018 Jun 1;114(7):965-978. (Commentary by Helen M. Phillips. Protein geranygeranylation: a possible new player in congenital heart defects. Cardiovasc Res. 2018;114:922-924)
3. Chen Jiang#, Fan Diao#, et al., Chao-Jun Li*. GGPP-mediated protein geranylgeranylation in oocyte is essential for the establishment of oocyte- granulosa cell communication and primary-secondary follicle transition in mouse ovary. PLoS Genetics, 2017, 13(1): e1006535.
4. Shan-Shan Lai,et al., Xiang Gao*, Chao-Jun Li*, Bin Xue* PP2Acα Positively Regulates Mice Liver Regeneration Termination through AKT/GSK3β/Cyclin D1 Pathway. J Hepatology 2016, 64(2):352-360
5. Shan Jiang#, Di Shen#, et al., Bin Xue*, and Chao-Jun Li*. GGPPS mediated Rab27A geranylgeranylation regulates β-cell dysfunction during type 2 diabetes development via affecting insulin granule docked pool formation. J Pathol, 2016; 238: 109-119. (Commentary by Kowluru A. A lack of "glue" misplaces Rab27A to cause islet dysfunction in diabetes. J Pathol. 2016; 238: 375–377)
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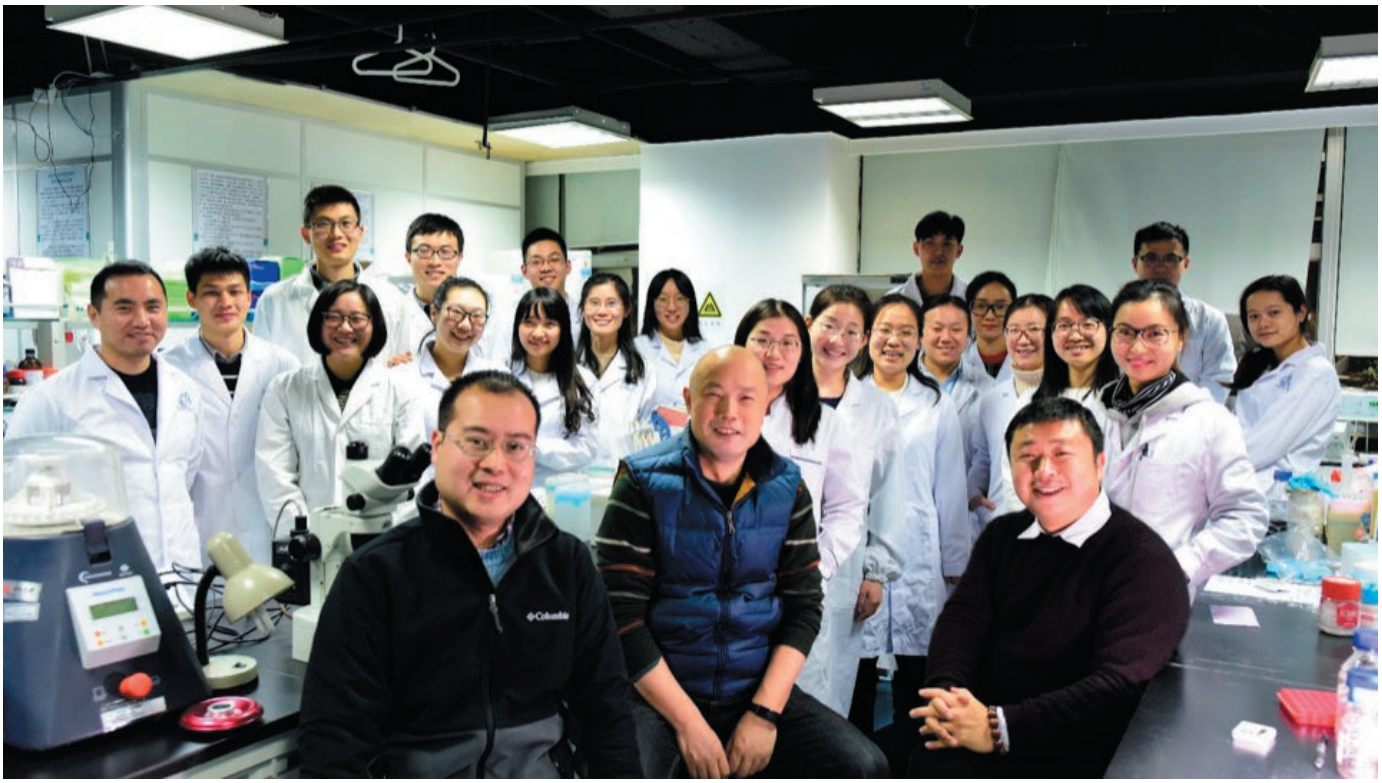

Group members

Group Leader
Chaojun Li

Associate professor

Bin Xue:
Ph.D of Nanjing Normal University, visiting scholar of Medical School of Harvard University (xuebin@nju.edu.cn)

Lei Fang:
Ph.D of Nanjing University at 2000, postdoctoral of University of California at Irvine, Assistant research scientist of Langone Medical Center of New York University (njfanglei@yahoo.com)

Research Associate

Yue Zhao:
Ph.D of Fudan University (zhyue@nju.edu.cn)

Graduate Students:
Jing-Zi Zhang (Ph.D.)
Di Shen (Ph.D.)
Rui-Lou Zhu (Ph.D.)
Jing Wu (Ph.D.)
Dan-Yang Chong (Ph.D.)
Yong-Juan Sang (Ph.D.)
Kang Li (Ph.D.)
Tong-Yu Zhang (Ph.D.)
Wei-Wei Chen (Ph.D.)
Feng Zheng (M.D.)
Yue Hua (M.D.)
Ruo-Yu Zhao (M.D.)
Meng-Fei Zhao (M.D.)
Qi Cheng (M.D.)
Hai-Quan Wang (M.D.)
Bing-Hao Wang (M.D.)
Xin-Ying Wang (M.D)
Yang-Qing Li (M.D.)
Dan-Dan Bu (M.D.)
Xiao-Yan Shao (M.D.)

Former graduate students

Xiu-Xing Wang (2012):
Assitant project scientist, Department of Medicine, UCSD. La Jolla, CA, USA

Ning Shen (2013):
Founder, Asia-vector Biotechnology Ltd. Shanghai, China.

Na Xu (2014):
Postdoc Fellow, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA.

Shan Jiang (2015):
Postdoc Fellow, Karolinska Institutet Solna, Sweden.Zongchao Sun Chenyun Ding

Fan Diao (2015):
Postdoc Fellow, McGill, Pharmacology & Therapeutics, Canada

WeiWei Tao (2015):
Postdoc Fellow, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA.

Chen Jiang (2015):
PhD. student, DFG-funded PhD program, Philipps-University Marburg, Germany

Shan-Shan Lai (2016, Postdoc):
Lecture, Nanjing Normal University

Dan-Dan Zhao (2016):
Research Assistant, Shanghai Chest Hospital, Shanghai

Yaling Qi (2017):
Research Assistant. Nanjing Medical School, Nanjing

Jia Liu (2018):
Project Manager. Geneseeq Technology Inc., Nanjing

Zhong Chen (2018):
Project Manager. GemPharmatech Co.,Ltd. Nanjing

Zhenji Gan, Ph.D.

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

Contact Information

Tel : +86-25-58641546 (Office) +86-25-58641569 (Lab)
Fax: +86-25-58641500
Email: ganzj@nju.edu.cn, ganzj@nicemice.cn

Energy metabolism and muscle fitness

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

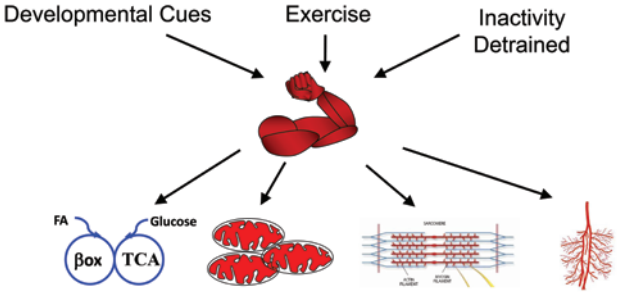

Fig 1. The muscle fitness is determined by developmental as well as physiological inputs. These inputs coordinately control the programs including fuel burning, mitochondrial ATP production, contraction, and angiogenesis.

Delineate the nuclear receptor/microRNA networks controlling muscle fitness.

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

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

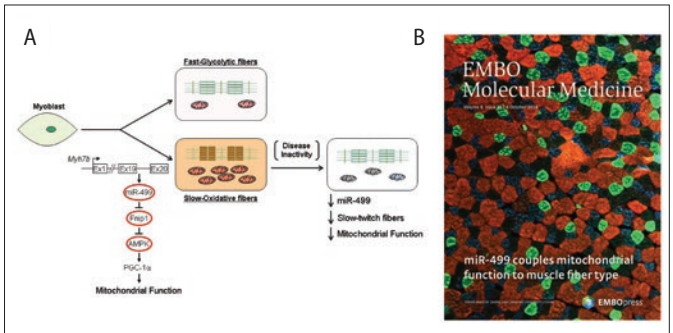

Fig 2. (A) The schematic depicts a proposed model for the miR-499/Fnip1/AMPK circuit that orchestrates mitochondrial function to match muscle contractile machinery during fiber type transition. (B) EMBO Mol Med. October 2016 Cover.

Skeletal muscle mitochondrial remodeling upon adaption to exercise and diseases.

Mitochondria are essential organelles that require continuous surveillance to maintain its functional integrity. The quality of mitochondria is particularly importance in skeletal muscle, the largest metabolic demanding tissue that depends critically on mitochondrial function, accounting for ~40% of total body mass. Skeletal muscle mitochondrial dysfunction has been implicated in the pathogenesis of many diseases including muscular dystrophy, atrophy, obesity, type 2 diabetes and aging-sarcopenia. Conversely, exercise counteracts the effects of many chronic diseases on skeletal muscle mitochondrial function. Recent studies have revealed a finely tuned regulatory network that orchestrates skeletal muscle mitochondrial biogenesis and mitochondrial maintenance in response to exercise and in disease states.

For example, mitophagy serves as a major quality-control mechanism for selective targeting and removal of damaged or dysfunctional mitochondria to ensure metabolic demands. There is increasing evidence also suggest that mitochondria might "communicate" with nucleus and mediate the beneficial effects of mild mitochondrial stress. However, the in vivo physiological relevance and molecular working mechanisms of mitochondrial quality control remain unclear. We are very interested in exploring the dynamic remodeling and molecular mechanism that underlying the adaptation of skeletal muscle mitochondria to exercise and in disease states (Fig. 3).

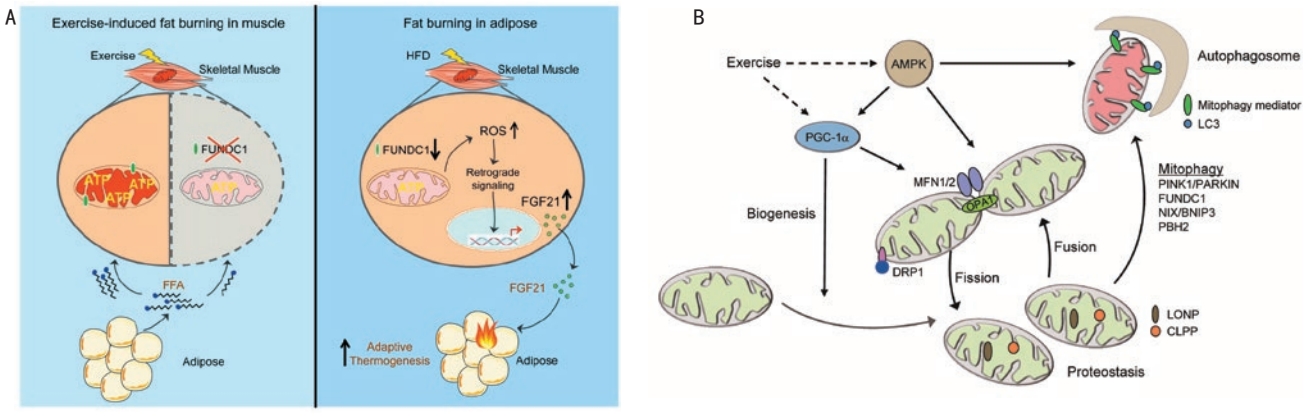

Fig 3. (A) The schematic depicts a proposed model for mitochondrial quality directs muscle-adipose dialog that regulates systemic metabolism. (B) Muscle mitochondria undergo extensive turnover and remodeling in response to a variety of physiological stimuli.

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1. Gan Z*, Fu T, Kelly DP*, Vega RB*. Skeletal muscle mitochondrial remodeling in exercise and diseases. Cell Research. 2018;0:1-12.
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Group members

Group Leader

Zhenji Gan

Postdoctoral Fellow

Jing Liu

Graduate students

Tingting Fu
Lin Liu
Liwei Xiao
Zhisheng Xu
Yujing Yin
Zongchao Sun
Chenyun Ding
Qiqi Guo
Wanping Sun
Yan Mao

Former Graduate Students

Jing Liu (Ph.D.)
Xijun Liang (Ph.D.)
Qian Zhou (MS)

Technical Assistant

Danxia Zhou

Zongde Zhang, Ph.D.

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

Contact Information

Tel: +86-25-58500905(office)

Email: zongdez@nicemice.cn

Microbiota induced immune system maturation, Dysbiosis-related diseases

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

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

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

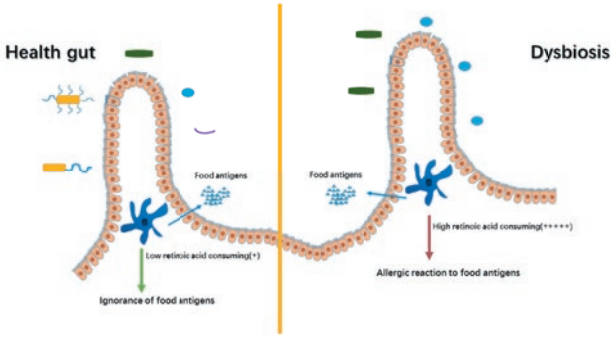

Figure 1. Microbiota repress food allergic challenge via modulating dendritic cells retinoic acid response

Microbiota upregulation of CTGF/CYR61 decorating High Endothelial Venules

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

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

Selected publications: (*corresponding author)

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Hong-Yu Wang, Ph.D.

Dr. Hong-Yu Wang gained a PhD in Plant Molecular Genetics from Saarland University in 2006 and following Postdoctoral Research posts at University of Dundee and the University of Oxford, joined Model Animal Research Center in 2012.

Contact Information

Tel: +86-25-58641589 (Office)
Fax: +86-25-58641500
Email: wanghy@nicemice.cn

The liver is a key organ in vertebrates, which has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of chemicals for digestion. Nonalcoholic fatty liver disease (NAFLD) is a range of condition caused by the hepatic fat accumulation, which is also considered the hepatic manifestation of metabolic syndrome affecting about one-third of the population worldwide. Up to 25% of NAFLD patients develop a progressive inflammatory and damaged liver disease termed non-alcoholic steatohepatitis (NASH) that may progress towards cirrhosis, hepatic carcinoma, and the need for liver transplantation. Yet, the pathogenesis of NAFLD/NASH has not been completely elucidated. However, insulin resistance, inflammatory cytokines, and oxidative stress are thought to be important in the development and/or progression of the disease. Lifestyle modification with exercise and diet has been the first step in NAFLD/NASH treatment.

Our laboratory aims to understand the molecular mechanisms of the development and progression of NAFLD/NASH. Lipidomics, biochemistry, cell biology and transgenics approaches are applied to identify novel components for diagnosis and intervention of NAFLD/NASH progressions.

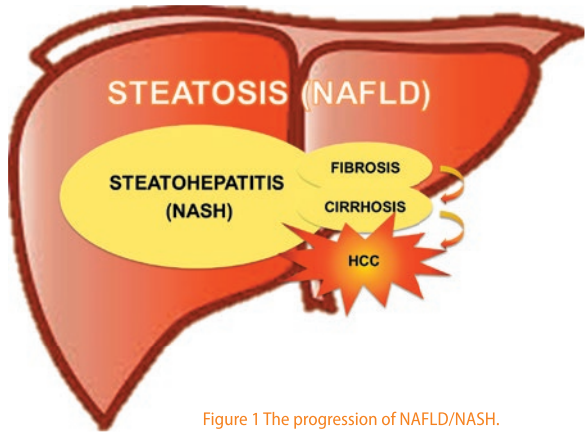

Figure 1 The progression of NAFLD/NASH.

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1. Chen Q, Rong P, Xu DJ, Chen L, Xie B, Sheng Y, Li P, Wang HY* and Chen S*. (2017) Muscle-specific Rab8a deletion impairs lipid uptake and storage in skeletal muscle and causes hyperlipidemia and hepatosteatosis in mice. Diabetes. 66(9):2387-2399.
2. Chen Q, Xie B, Zhu S, Rong P, Sheng Y, Ducommun S, Chen L, Quan C, Li M, Sakamoto K, MacKintosh C, Chen S* and Wang HY* (2017) A TBC1D1Ser231Ala knockin mutation partially impairs 5-aminoimidazole-4-carboxamide-1-ß-D-Ribofurano-side- but not exercise-induced muscle glucose uptake in mice. Diabetologia 60(2): 336-345
3. Wang HY*, Quan C, Hu B, Xie, BX, Du Y, Chen L, Yang W, Yang L, Chen Q, Shen B, Hu B, Zheng ZH, Zhu HB, Huang XX, Xu GW, Chen S. (2016) A lipidomics study reveals hepatic lipid signatures associating with deficiency of the LDL receptor in a rat model. Biol Open. Jul 15; 5(7): 979-86.
4. Wang H-Y, Ducommun S, Quan C, Xie BX., Li M., Wasserman DH, 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

Group members

PI

Hong-Yu Wang

Graduate students

Qian Ouyang

Shu Su

Yan LI Ph.D., M.D.

Dr. Yan LI acquired his Ph.D in 2012 from Singapore-MIT alliance program under the supervision of MIT Professor Jianzhu CHEN. He completed post-doctoral training with Prof. James DI SANTO in Institut Pasteur, Paris. He is currently appointed as a Professor in Model Animal Research Center of Nanjing University and a joint-PI in Drum Tower Hospital. He has established and extended a series of humanized immune system mouse models that allowed him to probe fundamental questions in human immunology, especially in relation to the area of immunotherapy and infectious diseases.

Contact Information

Tel (Office): 025-58500960
Tel (Lab): 025-58500959
Email: yanli@nicemice.cn

Next-generation humanized mouse models for translational medicine

In past 10 years, the development of 'humanized' mice bearing human immune systems (HIS mice) has greatly advanced our understanding of human innate and adaptive immunity behind infectious diseases, vaccines and cancers. It is now possible to generate mice with robust, stable human hematopoiesis that includes not only multiple lymphocyte subsets but also diverse myeloid cell lineages. Our lab aims to develop next-generation of HIS mice to overcome remaining limitations of current models, and apply HIS mice in diverse disciplines of translational medicine.

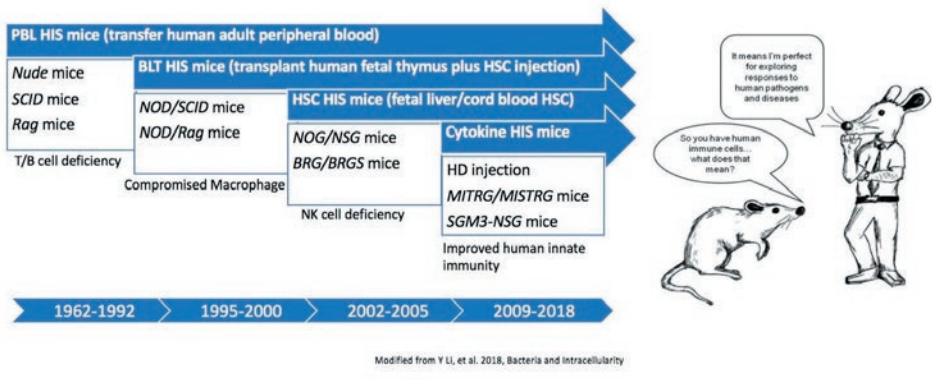

Figure 1. The development of HIS mouse models

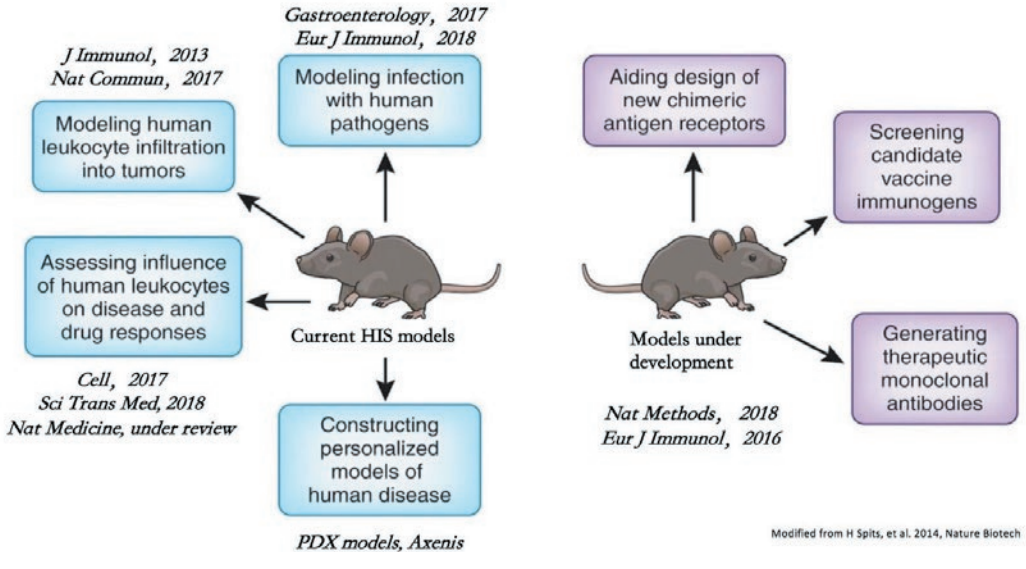

Figure 2. Application of HIS mice in basic research and translational medicine

Selected Publications

1. Yan Li, Guillemette Masse Ranson, Zacarias Garcia, Timothee Bruel, Ayrin Kok-Harunova, Helene Strick-Marchand, Gregory Jouvion, Nicolas Serafini, Ai Ing Lim, Mathilde Dusseaux, Thierry Hieu, Frank Bourgade, Antoine Toubert, Daniela Finke, Oliver Schwartz, Phillippe Bousso, Hugo Mouquet and James P. Di Santo. (2018) A human immune system mouse model with robust lymph node development. Nature Methods DOI: 10.1038/s41592-018-0071-6.
2. Emmanuel Clave, Itauá Leston Araujo, Cécile Alanio, Etienne Patin, Jacob Bergstedt, Alejandra Urrutia, Silvia Lopez-Lastra, Yan Li, Bruno Charbit, Cameron Ross MacPherson, Milena Hasan, Breno Luiz Melo-Lima, Noemie Saut, Marine Germain, David-Alexandre Tregouet, Pierre-Emmanuel Morange, Magnus Fontes, Darragh Duffy, James P. Di Santo, Lluis Quintana-Murci, Matthew L.Albert, Antoine Toubert, for The Milieu Intérieur Consortium. (2018) Human thymopoiesis is influenced by a common genetic variant within the TCRA-TRCD locus. Science Translational Medicine. DOI: 10.1126/scitranslmed.aao2966
3. Yan Li and James P. DI Santo. (2018) Yin and yang of regulatory T cells in immunotherapy. Oncotarget DOI: 10.18632/oncotarget.24394.
4. Yan Li, Helene Strick-Marchand, Ai Ing Lim, Jiazi Ren, Guillemette Masse-Ranson, Dan Li, Gregory Jouvion, Lars Rogge, Sophie Lucas, Bin Li and James P. Di Santo. (2017) Regulatory T cells control toxicity in a humanized model of IL-2 therapy. Nature Communications DOI: 10.1038/s41467-017-01570-9.
5. Mathilde Dusséaux, Guillemette Masse-Ranson, Sylvie Darche, Yan Li, Elodie Beaumont, Lise Rivière, Christine Neuveut, Patrick Soussan, Philippe Roingeard, Dina Kremsdorf, James P. Di Santo, Helene Strick-Marchand. Viral load affects the immune response to HBV in mice with humanized immune system and liver. Gastroenterology. (2017) DOI: 10.1053/j.gastro.2017.08.034.
6. Ai Ing Lim, Yan Li, Silvia Lopez-Lastra, Ralph Stadhouders, Franziska Paul, Armanda Cassrouge, Nicolas Serafini, Anne Puel, Laura Surace, Guillemette Masse-Ranson, Helene Strick-Marchand, Lionel Le Bourhis, Roberto Cocchi, Davide Topazio, Paolo Graziano, Lucia Anna Muscarella, Lars Rogge, Jean-Michel Sallenave, Matthieu Allez, Thomas Graf, Rudi W. Hendriks, Jean-Laurent Casanova, Ido Amit, Hans Yssel and James P. Di Santo. (2017) Systemic human ILC precursors provide a substrate for tissue ILC differentiation, Cell. DOI: 10.1016/j.cell.2017.02.021.
7. Silvia Lopez-Lastra, Guillemette Masse-Ranson, Oriane Fiquet, Sylvie Darche, Nicolas Serafini, Yan Li, Mathilde Dusséaux, Helene Strick-Marchand and James P. Di Santo. (2017) A functional DC crosstalk promotes human ILC homeostasis in humanized mice. Blood Advances. DOI: 10.1182/ bloodadvances.2017004358.
8. Yan Li, Jean-Jacques Mention, Nathalie Court, Antoine Toubert, Hergen Spits, Nicolas Legrand, Erwan Corcuff, Helene Strick-Marchand and James P. Di Santo. (2016) A novel Flt3-deficient HIS mouse model with selective enhancement of human DC development. European Journal of Immunology. DOI: 10.1002/eji.201546132.

Group members

PI

Yan LI

Graduate student

Wei LIU

Siqi LI

Shujie Zhang

Technician

Wei ZHAO

Cancer and Stem Cell Biology

Geng Liu, Ph.D.

Geng Liu received his B.S. degree in Biochemistry from Wuhan University, China and his Ph.D. degree in Gene & Development from University of Texas Graduate School of Biomedical Sciences at Houston in 1999. After his postdoctoral training at University of Texas M.D. Anderson Cancer Center, Dr. Geng Liu joined the Model Animal Research Center of Nanjing University as a principal investigator and professor of Genetics in 2006.

Contact Information

Tel : 025-58641515

Email: liugeng@nicemice.cn

Stress responses, metabolism and tumorigenesis

Our laboratory is interested in studying the determinants of cell behaviors and their close connections with stress responses and cellular metabolism in the contexts of tissue homeostasis as well as cancer. Integral to their functions, various cell behaviors are dictated by extrinsic and intrinsic stimuli through a network of signaling mechanisms. We investigated how stress response as mediated by the p53 signaling pathway regulated cell behaviors including cell proliferation, cell competition, inflammatory response and Epithelial-Mesenchymal transition. Another research direction is on cellular metabolism. While cellular metabolisms are required for the execution of proper cell functions, they could also serve as a signaling module in adapting the cells to certain behaviors. Dissecting the intricate interplay between cell behaviors, stress responses and metabolism may allow us to fully understand the complex cell behaviors in many fundamental processes including development, ageing and tumorigenesis.

1. p53 stress response pathway influences cell behaviors in distinctive manners

p53 is extremely important for stress response and tumor suppression as exemplified by its mutations found in over 50% of human cancers. p53 protein is undetectable in normal tissues. With the newly established BAC transgenic p53 reporter mice, we revealed a regulatory mechanism controlling p53 expression and activity selectively in the proliferating cellular compartments during mouse development, postnatal growth, tissue homeostasis, regeneration and tumorigenesis (Chen, et al., 2015). The close monitoring of cellular proliferation state by p53 also serves as a base to generate genetic tools in studying the cardiomyocyte proliferation during heart regeneration (Xiao, et al., 2017).

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). p53 also play a crucial role in macrophage polarization in the tumor microenvironment to affect tumorigenesis in a non-cell autonomous manner (He, et al., 2015). Our more recent study found that mild p53 activation in cells renders them less competitive in multi-cellular context during mouse embryogenesis, possibly contributing to the control of tissue fitness (Fig.1, Zhang, et al, 2017). These results indicate that p53 signaling pathway critically and delicately influence cell behaviors and functions in distinctive manners.

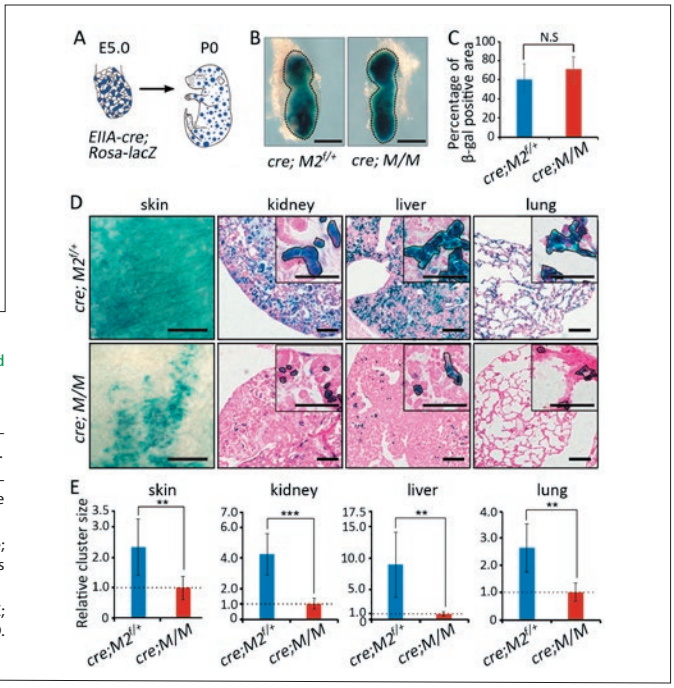

Figure 1. Cells with the $Mdm2^{+/-}$ $Mdm4^{+/-}$ genotype were out-competed in EIIA-cre induced mosaic embryos.

(A) A schematic presentation of the mosaic mice.

(B) Whole-mount X-gal staining of E6.5 EIIA-cre; Rosa-lacZ; $Mdm2^{flox/+}$ ($M2^{f/+}$) and EIIA-cre; Rosa-lacZ; M/M embryos. Embryonic tissues were marked by dotted lines. Scale bars represent 1mm.

(C) Percentages of β-gal expressing cells in E6.5 EIIA-cre; Rosa-lacZ; $Mdm2^{flox/+}$ ($M2^{f/+}$) and EIIA-cre; Rosa-lacZ; M/M embryos (n=6). Data are presented as Mean±SD. Statistical significance was determined by Student's two-tailed t test. N.S. means not significant.

(D) X-gal staining of tissues from neonatal EIIA-cre; Rosa-lacZ; $Mdm2^{flox/+}$ ($M2^{f/+}$) and EIIA-cre; Rosa-lacZ; M/M mice. Insets indicated higher magnification. Areas marked with dotted lines indicated cell clusters. Scale bars represent 100μm.

(E) Quantifications of the size of cell clusters in skin, kidney, liver and lung of EIIA-cre; Rosa-lacZ; M/M and EIIA-cre; Rosa-lacZ; $Mdm2^{flox/+}$ ($M2^{f/+}$) mice (n=4). Data are presented as Mean±SD. **p≤0.01, ***p≤0.001 (two-tailed t test).

2. Cellular metabolism and its link to cell behavior and stress responses in vivo

To begin studying the influence of cellular metabolism on cell behaviors and function, we are currently in the process of addressing how different metabolic preferences link to or affect cell behaviors and systemic homeostasis in a multitude of in vivo contexts. We have established a series of BAC transgenic mice expressing key metabolic enzymes involved in glycolysis, glutaminolysis, fatty acid synthesis and one carbon metabolism in a controlled manner. Our preliminary results showed that cellular metabolisms could be manipulated in vivo and may have great impact on either cell behavior or systemic homeostasis. These new genetic tools will greatly facilitate the analysis of metabolic advantage, cooperation, switch, adaptation and homeostasis in various contexts in the future. In addition, cell metabolism is regulated by a complex network of signaling pathways including those of stress responses. We are interested in studying the metabolic heterogeneity within the tissues and their possible link to stress response in physiological contexts.

Publications

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3. Wang J, Ye H, Zhang D, Cheng K, Hu Y, Yu X, Lu L, Hu J, Zuo C, Qian B, Yu Y, Liu S, Liu G, Mao C, Liu S (2017) Cancer-derived circulating MicroRNAs promote tumor angiogenesis by entering dendritic cells to degrade highly complementary MicroRNAs. Theranostics. 2017 Mar 24; 7(6):1407-1421.
4. Zhang CX, Zhang Q, Xie YY, He XY, Xiang C, Hou XS, Zhou Y, Chen L, Zhang GX and Liu G* (2016) MDM 2 actively suppresses p53 activity in oocytes during mouse folliculogenesis. Am J Pathol. 2017 Feb; 187(2):339-351. Epub 2016 Nov 29.
5. 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 Rep. 2015 Nov 3; 13(5):888-97.
6. Chen L, Zhang GX, He XY, Zhang CX, Xie YY and Liu G* (2015) BAC transgenic mice provide evidence that p53 expression is highly regulated in vivo. Cell Death Dis. 2015 Sep 17; 6: e1878.
7. 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.
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Group members

Postdoctoral fellow

Xie Yinyin

Graduate students

Xiang Cong
Liu Lulu
Hou Xiaoshuang
Sun Aina
Hou Weikang
Zhang Yannan
Peng Yamei
Zheng Fangfang

Technical staff

Ren Yan

Former lab members

Zhang Qin
Zhang Chenxi
Chen Lai
Zhang Guoxin
He Xueyan
Zhou Ying

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.

Contact Information

Tel : +86-25-58641535 (Office) +86-25-58641542 (Lab)
Fax: +86-25-58641500
Email: yanjun@nicemice.cn ; yanjun@nju.edu.cn

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

1)It is urgent to distinguish and dissect the mechanism indolent and lethal prostate cancer (PCa), particular in castration resistance stage. c-Myc is an oncogene, frequently amplified and/or overexpressed in aggressive PCa. However, it is only detected overexpressed at protein level, without obvious gain of DNA copy or elevated mRNA level in some PCa patients. Nowadays, we reconciled previous discrepancies on the altered expression level of heterochromatin protein 1γ (HP1γ) in PCa and characterized its oncogenic role and a poor prognostic biomarker in PCa patients. Notably, we identified a novel c-Myc/HP1γ/miR-451a regulatory circuitry to maintain c-Myc overexpression in the aggressive PCa patients, which is confirmed in a public TCGA database (Fig. 1). Our data suggesting targeting either of the components in this circuitry will interrupt this vicious circle (Chang C, et al. Oncogene 2018).

2)Metastatic castration resistant prostate cancer (mCRPC) is a devastating stage for PCa patients, without many therapeutic approaches for clinicians. The development of resistance to the 2nd generation of androgen receptor (AR) antagonist, Enzalutamide, leads to the amplification, genetic mutation or alternative splicing of AR. Therefore, it is urgent to develop novel agents to overcome enzalutamide resistance. In collaboration with the group from Northwestern Forest University, we characterized triptolide, one chemical derived from the Chinese herb thunder god vine, possesses anti-cancer effects against CRPC cells by targeting XPB at nM level to suppress both AR and AR-V7 transcription activities. The combined treatment of triptolide and enzalutamide suppressed CRPC cell survival in vivo, without showing obvious side effects (Han Y, et al, Theranostics 2017). We also screened out a chemical library of spirocyclopropyl oxindoles and found several potential novel chemicals which target AR and AR-V7 signaling, as well as NFκB signaling (Xu P, et al, Nat Commun 2017 and J Org Lett 2018.). The further study is ongoing.

3)As a small population in solid tumor, cancers stem-like cells (CSCs) are resistant to conventional chemotherapeutic agents. To identify such molecular mechanisms, we established bladder cancer xenograft model, treated with gemcitabine in a clinical regimen. We identified the increased CSC population percentage in chemo-resistant xenografts with the dysregulation of TGFβ1/lncRNA-LET, accounting for such chemo-resistance. Treatment with a clinical trial TGFβRI inhibitor, LY2157299, significantly delayed the chemoresistance to gemcitabine. Mechanistically, the reduced lncRNA-LET by TGFβ1 stabilizes NF90 and interferes the biogenesis of tumor suppressive miR-145, eventually leading to the increase of CSC population, which was proved in human UBC samples (Fig. 2; Zhuang J, et al, Theranostics 2017). Moreover, we also identified Wnt7a promotes UBC metastasis through canonical Wnt pathway, whereas its expression is inhibited by a tumor-suppressive miR-370-3p (Fig. 3; Huang X, et al. J Biol Chem 2018.).

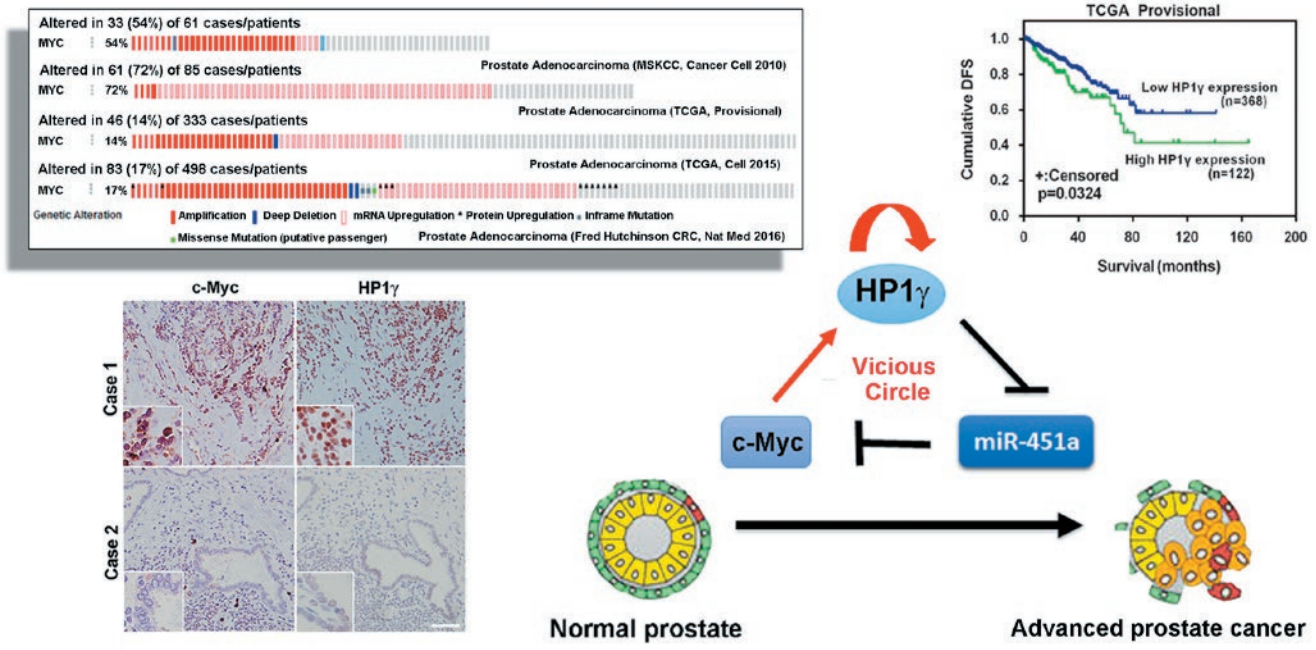


Fig 1. A vicious regulatory circuitry of c-Myc/HP1γ/miR-451a determines prostate cancer aggressiveness (Chang C, et al. Oncogene 2018).

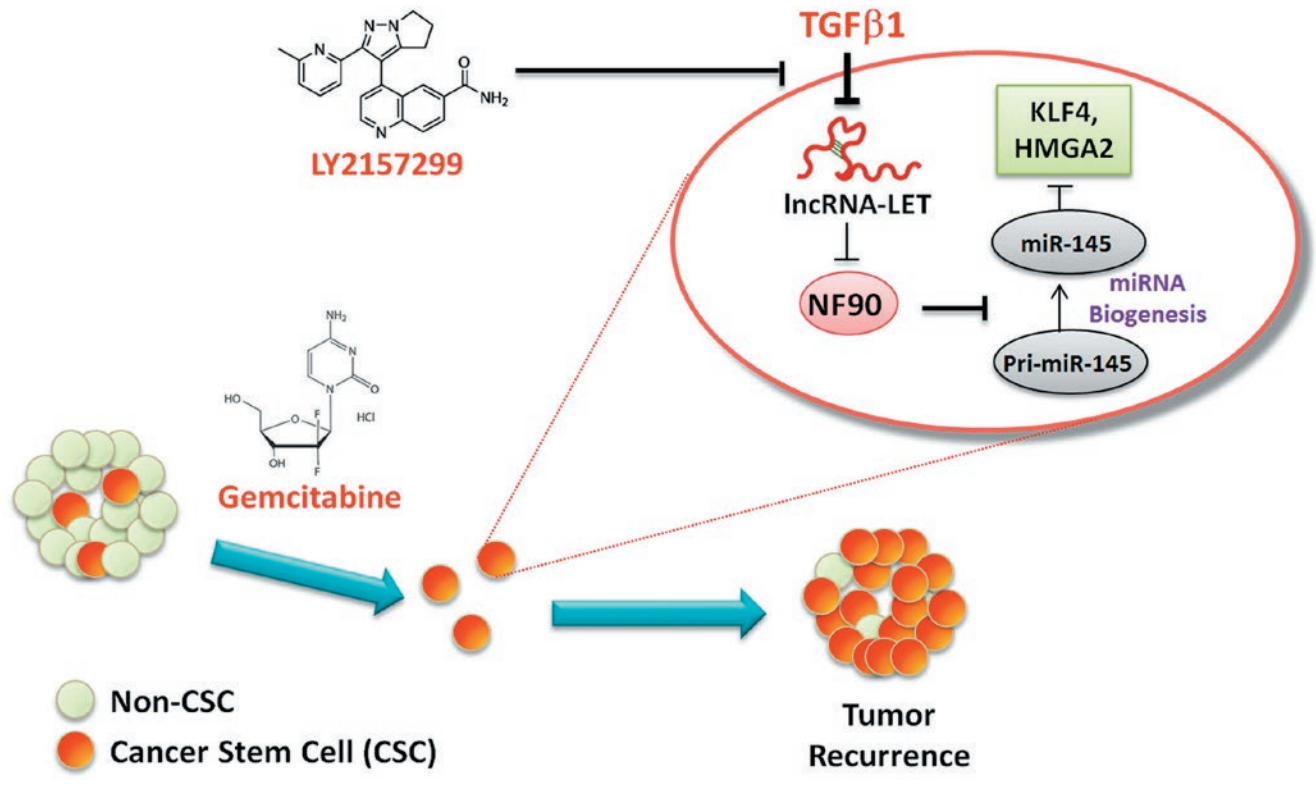


Fig 2. Suppression of bladder cancer stemness through targeting TGFβ1 signaling enhances chemotherapy to Gemcitabine (Zhuang J, et al. Theranostics 2017).

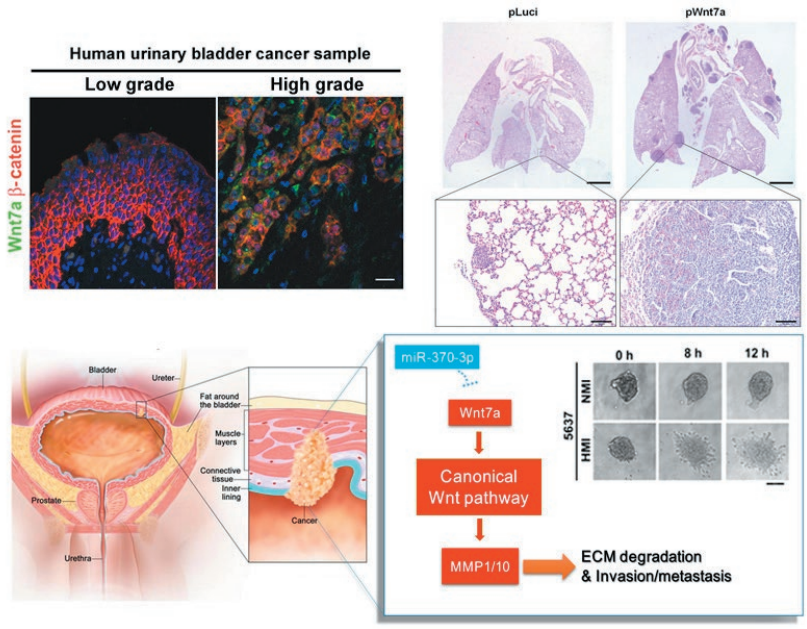


Fig 3. Wnt7a promotes bladder cancer metastasis through canonical Wnt pathway, which is inhibited by miR-370-3p (Huang X, et al. J Biol. Chem. 2018).

Selected publications(* corresponding author)

1. Xu PW, Chen C, Liu JK, Song YT, Zhou F*, Yan J*, Zhou J*. One-pot Sequential [3+3] Dipolar Cycloaddition of aldehyde or ketone, hydroxylamine with spirocyclopropyl oxindole. J. Org. Chem. 2018;83(20):12763-12774.
2. Fang T‡, Liu D‡, Ning HM‡, Liu D, Sun JY, Huang XJ, Dong Y, Geng M, Yun SF*, Yan J*, Huang R*. Modified citrus pectin inhibited bladder tumor growth through downregulation of galectin-3. Acta Pharmacol Sin. 2018 May 16. doi: 10.1038/s41401-018-0004-z.
3. Huang X‡, Zhu H‡, Gao Z, Li J, Zhuang J, Dong Y, Shen B, Li M, Zhou H, Guo H*, Huang R*, Yan J*. Wnt7a activates canonical Wnt signaling, promotes bladder cancer cell invasion, and is suppressed by miR-370-3p. J Biol Chem. 2018;293: 6693.
4. Chang C, Liu J, He W, Qu M, Huang X, Deng Y, Shen L, Zhao X, Guo H, Jiang J, Fu XY, Huang R, Zhang D, Yan J*. A regulatory circuit HP1γ/miR-451a/c-Myc promotes prostate cancer progression. Oncogene 2018; 37:415-426.
5. Zhuang J, Shen L, Yang L, Huang X, Lu Q, Cui Y, Zheng X, Zhao X, Zhang D, Huang R, Guo H*, Yan J*. TGFβ1 promotes gemcitabine resistance through regulating the lncRNA-LET/NF90/miR-145 signaling axis in bladder cancer. Theranostics 2017;7:3053-3067.
6. Xu P, Liu J, Shen L, Cao ZY, Zhao XL, Yan J*, Zhou J*. Diastereo- and enantioselective [3 + 3] cycloaddition of spirocyclopropyl oxindoles using both aldonitrones and ketonitrones. Nat Commun. 2017;8:1619.

Group members

Techinician Xiaohong Yu

Graduate students

Yangyan Cui
Junzun Li
Lan Shen
Zemin Gao
Jiakuan Liu
Meiqian Li
Jiaxuan Li
Zesheng Xue

Trainees in collaboration

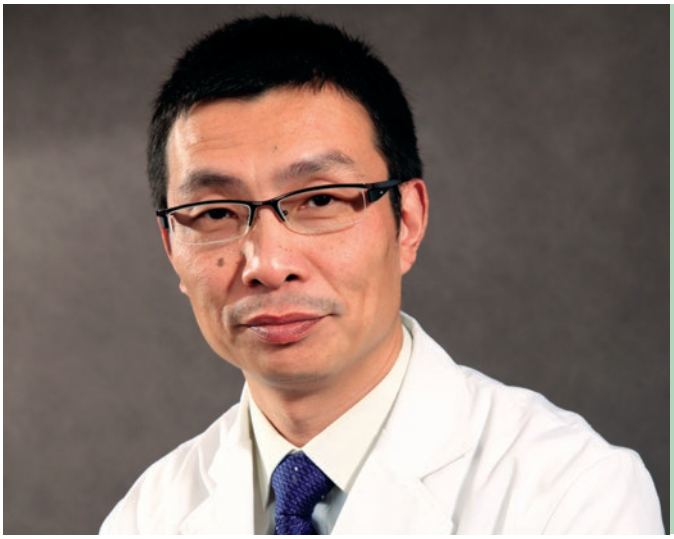
Lin Yang
Zhengnan Huang
Yilin Yan
Qinghe Zeng

Undergraduate student

Yidan Zhang

Lab alumni

Wei Zhao, Ph.D.
Cunjie Chang, Ph.D.
Xiaojing Huang, Ph.D.
Sumiya Dalangood, MS
Junlong Zhuang, M.D.
Yangyang Han, Ph.D.
Lin Yang, M.D.



Qing Jiang Ph.D., M.D.

Chief physician / Professor / Doctoral supervisor; The head of the department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital/ The vice president of school of medicine, Nanjing University/ Director, institute of medical 3D printing, Nanjing University. Prof. Jiang, the first sports medicine clinical doctor cultivated by China, had been engaged in orthopedic and sports medicine clinical and basic research since 1989 and got the 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. Prof. Jiang won the National Science Fund for Distinguished Young Scholars in 2011. The department of Sports Medicine and Adult Reconstructive Surgery is the only joint disease diagnosis and treatment center identified by Jiangsu Provincial Health Department, and also the training base of artificial joint and arthroscopic techniques in Jiangsu province. Qing Jiang's team 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), and published 232 Chinese core articles, 118 SCI articles, included Nature Medicine, Nature Genetic, ACS NANO, Advanced Functional Materials and so on. Prof. Jiang is the first domestic scholars who hold the post of committee member of the OARSI, and he is the vice chair of China branch of basic research branch of SICOT, the vice chair of China branch of ICRS, the vice chairman of sports medicine branch of Chinese medical association, the chairman of sports medicine branch of Jiangsu province, vice chairman of orthopedics branch, vice chairman of trauma branch, the head of Nanjing Drum Tower hospital orthopedics laboratory of ICMRS, etc.

Contact Information

Tel : 025-83106666

Email: qingj@nju.edu.cn

Skeletal System Disease

Cartilage repairation. Microfracture does not properly repair full-thickness cartilage defects. The purpose of this study was to evaluate the effect of intraarticular injection of the small-molecule compound kartogenin (KGN) on the restoration of a full-thickness cartilage defect. To confirm that KGN can induce human MSCs into chondrocytes, we compared the isolated SMSCs from the synovium tissue and the used pellets culture system with or without KGN. The average diameter of the KGNNP was 270 nm, identified by dynamic light scattering (DLS), which was consistent with the result of SEM imaging. KGN nanoparticles was quickly formed through a free-radical polymerization with exposure to UV light for 1 min, in the presence of cross-linker (N,N-methylenebis(acrylamide) (MBA)) and photoinitiator (Shi 2016). We also used 3D scanning and 3D printing to repair the bone and cartilage defects. We designed a polyvinyl alcohol (PVA) alginate based hydrogel and evaluated its cytocompatibility and printability. The mechanical strength, cytocompatiblity, crosslinking time, and printability were remarkably improved with the use of PVA (Figure 1) (Li 2018). Our data suggest that Hybrid bioink is more appropriate for precise 3D bioprinting due to its rapid prototyping capability and better cytocompatibility. The tissue bank for cartilage and ligament has been established and enlarged.

Rare skeletal diseases usually are misdiagnosed, so that patient cannot get the optimal treatment. Genetic factors such as gene mutations play a considerable role in etiology and pathogenesis of these rare and developing diseases of skeletal system. We performed genetic testing for the patient by using the next generation sequencing and direct nucleotide sequencing. We checked the target mutations in the proband's family members and healthy individuals. So far we detected 5 novel mutations of WISP3 that responsible for Progressive pseudorheumatoid dysplasia, 1 novel mutation in CHST3 that are responsible for Spondyloepiphyseal dysplasia with congenital joint dislocations, 2 novel mutations in HSPG2 for Schwartz-Jampel Sydrome, and so on. The DNA bank for rare skeletal diseases is still enlarging.

Osteoarthritis (OA) is a progressive degenerative disease of the joints that is associated with both joint injury and ageing. We found that shorter patients had reduced Egr1 expression levels in the hypertrophic cartilage zone of the femoral head. Egr1 knockout (KO) mice exhibited reduced body size and reduced bone volume. The extracellular matrix of Egr1 KO mice exhibited a relatively limited degree of mineralization, and reduced cell apoptosis levels. After transfecting the iMACs with dominant-negative Egr1 adenoviruses to inhibit Egr1, the enzymes of Adamst4, Adamst5, Mmp3 and Mmp13 were significantly upregulated. Our results suggested that Egr1 has an important regulatory effect on the dynamic equilibrium of the chondrocyte extracellular matrix, which may be achieved through the PPARγ/RUNX2 signaling pathways (Lu & Shi, 2018). We also noticed that Zhuangguguanjie formulation (ZG) can provide noticeable relief from joint pain in patients suffering from knee osteoarthritis (OA). However, the underlying mechanism has not been fully described. DMM mice indicated reduced cartilage destruction and lower blood serum biomarkers of OA (COMP1 and CTX-1) following ZG treatment (Figure 2). The femoral condyle and tibial plateau histological scores were significantly reduced following ZG treatment of the DMM mice. ZG could markedly downregulate the expression of OA-related genes namely, ADAMTS5, MMP3 and MMP13, while it simultaneously upregulated collagen II as demonstrated by in vitro assays. We found that ZG is capable of preventing and/or reducing the progression of OA by inhibiting chondrocyte apoptosis via the p-AKT/Caspase 3 pathway (Lu & Shi, 2018).

Deep vein thrombosis (DVT) remains to be major clinical problem despite decades of research effort. A total of 402 patients were enrolled, with 229 patients undergoing total knee arthroplasty (TKA) and 173 patients undergoing total hip arthroplasty (THA) to determine the association between preoperative solealvein (SV) diameter and deep vein thrombosis (DVT) following total joint arthroplasty (TJA). We found that SV diameter was anindependentriskfactorfortotalandsymptomat icDVTafterTJA.PreoperativeultrasoundscreeningoftheSVdiametermay be beneficial for the prevention of postoperative DVT (Yao 2018). We evaluated the effects of NO microbubbles in an inferior vena cava (IVC) and left common iliac vein (LCIV) ligation-induced rat DVT model. We have demonstrated a clear effect of NO microbubbles on DVT resolution. Both thrombus weight and thrombus size (thrombus weight/thrombus length) significantly decreased in NO microbubbles group at day 8, suggesting that NO microbubbles had accelerated the progression of thrombolysis.

Developmental dysplasia of the hip (DDH) is the most frequent inborn deformity of the locomotors apparatus. Genetic factors play a considerable role in pathogenesis of DDH. At current stage, we have performed GWAS study of DDH, and detected novel genes and signaling pathways, such as HSPA8, RASAL1, SDSL and so on (Yan 2018). 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. The association between PAPPA2 and DDH should be evaluated by additional studies.

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

1. The research on whole exome sequencing of familiar Developmental dysplasia of the hip. (Projects of International Cooperation and Exchanges NSFC 81420108021)

2. The mechanism study of the cartilage and subchondral bone defect reconstruction using a hydrogel with sustained release of small molecule kartogenin (Major projects of NSFC 8173000209)

3. The perioperative personalized diagnosis and treatment of osteoarthritis. (Jiangsu Provincial Key Research and Development Fundation BE2016608)

4. The study of repair cartilage defect by using hyaline hydrogel with sustained small molecule BIO. (Excellent Young Scholars NSFC 81622033)

5. Tendons outside source of stem cells secrete body by passing mirnas injured tendon repair. (NSFC 81702151)

6. 3D printing more peptide base the numerical modeling and optimization of the subchondral bone and animal studies for the treatment of net focal cartilage injury Natural Science Foundation of Jiangsu Province, China (SBK2017040751)

7. National Science Foundation of China(81871832), Effect and mechanism of exosomes in circulating blood on venous thrombosis during perioperative orthopedics

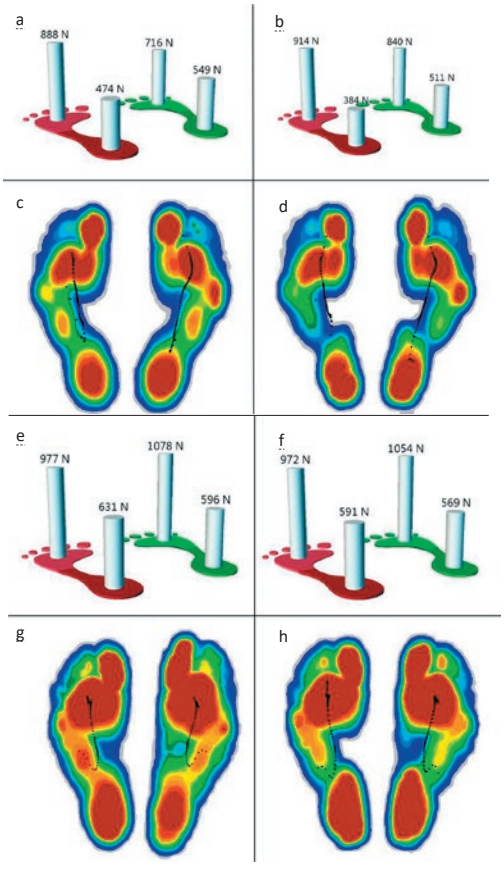


Fig. 1 Plantar pressure analysis during walking (a–d) and jogging (e–h). a, e Overall load of forefoot and hind foot with bare foot. b, f Overall load of forefoot and hind with heel cup. The load increased on forefoot and decreased on hind foot. c, g Stress nephogram of plantar tissue with bare foot. d, h Stress nephogram of plantar tissue with heel cup. The region with orange on mid foot was replaced by green, indicating that the load in this region was reduced. The color that changed from red to blue represented the stress variation from large to small.

8. National Science Foundation of China(81672239), Proteomics of knee arthroplasty and deep venous thrombosis of lower extremities

9. National Science Foundation of China(81702151), Exosomes derived from tendon stem cells facilitate repair of damaged tendons by delivering microRNA

10.National Science Foundation of China (8180090535), A study on the application of hollow porous magnetic nanoparticles to target aggregation and control of the release of icariin to promote fracture healing in mice

11.National Science Foundation of China(81802196), Studies on the role and mechanism of biological clock gene Bmal1 in regulating the autophagy rhythm of chondrocytes in maintaining cartilage homeostasis with trehalose

12.Social Development Project of Jiangsu Provincial Science and Technology Department(BE2016609), Sustained release of small molecule drug KGN to treat soft injury

13.Six Talent Peaks Project of Jiangsu Province(WSW-061), Peptide hydrogel repair of sustained-release small molecule organic compound BIO

14.Science and Technology Development projectof Nanjing (201605020), Clinical study on femoral extramedullary localization device for knee joint replacement

15.Natural Science Foundation of Jiangsu Province,China(BK2017040751), Three Digital modeling and optimization of polypeptide subchondral bone and animal study on the treatment of focal cartilage injury

16.Natural Science Foundation of Jiangsu Province,China (BK20180127), Study on the role of natural carbohydrate trehalose in maintaining cartilage homeostasis by regulating autophagy rhythm through cartilage cell clock gene Bmal1

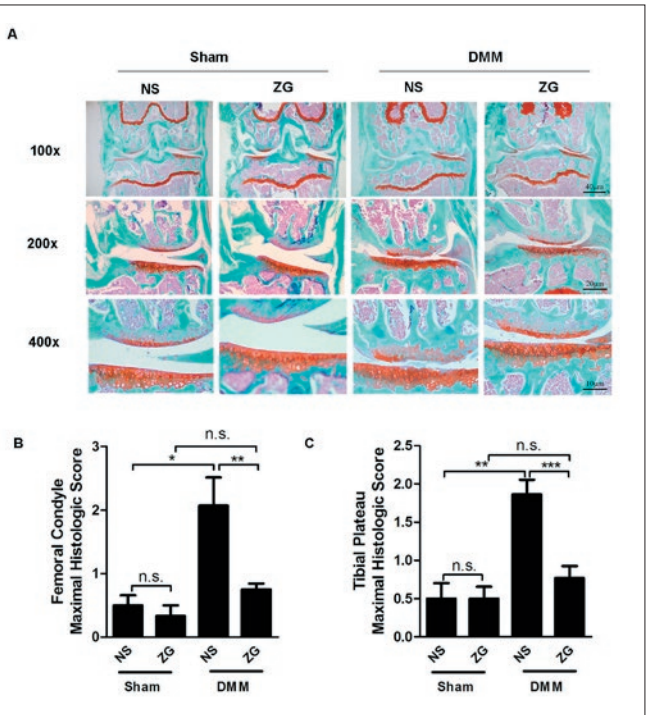


Figure 2. ZG inhibits cartilage degeneration in DMM mice at the 6th week. A. Joint histology in the DMM model. Knee joint tissue sections were derived from DMM mice following 6 weeks of ZG and NS treatments (NS). Joint sections were stained with Safranin-O/fast green. Representative images of mouse joints are shown (n=8 mice per time point). B. Maximal histological scores across the femoral condyle. C. Maximal histological scores across the tibial plateau. *P<0.05, **P<0.01, ***P<0.001, n.s, not statistically significant.

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1. Nakajima M, Shi D, Dai J, et al. (2011) Replication studies in various ethnic populations do not support the association of the HIF-2α SNP rs17039192 with knee osteoarthritis. Nat Med. 17(1):26-7. (IF= 30.357)
2. Shi D, Xu X, Ye Y, et al. (2016) Photo-cross-linked scaffold with kartogenin-encapsulated nanoparticles for cartilage regeneration. Acs Nano, 2016, 10(2):1292. (IF= 13.334)
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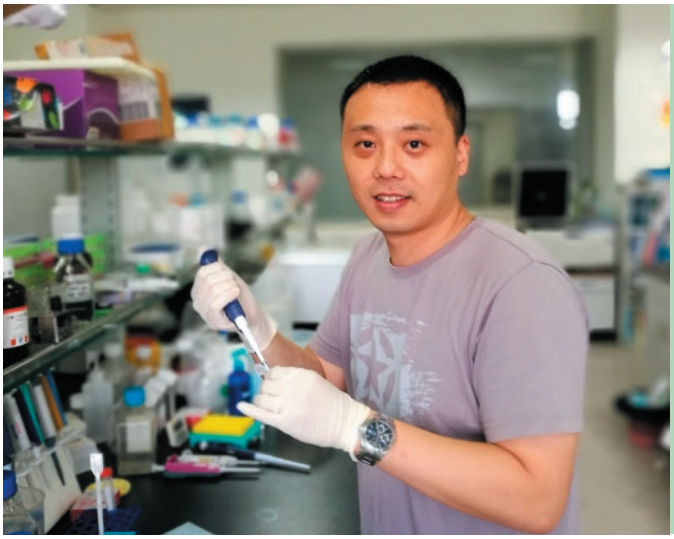
5. Lu K, Shi T, Li L, Zhang K, Zhu X, Shen S, et al. (2018). Zhuangguguanjie formulation protects articular cartilage from degeneration in joint instability-induced murine knee osteoarthritis. Am J Transl Res, 10(2), 411-421. (IF= 3.061)
6. Lu K, Shi T, Shen S, et al. (2018). Egr1 deficiency disrupts dynamic equilibrium of chondrocyte extracellular matrix through ppary/runx2 signaling pathways. American Journal of Translational Research, 10(6), 1620. (IF= 3.061)
7. Yan W, Hao Z, Tang S, et al. A genome-wide association study identifies new genes associated with developmental dysplasia of the hip. Clinical Genetics accepted (IF=3.512).
8. Yao Y, Qiao L, Song K, et al. (2018). Preoperative evaluation of soleal vein diameter by ultrasound is beneficial for prophylaxis of deep vein thrombosis after total knee or hip arthroplasty. BioMed Research International. Online. (IF=2.583)

Group members

Graduated Doctors	Graduated Masters	Technicians
Ke Lu	Xiaoxiao Song	Sheng Shen
Sicong Min	Liang Qiao	Lan Li
	Qiangqiang Li	Wenjin Yan
	Cheng Yao	Rui Wu
	Fei Yu	Peng Wang
		Qiting Ge
		Jing Jin



Jianghuai Liu, Ph.D.

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

Contact Information

Tel : 86-25-58641599
Fax: 86-25-58641500
Email: liujianghuai@nicemice.cn

Harnessing macrophage functions for treatment of cancer

Our immune system is programmed to exert very diverse functions that range, for instance, from defending against foreign pathogens to regulation of metabolic homeostasis. Such diversity is mediated, at least in part, by the functional and populational plasticity of macrophages. These are the innate immune cells, as suggested by the great Ilya Metchnikoff (1845-1916) from an evolutionary perspective, that help to maintain the organismal "harmony". Dysregulation within the macrophage compartment is known to underlie many disease states. However, therapeutic targeting of macrophages has not become a common practice, likely reflecting a lack of understandings to macrophage heterogeneity in disease. We use mouse models to investigate the regulation of macrophages in inflammation and cancer. We also aim to develop novel tools to target or rewire such cells in pathway- or subset-specific manner. Ultimately, we hope that our preclinical studies may in some way impact the management of human diseases.

1. A "checkpoint" in type I IFN-mediated stimulation of anti-tumor immunity:

Initially identified as a key anti-viral cytokine, the type I IFN (IFN-I) was later shown to exhibit antitumor activities. This led to its clinical applications in treatment of several cancers. However, against solid malignancies, IFN-I-based treatment strategies have shown only sub-optimal therapeutic effects. As tumor microenvironment can affect treatment outcomes, we examined the regulation of tumor-associated monocytes and macrophages in a mouse model of IFN-I-based therapy.

We found that poly(I:C)-IFN treatment could slow the conversion of monocytes to tumor-associated macrophages (TAMs) (Fig. 1A). We further uncovered a mir-155-CSF1R inhibitory circuit that underlies such an activity (Fig. 1B). By treatment of the mice using poly(I:C) in combination with a CSF1R inhibitor, to further reduce the number of TAMs, we validated that the IFN-I-mir-155-CSF1R inhibitory axis contributes to limiting tumor progression (Fig. 1C).

Remarkably, further analyses of gene expression profile of IFN-treated differentiating monocytes reveal a strong induction of Arg1 (encoding arginase-1) in addition to other classical IFN targets (Fig. 2A). Furthermore, TAMs from poly(I:C)-treated mice were found to feature high ARG1 expression (Fig. 2B). Given the general pro-tumoral role by myeloid-derived arginase, these striking results implicated that IFN-I paradoxically engaged an arginase-dependent pro-tumoral program in the TAM compartment. Indeed, inhibition of arginase activity could synergize with poly(I:C) to thwart tumor growth in mice (Fig. 2C). Our results suggest IFN-ARG1 immunosuppressive axis as a critical"checkpoint" mechanism limiting the efficacies of IFN-based therapies. A graphic summary for this part of our research is provided in Fig. 2D. Combination of IFN-I-based cancer therapies with reagents that target various molecules enabling such "checkpoint" is likely to be a key step for better treatment effects. Our work may have also provided some general insights to the long-held vision of attacking cancer via stimulation of innate immunity.

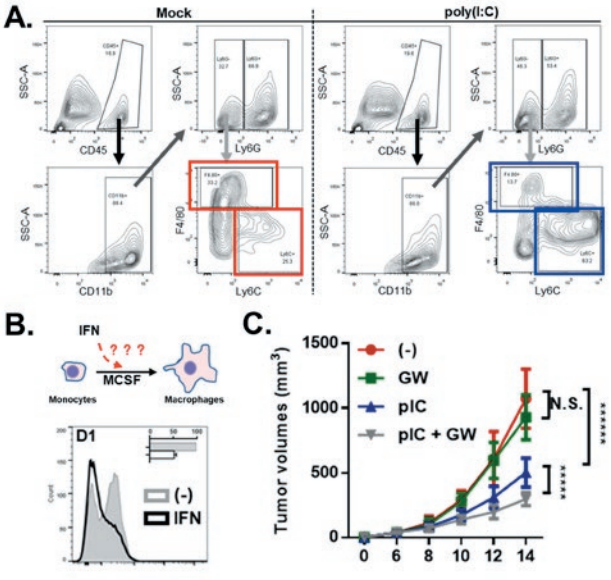


Figure 1: Type I IFN-based therapy affects monocyte differentiation toward tumor-associated macrophages (TAMs). (A)

Treatment of tumor-burden mice (LLC model) with an IFN-I inducer (poly(I:C)) led to impediment of monocyte-to-TAM differentiation. Tumors were harvested and segregated into single cells. Monocytes and TAMs were distinguished by their characteristic Ly6ChighF4/80low and F4/80high immunophenotypes, respectively. (B) IFN-I inhibited cell surface CSF1R levels in cultured differentiating monocytes. (C) Treatment of the mice using poly(I:C) in combination with a CSF1R inhibitor, to further reduce the number of TAMs, led to enhanced control of tumor progression.

2. Development of synthetic biological tools interfacing with the immune system:

The recently emerging discipline of synthetic biology centers on construction of synthetic gene circuits that can drive new biological behaviors. Based on the cutting-edge CRISPR/Cas9 technology, we are developing tools to synthetically connect the endogenous transcriptional inputs to either reporters or to perturbation of gene expression. We envision that such new tools will facilitate future immunological research.

Fig. 2

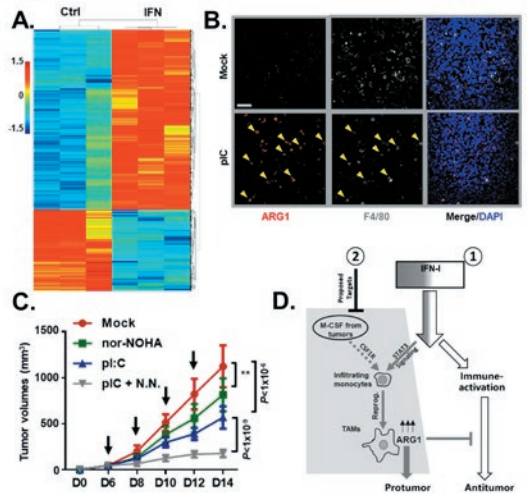


Figure 2: An unexpected IFN-I-ARG1 axis may represent a "checkpoint" for IFN-I-based cancer therapies. (A)

Gene expression profiling of IFN-I-treated differentiating monocytes (under M-CSF stimulation) helped to reveal a strong induction of Arg1. (B) TAMs from poly(I:C)-treated mice (LLC model) feature high expression of ARG1. F4/80 is macrophage marker. (C) Poly(I:C) and arginase inhibitor showed synergistic effect on control of tumor progression. (D) A schematic summary for the dichotomous actions by IFN-I in tumor-associated monocytes and macrophages.

Selected publications: (*corresponding author)

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7. 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 (2011), 6: e27464.

Group members

Technical assistant:

Yan-lan Cao

Graduate students and trainees:

Dr. Yuan-yuan Tong

Qing-zhou Meng

Pan-pan Guo

Ya-feng Wang

Li-min Yang

Meng-fan Zhang

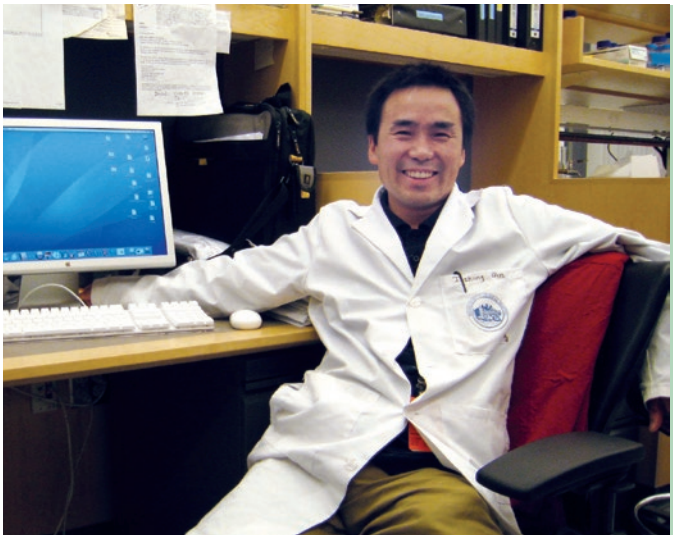
Gui-quan Zhang

Lab alumni:

Dr. Hui Jiang (Molecular Infection Medicine Sweden)

Dr. Yi Lu (ShanhaiTech University)

Ms. Man Sun (ShanghaiTech University)



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.

Contact Information

Tel: +86-25-58641504

Email: qinjz@nicemice.cn

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.

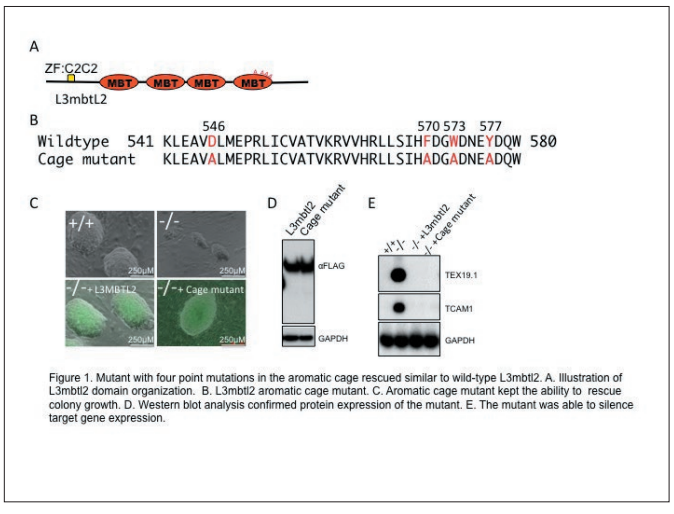


Figure 1. Mutant with four point mutations in the aromatic cage rescued similar to wild-type L3mbtl2. A. Illustration of L3mbtl2 domain organization. B. L3mbtl2 aromatic cage mutant. C. Aromatic cage mutant kept the ability to rescue colony growth. D. Western blot analysis confirmed protein expression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

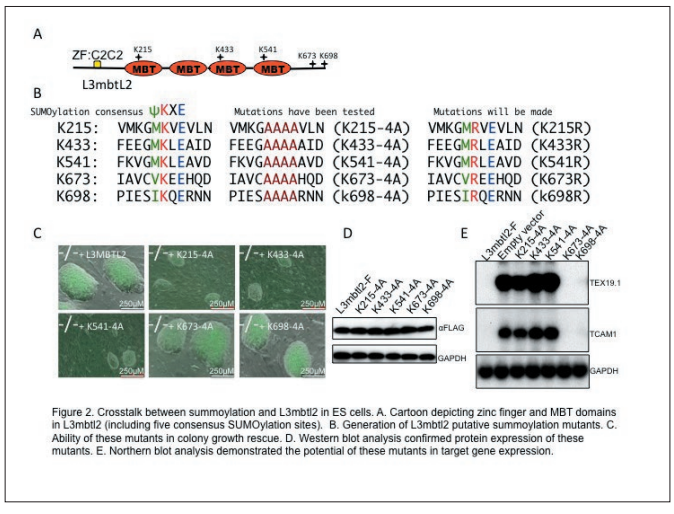


Figure 2. Crosstalk between summoylation and L3mbtl2 in ES cells. A. Cartoon depicting zinc finger and MBT domains in L3mbtl2 (including five consensus SUMOylation sites). B. Generation of L3mbtl2 putative summoylation mutants. C. Ability of these mutants in colony growth rescue. D. Western blot analysis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

Selected publications:

1. Zhao W., Liu M., Ji H., Zhu Y., Wang C., Huang Y., Ma X., Xing G., Xia Y., Jiang Q., Qin J*. 2018. The polycomb group protein Yaf2 regulates the pluripotency of embryonic stem cells in a phosphorylation-dependent manner. J Biol Chem.293(33):12793-12804.
2. Huang, Y., Zhao, W., Wang, C., Zhu, Y., Liu, M., Tong, H., Xia, Y., Jiang, Q., and Qin, J*. (2018) Combinatorial Control of Recruitment of a Variant PRC1.6 Complex in Embryonic Stem Cells. Cell reports 22, 3032 -3043.
3. Liu W., Chen B., Wang Y., Meng C., Huang H., Huang XR., Qin J., Mulay SR., Anders HJ., Qiu A., Yang B., Freeman GJ., Lu HJ., Lin HY., Zheng ZH., Lan HY., Huang Y., Xia Y*. 2018. RGMb protects against acute kidney injury by inhibiting tubular cell necroptosis via an MLKL-dependent mechanism. Proc Natl Acad Sci U S A 115(7):E1475-E1484.
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5. Zhao, W., Huang, Y., Zhang, J., Liu, M., Ji, H., Wang, C., Cao, N., Li, C., Xia, Y., Jiang, Q., and Qin, J*. (2017) Polycomb group RING finger proteins 3/5 activate transcription via an interaction with the pluripotency factor Tex10 in embryonic stem cells. J Biol Chem 292, 21527 -21537.
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Group members

Group Leader
Jinzhong Qin

Technical Assistants
Shuang Liu

Graduate Students
Yikai Huang
Mengjie Liu
Congcong Wang
Yaru Zhu
Lixia Dong

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.

Contact Information

Tel : +86-25-89686635 (Office) +86-25-89682995 (Lab)
Fax: +86-25-89684060
Email: ppshen@nju.edu.cn

Interactions between TAMs and tumor cells

Tumor associated macrophages (TAMs) are increasingly viewed as a target of great relevance in the tumor microenvironment, because of their vital role in cancer progression and metastasis. However, the endogenous regulatory mechanisms underlying the interactions between TAMs and cancer cells remain largely unknown. With this as one of our goals, we are currently studying the actions and related regulatory mechanisms of certain soluble factors secreted by TAMs as tumor growing. It has been found that factor 1 derived from TAMs in triple-negative breast cancer (TNBC) microenvironment induces the CDKn activation in BC cells. CDKn activation then triggers a series of molecular cascades, eventually, mediates tumor malignant progression. Importantly, CDKn activation are correlated with disease progression in TNBC patients. Inhibiting CDKn activation via specific chemical inhibitors, suppresses tumor metastatic progression significantly. Meanwhile, targeting factor 1 by specific antibody interrupts the communication between TAMs and TNBC cells, consequently, reverses immunosuppressive tumor immune status.

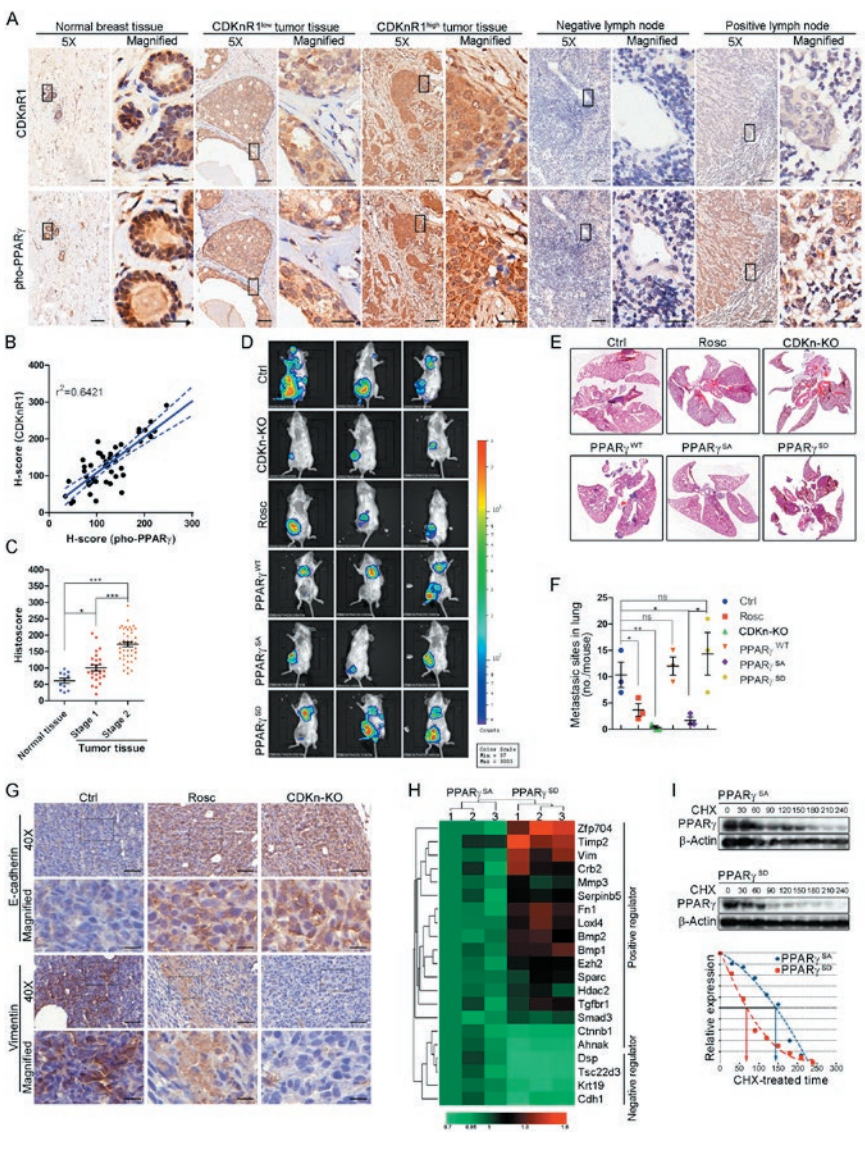

Fig.1 Blockade of CDKn activation attenuated metastatic progression of TNBC via inhibiting EMT.

Developing the Clinical diagnostic techniques:

We have established an ultrasensitive immunosensor based on the gold nanoparticles-enhanced tyramine signal amplification (AuNPs-TSA) for the detection of PCT. By using a novel electrode modified by graphene oxide nanosheets/GNP nanocomposite, we have achieved an obvious signal amplification. This designed immunosensor exhibits a wide dynamic detection range from 0.05 ng mL^{-1} to 100 ng mL^{-1} and with an ultralow detection limit of 0.1 pg mL^{-1}. Moreover, we have successfully utilized this immunosensor to quantify the concentration of PCT in human serum samples, and the results suggest its potential use in clinical application. Based on above mentioned design strategy, we are developing a serious of new sensors for further detection in human specimens.

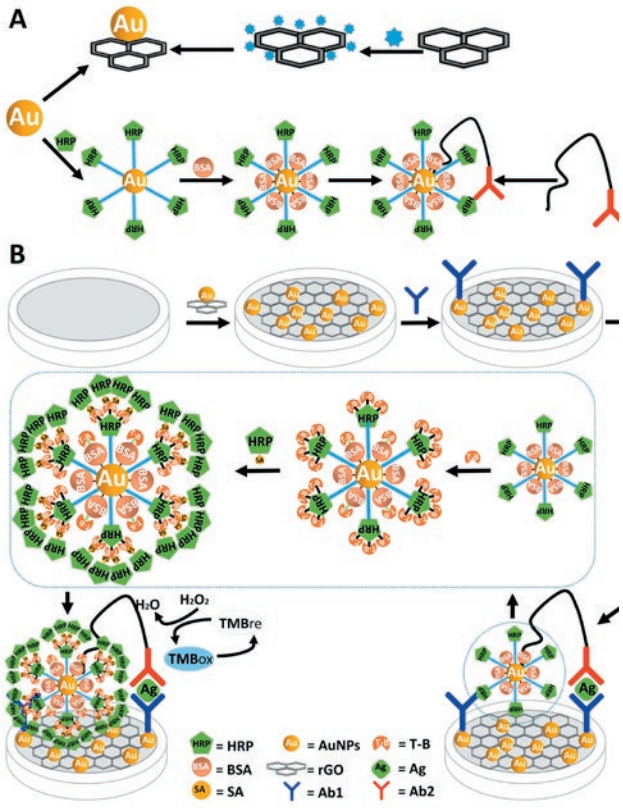

Fig.2 Schematic illustration of an immunosensor for detection of PCT.

Developing Immunocellular Therapeutic techniques

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

Selected publications

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10. 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.

Group members

Group leader

Pingping Shen

Graduate students

Jun Cui
Ying Liu
Qian Shi
Ting Chen
Xiaofeng Bao
Yuanyuan Su
Yuanyuan Wu
Jiafa Xu
Yinna Wei
Haocheng Wu
Weiwei Yang
Tingzhe Sun
Xiaojuan Pang
Xiujing Feng
Zhiyuan Niu
Yongfang Yao
Yuncheng Bei

Students in lab

Wentong Fang
Nan Cheng
Pei Liu
Yuxin Shu
Jingfa Zhao
Nanfei Yang
Wenlong Zhang
Luchen Sun
Xiang Wu

Teachers

Yahong Huang
Yan Lu

Technician

Wei Zheng

After two years in operation, the Core Facilities of MARC have begun to take shape. We have been equipped with more than 20 state of the art instruments and provided over 45,000 hours service within or outside MARC research community.

So far, we have set up Microscopy and Imaging Core, Flow Cytometry Core, Molecular and Metabolomics Core, providing a diverse range of resources and services, including high resolution imaging, flow cytometry, protein and gene expression profiling, and metabolic analysis. The featured instruments are listed below and more resources could be found on our website. http://core.nicemice.cn:8081/default.htm

Microscopy and Imaging Core

► GE DeltaVision OMX

True 3D structured illumination imaging enables resolution improvements to 120 nm in XY and 340 nm in Z, providing an overall eight-fold improvement in volume resolution

Simultaneous photoactivation and sample imaging for fast photokinetic applications in TIRF mode(e.g. caged-calcium release or PA-GFP activation)

Ultra-fast widefield imaging at 150 fps depending on exposure time

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TruLight illumination system delivers exceptional signal-to-noise performance and five times more light to the sample compared to previous illuminator assembly, enabling detection of small, dim objects such as organelles and microbial particles

Deconvolution improves contrast and resolution compared to raw data images without sacrificing data integrity.

UltimateFocus automatically maintains the sample z-position regardless of mechanical or thermal changes that can impact experiment.

Cell tracking function automatically repositions the stage to accurately follow cells as they move during time-lapse experiments.

► Zeiss LSM 880

Increase the resolution with Airyscan to resolve 140 nm laterally and 400 nm axially at 488 nm, achieving 1.7× higher resolution for photon or multi photon experiments

Working with thicker samples such as tissue sections or whole animal mounts that need a higher penetration depth, in such situation where widefield-based superresolution techniques would struggle.

Using the Fast module to image with up to 27 frames per second at 480 × 480 pixels

Flow Cytometry Core

► BD FACS LSRFortessa

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

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

► BD FACSAaria III

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

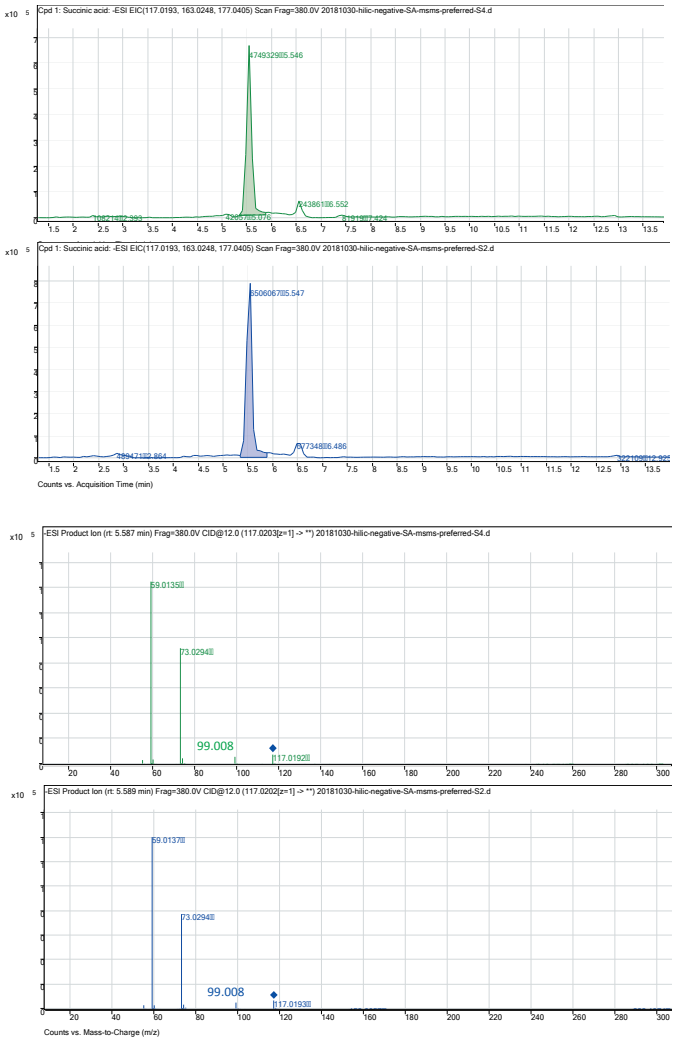
At 70 psi and 90 kHz with an average threshold rate of 25,000 events per second, a four-way sort achieved a purity of >98% and a yield >80% of Poisson's expected yield. Higher threshold rates up to 70,000 events per second can be achieved without affecting purity.

Proteomics core and metabonomics core

► Agilent 6550 iFunnel Q-TOF

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

Determination of Succinic Acid in cell lysate using LC-MS/MS. The detection of SA was confirmed by production iron of the compound (parent iron 117.0194; production iron 73, 99). The treated sample(S2) displayed high level of succinic acid compared to control sample(S4)



- METLIN Personal Metabolite Database Software

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

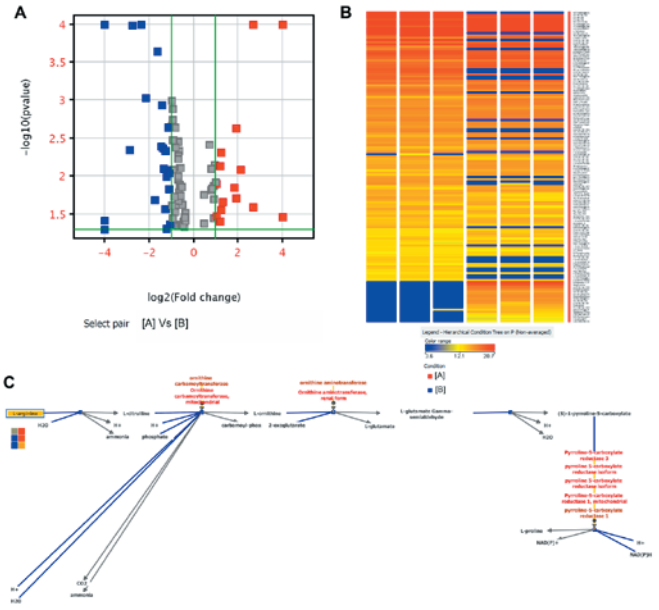
- Spectrum Mill

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

- Mass Profiler Professional Software

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

Metabolites analysis of brain tumor. Two diffierent brain tumor were compared for metabolites. (A) Volcano plot shows the metabolites difference in two different brain tumor. (B) The metabolites comparision is displayed in heatmap, in which L-arginine is elevated in one brain tumor. (C) The L-arginine metabolic pathways.



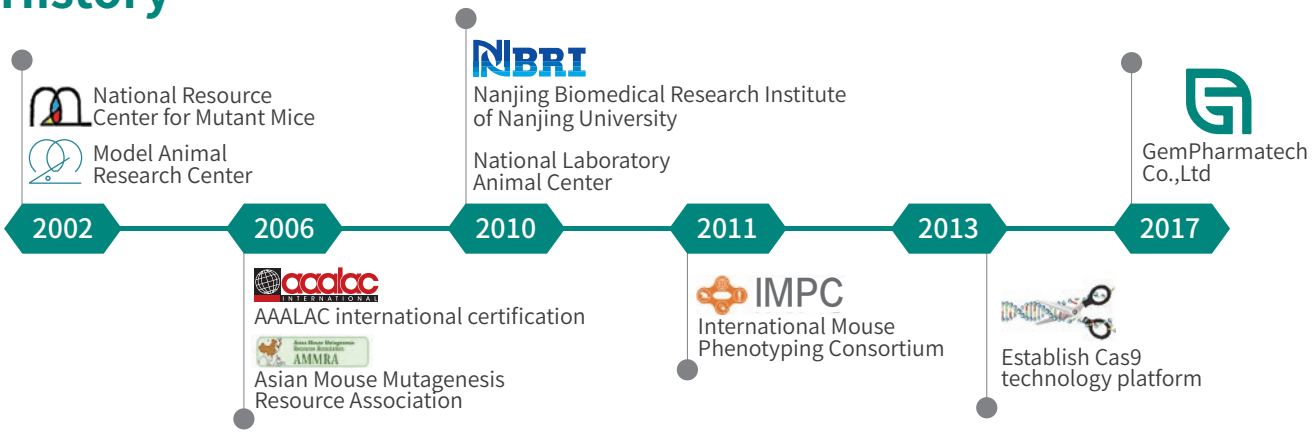
As shown in the figures, the services provided by Metabonomics Core include targeted or non-targeted metabolites identification. We also plan to establish metabolite library containing over 600 metabolites for targeted metabolomics study.

We will work hard to service efficient and effective technological support for our users, and develop new methods based on our equipment to assist research study. It will enhance effective usage of resources and maximize the use of expensive instruments required for these advanced technology platforms. It is expected to further promote and facilitate multi-disciplinary research studies and collaboration among the research community.

National Resources Center for Mutant Mice

Certified by the Ministry of Science and Technology of the People's Republic of China.
National Science and Technology Basic Conditions Service Platform.Ranked World third largest disease model resources center.
Elite one-stop service center for production, distribution, and phenotyping of gene-modified mouse models.

History



Highlights

Dalmatian Mouse Action

Dedicated in generating >20000 conditional knock out models and becoming world' s largest repository for CKO mice models.

Over 1200 cases of CKO mouse model, fast delivery in 90 days

Hot gene spots selected covering research areas of tumor, metabolism, immune response, developmental biology, DNA and protein modification etc.

Rich selection
First CKO project generating 1200 mouse models and associated sperm bank is finished. Have initiated the 2nd phase which will include 5000 more strains.

More convinient
Direct online ordering process.
(www.gempharmatech.com)

Faster delivery
Delivery cycle of CKO mouse model has been shortened to 90 days.

Lower price
Much lower than the model customization.

Resource

World thrid of Mouse Disease Model Resources(>8000)

Unique member in China of International Mouse Phenotyping Consortium (IMPC)

Technology

World Third of Mouse Model Innovation capability

The world' s leading TG/KO technology platform

>2000 new strains developed per year

Scale

80000 IVC cages in 10000 m2 SPF facility

China' s largest lab mouse supplier

Provide more than 700 domestic and foreign organizationswith over 100,000 mouse each year.

Resources & services

Model animal distribution

Humanized mouse model
Immunodeficient model
Diabetes model
Atherosclerosis model
Tumor model
Cre and reporter model
Other mouse model

Genetic engineering mouse customization

CRISPR/Cas9 technology
Traditional ES technology
Transgenic technology

Mouse phenotype service

Tumor platform
Metabolic platform
Mouse phenotype screening service
Pathological platform

Breeding & Conservation service

Cryopreservation
Breeding

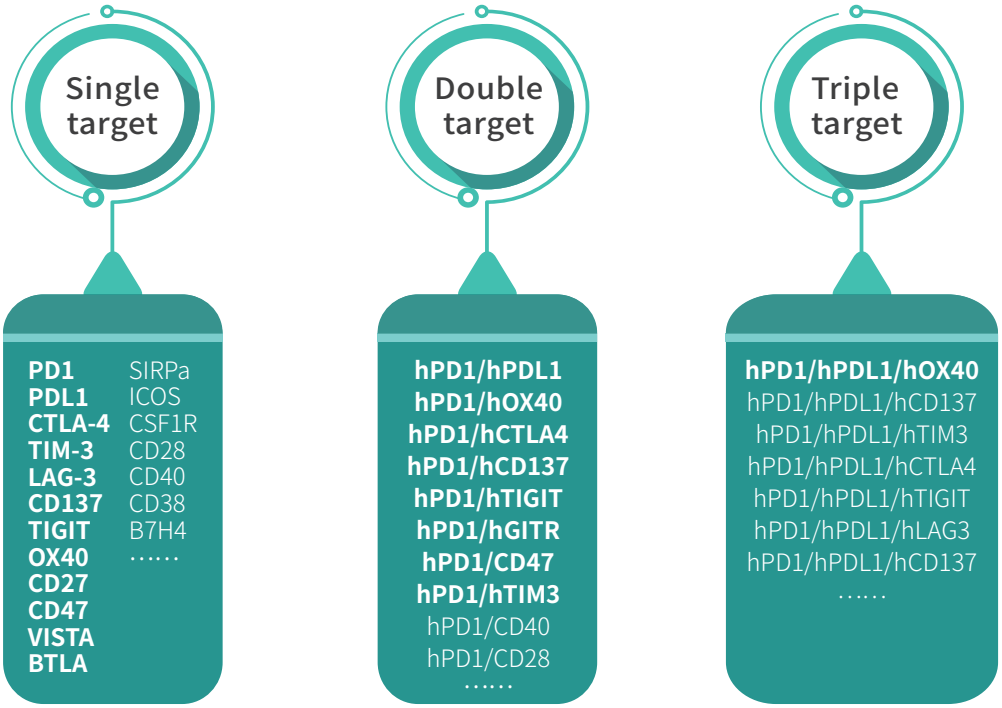
Veterinary service

Testing service
Veterinary inspection service

Resource sharing

Immune checkpoint humanized mouse models

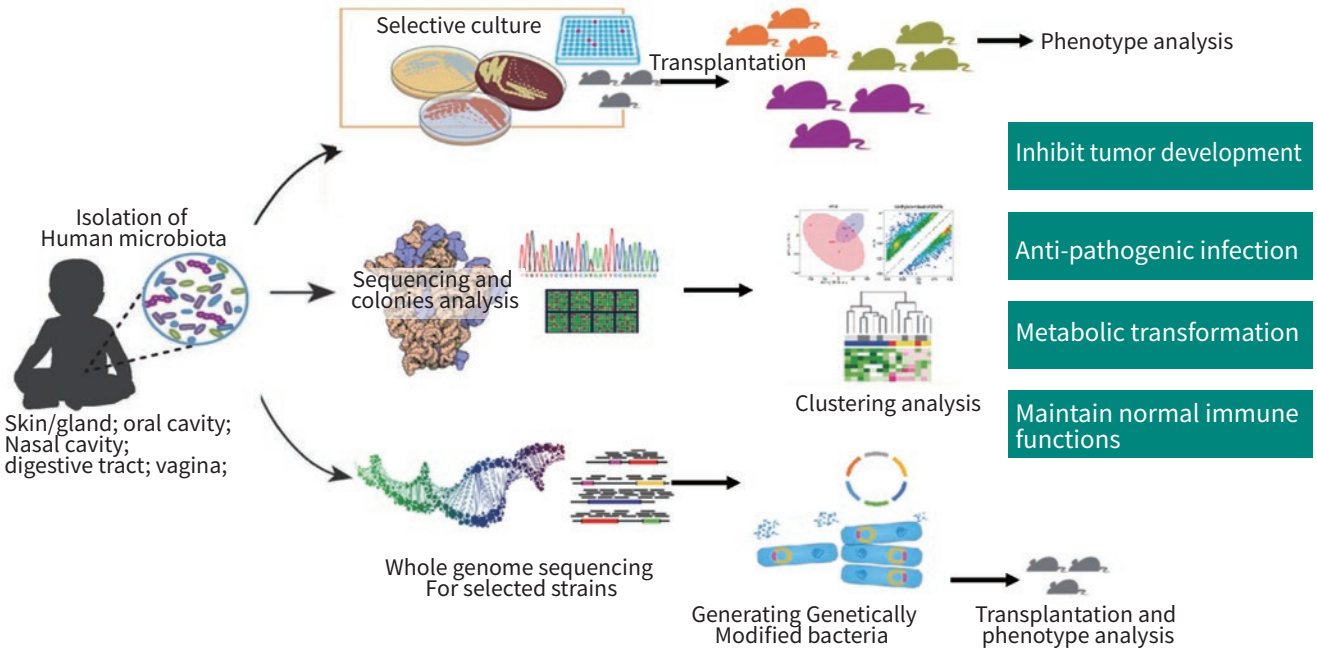
Gempharmatech is the world' s largest resource center and provider for immune checkpoint humanized mouse models. It covers all known immune checkpoint targets including mice with single, double or triple humanized immune-checkpoint-targets. Most important, each model was developed in both C57BL/6 and BALB/c backgrounds. These models are ideal for efficacy and safety evaluation of antibodies and anti-carcinoma small molecules.



Dual version : BALB/c and C57BL/6 background.

Situational implantation model library of microbial flora

Establishment of sterile mouse platform. Widening aseptic animals.Construct and perfect the experiment platform of Humanized microorganism.

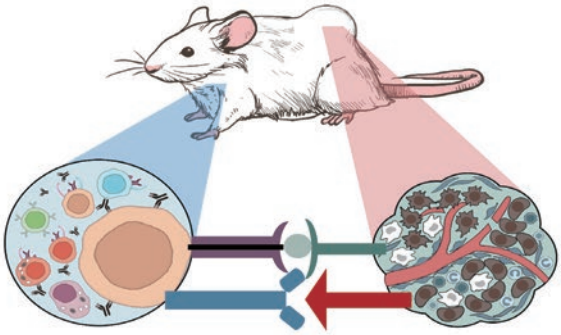


Immune reconstitution and patient derived xenograft (PDX) models

Over 600 PDX tumors will be available in 2019

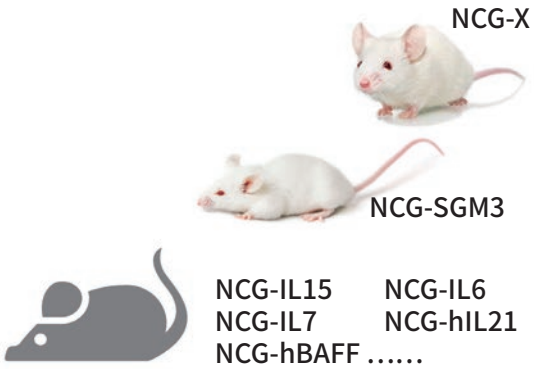
CD34+ cord hematopoietic stem cell (HSC) reconstituted model is available

Peripheral Blood Mononuclear Cell (PBMC) reconstituted model is available



Human immune system Patient derived tumor

Used for in vivo efficacy and safety study of CAR-T cells, macro- and small molecules targeting immune system and(or) tumor cells.



Cytokine humanized strains based on NCG background

Reduce the cytokine release storm (CRS)

Facilitate the immune reconstitution

Increase the engraftment of AML and CML

Contact us

National Resources Center for Mutant Mice

services@gempharmatech.com
www.gempharmatech.com
+86 025 58641580 / 58641559 / 58641570
12 Xuefu Road,Jiangbei New Area Nanjing, P.R.China

Beijing Office
WU Pengfei +86 18701561819
Room 1701, building A, tianzuo international building, no.12 zhongguancun south street, haidian district, Beijing, P.R.China

Shanghai Office
HAN Yang +86 13920237505
Building 1 Jinou Wanguo Plaza, Apt 2201, Alley 358,Fengyang Road, Huangpu District, Shanghai, P.R.China

Chengdu Office
SHI Peiliang +86 028 82600716
1588, 3rd section of Guanghua Avenue, Wenjiang Area, Chengdu, Sichuan, P.R.China

Guangzhou Office
WEI Baoli +86 13725331344
Room 937, Garden Mansion, 368 East Road Huanshi, Yuexiu District, Guangzhou, P.R.China

Publications in 2018

1.	Gan ZJ, Fu TT, Kelly DP, Vega RB (2018) Skeletal muscle mitochondrial remodeling in exercise and diseases. Cell Research 28: 969-980
2.	Duan GF, Ye YX, Xu S, Tao WC, Zhao SP, Jin TC, Nico RA, Shi YS, Sheng NY (2018) Signal peptide represses GluK1 surface and synaptic trafficking through binding to amino-terminal domain. Nature Communications 9: 4879
3.	Wang P, Zhao W, Sun J, Tao T, Chen X, Zheng YY, Zhang CH, Chen Z, Gao YQ, She F, Li YQ, Wei LS, Lu P, Chen CP, Zhou J, Wang D-Q, Chen L, Shi X-H, Deng L, Zhuge R, Chen HQ, Zhu MS (2018) Inflammatory mediators mediate airway smooth muscle contraction through a G protein-coupled receptor-transmembrane protein 16A-voltage-dependent Ca2+ channel axis and contribute to bronchial hyperresponsiveness in asthma. Journal of Allergy and Clinical Immunology 141: 1259-1268
4.	Kang D, Wang D, Xu J, Quan C, Guo X, Wang H, Luo J, Yang Z, Chen S, Chen J (2018) The InR/Akt/TORC1 Growth-Promoting Signaling Negatively Regulates JAK/STAT Activity and Migratory Cell Fate during Morphogenesis. Developmental Cell 44: 524-531
5.	Huang H, Xu C, Wang Y, Meng C, Liu W, Zhao Y, Huang X-R, You W, Feng B, Zheng Z-H, Huang Y, Lan H-Y, Qin J, Xia Y (2018) Lethal (3) malignant brain tumor-like 2 (L3MBTL2) protein protects against kidney injury by inhibiting the DNA damage-p53-apoptosis pathway in renal tubular cells. Kidney International 93: 855-870
6.	Huang Y, Zhao W, Wang C, Zhu Y, Liu M, Tong H, Xia Y, Jiang Q, Qin J (2018) Combinatorial Control of Recruitment of a Variant PRC1.6 Complex in Embryonic Stem Cells. Cell Reports 22: 3032-3043
7.	Fu T, Xu Z, Liu L, Guo Q, Wu H, Liang X, Zhou D, Xiao L, Liu L, Liu Y, Zhu M-S, Chen Q, Gan Z (2018) Mitophagy Directs Muscle-Adipose Crosstalk to Alleviate Dietary Obesity. Cell Reports 23: 1357-1372
8.	Chang C, Liu J, He W, Qu M, Huang X, Deng Y, Shen L, Zhao X, Guo H, Jiang J, Fu XY, Huang R, Zhang D, Yan J (2018) A regulatory circuit HP1 gamma/miR-451a/c-Myc promotes prostate cancer progression. Oncogene 37: 415-426
9.	Zhou ZZ, Yao X, Pang S, Chen P, Jiang WR, Shan ZL, Zhang Q (2018) The deubiquitinase UCHL5/UCH37 positively regulates Hedgehog signaling by deubiquitinating Smoothened. Journal of molecular cell biology 10: 243-257
10.	Zou JH, Lai BB, Zheng MZ, Chen Q, Jiang SJ, Song AY, Huang Z, Shi PL, Tu X, Wang D, Lu LR, Lin ZY, Gao X (2018) CD4+T cells memorize obesity and promote weight regain. Cellular & Molecular Immunology 15: 630-639
11.	Liu J, Jiang S, Zhao Y, Sun Q, Zhang JZ, Shen D, Wu J, Shen N, Fu X, Sun XT, Yu DC, Chen J, He J, Shi TT, Ding YT, Fang L, Xue B, Li CJ (2018) Geranylgeranyl diphosphate synthase (GGPPS) regulates non-alcoholic fatty liver disease (NAFLD)-fibrosis progression by determining hepatic glucose/fatty acid preference under high-fat diet conditions. Journal of Pathology 246: 277-288
12.	Zhu H, Xue CB, Yao M, Wang HK, Zhang P, Qian TM, Zhou SL, Li SY, Yu B, Wang YJ, Gu XS (2018) miR-129 controls axonal regeneration via regulating insulin-like growth factor-1 in peripheral nerve injury. Cell Death & Disease 9: 720
13.	Wu DM, Wang S, Wen X, Han XR, Wang YJ, Shen M, Fan SH, Zhang ZF, Shan Q, Li MQ, Hu B, Lu J, Chen GQ, Zheng YL (2018) LncRNA SNHG15 acts as a ceRNA to regulate YAP1-Hippo signaling pathway by sponging miR-200a-E3p in papillary thyroid carcinoma. Cell Death & Disease 9: 947
14.	Chen Z, Xu N, Chong DY, Guan S, Jiang C, Yang ZZ, Li CJ (2018) Geranylgeranyl pyrophosphate synthase facilitates the organization of cardiomyocytes duringmid-gestation through modulating protein geranylgeranylation inmouse heart. Cardiovascular Research 114: 965-978
15.	Zhang LL, Yang YX, Li BB, Scott IC, Lou X (2018) The DEAD-box RNA helicase Ddx39ab is essential for myocyte and lens development in zebrafish. Development 145: dev161018
16.	Wang H, Qiu ZQ, Xu ZH, Chen SJ, Luo J, Wang XB, Chen J (2018) aPKC is a key polarity determinant in coordinating the function of three distinct cell polarities during collective migration. Development 145: dev158444
17.	Lin Z, Liu F, Shi P, Song A, Huang Z, Zou D, Chen Q, Li J, Gao X (2018) Fatty acid oxidation promotes reprogramming by enhancing oxidative phosphorylation and inhibiting protein kinase C. Stem Cell Research & Therapy 9: 47
18.	Wu J, Tao WW, Chong DY, Lai SS, Wang C, Liu Q, Zhang TY, Xue B, Li CJ (2018) Early growth response-1 negative feedback regulates skeletal muscle postprandial insulin sensitivity via activating Ptp1b transcription. Faseb Journal 32: 4370-4379
19.	Zhou L, Fang Y, Wang F, Dong X, Jiang L, Liu C, Zhao Q, Li K (2018) Generation of all-male-like sterile zebrafish by eliminating primordial germ cells at early development. Scientific Reports 8: 1834
20.	Lai B, Zou J, Lin Z, Qu Z, Song A, Xu Y, Gao X (2018) Haploinsufficiency of hnRNP U Changes Activity Pattern and Metabolic Rhythms. American Journal of Pathology 188: 173-183
21.	Li L, Yang LF, Yu F, Shi JP, Zhu LY, Yang XF, Teng HJ, Wang XS, Jiang Q (2018) 3D printing individualized heel cup for improving the self-reported pain of plantar fasciitis. Journal of Translational Medicine 16: 167
22.	Xu PW, Chen C, Liu JK, Song YT, Zhou F, Yan J, Zhou J (2018) One-Pot Sequential 3+3 Dipolar Cycloaddition of Aldehyde or Ketone and Hydroxylamine with Spirocyclopropyl Oxindole. Journal of Organic Chemistry 83: 12763-12774
23.	Wang Q, Tang J, Jiang S, Huang Z, Song A, Hou S, Gao X, Ruan H-B (2018) Inhibition of PPARgamma, adipogenesis and insulin sensitivity by MAGED1. The Journal of endocrinology 239: 167-180

24.	Zhao WK, Liu MJ, Ji HJ, Zhu YR, Wang CC, Huang YK, Ma XQ, Xing GD, Xia Y, Jiang Q, Qin JZ (2018) The polycomb group protein Yaf2 regulates the pluripotency of embryonic stem cells in a phosphorylation-dependent manner. Journal of Biological Chemistry 293: 12793-12804
25.	Yue Y, Jiang M, He L, Zhang Z, Zhang Q, Gu C, Liu M, Li N, Zhao Q (2018) The transcription factor Foxc1a in zebrafish directly regulates expression of nkx2.5, encoding a transcriptional regulator of cardiac progenitor cells. Journal of Biological Chemistry 293: 638-650
26.	Huang X, Zhu H, Gao Z, Li J, Zhuang J, Dong Y, Shen B, Li M, Zhou H, Guo H, Huang R, Yan J (2018) Wnt7a activates canonical Wnt signaling, promotes bladder cancer cell invasion, and is suppressed by miR-370-3p. Journal of Biological Chemistry 293: 6693-6706
27.	Ma D, Tu C, Sheng Q, Yang Y, Kan Z, Guo Y, Shyr Y, Scott IC, Lou X (2018) Dynamics of Zebrafish Heart Regeneration Using an HPLC-ESI-MS/MS Approach. Journal of Proteome Research 17: 1300-1308
28.	Wu DM, Hong XW, Wang LL, Cui XF, Lu J, Chen GQ, Zheng YL (2018) MicroRNA-17 inhibition overcomes chemoresistance and suppresses epithelial-mesenchymal transition through a DEDD-dependent mechanism in gastric cancer. International Journal of Biochemistry & Cell Biology 102: 59-70
29.	Fang T, Liu D-D, Ning H-M, Dan L, Sun J-Y, Huang X-J, Dong Y, Geng M-Y, Yun S-F, Yan J, Huang R-M (2018) Modified citrus pectin inhibited bladder tumor growth through downregulation of galectin-3. Acta pharmacologica Sinica
30.	Lu K, Shi T, Li L, Zhang K, Zhu X, Shen S, Yu F, Teng H, Gao X, Ju H, Wang W, Jiang Q (2018) Zhuangguguanjie formulation protects articular cartilage from degeneration in joint instability-induced murine knee osteoarthritis. American Journal of Translational Research 10: 411-421
31.	Dai HR, Zhou Y, Tong C, Gu YL, Shi FX, Wang Y, Shen PP (2018) Restoration of CD3(+)CD56(+) cell level improves skin lesions in severe psoriasis: A pilot clinical study of adoptive immunotherapy for patients with psoriasis using autologous cytokine-induced killer cells. Cytotherapy 20: 1155-1163
32.	Wu DM, Wen X, Wang YJ, Han XR, Wang S, Shen M, Fan SH, Zhuang J, Zhang ZF, Shan Q, Li MQ, Hu B, Sun CH, Lu J, Chen GQ, Zheng YL (2018) Effect of microRNA-186 on oxidative stress injury of neuron by targeting interleukin 2 through the janus kinase-signal transducer and activator of transcription pathway in a rat model of Alzheimer's disease. Journal of Cellular Physiology 233: 9488-9502
33.	Hu J, Shi Y, Xia M, Liu Z, Zhang R, Luo H, Zhang T, Yang Z, Yuan B (2018) WDR1-regulated actin dynamics is required for outflow tract and right ventricle development. Developmental Biology 438: 124-137
34.	Hu XC, Ding Z, Hong ZW, Zou ZC, Feng YM, Zhu RL, Ma JZ, Ge X, Li CJ, Yao B (2018) Spermatogenesis improved by suppressing the high level of endogenous gonadotropins in idiopathic non-obstructive azoospermia: a case control pilot study. Reproductive Biology and Endocrinology 16: 91
35.	Yao Y, Qiao L, Song K, Xu XQ, Shi DQ, Xu ZH, Dai J, Qin JH, Chen DY, Jiang Q (2018) Preoperative Evaluation of Soleal Vein Diameter by Ultrasound Is Beneficial for Prophylaxis of Deep Vein Thrombosis after Total Knee or Hip Arthroplasty. BioMed research international: 3417648
36.	Yang G-M, Sun J, Pan Y, Zhang J-L, Xiao M, Zhu M-S (2018) Isolation and identification of a tribenzylisoquinoline alkaloid from Nelumbo nucifera Gaertn, a novel potential smooth muscle relaxant. Fitoterapia 124: 58-65
37.	Dai B, Li Q, Song X, Ge Y, Wu J, Zhang K, Wang C, Zhang Y, Teng H, Li C, Jiang Q (2018) Knockdown of Ggps1 in chondrocyte expedites fracture healing by accelerating the progression of endochondral ossification in mice. Journal of Bone and Mineral Metabolism 36: 133-147
38.	Lu WL, Zhou S, Xue L, Dai BY, Qiao L, Chen DY, Xu ZH, Yao Y, Teng HJ, Jiang Q (2018) High preoperative serum leptin level is an independent risk factor for deep vein thrombosis after total knee arthroplasty in osteoarthritis patients: A prospective and cross-sectional study. Medicine 97: e10884
39.	Yao C, Zhang Z, Yao Y, Xu X, Jiang Q, Shi D (2018) Predictive value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio for acute deep vein thrombosis after total joint arthroplasty: a retrospective study. Journal of Orthopaedic Surgery and Research 13: 40
40.	Dai J, Zhou S, Ge QT, Qin JZ, Chen DY, Xu ZH, Shi DQ, Li JX, Ju HX, Cao Y, Zheng MH, Li CJ, Gao X, Teng HJ, Jiang Q (2018) Bi-directional regulation of cartilage metabolism by inhibiting BET proteins-analysis of the effect of I-BET151 on human chondrocytes and murine joints. Journal of Orthopaedic Surgery and Research 13: 118
41.	Dai J, Ying P, Shi D, Hou H, Sun Y, Xu Z, Chen D, Zhang G, Ni M, Teng H, Wang Y, Jiang Q (2018) FTO variant is not associated with osteoarthritis in the Chinese Han population: replication study for a genome-wide association study identified risk loci. Journal of Orthopaedic Surgery and Research 13: 65
42.	Sun J, Yang GM, Tao T, Wei LS, Pan Y, Zhu MS (2018) Isometric Contractility Measurement of the Mouse Mesenteric Artery Using Wire Myography. Jove-Journal of Visualized Experiments: e58064
43.	Lu K, Yu F, Shi TS, Shen SY, Li L, Li YX, Jiang JX, Gao X, Ju HX, Wang W, Teng HJ, Jiang Q (2018) Seasonal variance of serum biomarker levels in osteoarthritis patients: a cross-sectional study of 104 patients. International Journal of Clinical and Experimental Medicine 11: 10774-10780

Seminar in 2018

	Date	Speaker	Title	Unit
1	20180326	Moshi Song	Mitochondrial quality control and cardiac homeostasis	Institute of Zoology, Chinese Academy of Sciences
2	20180329	Shi-Qing Cai	The neurobiological basis of healthy aging	Institute of Neuroscience, Chinese Academy of Sciences
3	20180419	Yue Qin	The neurobiological basis of healthy aging	Soochow University
4	20180420	Thomas Worzfeld	Semaphorin-plexin signaling in epithelial regeneration and cancer	Institute of Pharmacology,University of Marburg
5	20180508	Fan Zhang	Host genetics shapes Caenorhabditis elegans gut microbiome	University of Maryland, College Park
6	20180510	Zilong Qiu	The genetic basis and neural circuitry for autism spectrum disorders	Institute of Neuroscience, Chinese Academy of Sciences
7	20180510	Pingsheng Liu	Lipoprotein and metabolic diseases	The Institute of Biophysics, Chinese Academy of Sciences
8	20180511	Guo Huang	Molecular control of organ regeneration in development and evolution	Investigator of Cardiovascular Research Institute UCSF
9	20180525	Yan LI	humanized mouse models for evaluation and development of immunotherapies	Institut Pasteur
10	20180531	Jinke Cheng	Protein Modification and Signaling	Shanghai Jiao Tong University
11	20180615	Jingwei Xiong	Using model organisms to gain novel insights into heart regeneration	Institute of Molecular Medicine, Peking University
12	20180621	Ye Tian	Mitochondrial stress and aging	Institute of Genetics and Developmental Biology, Chinese Academy of Sciences
13	20180625	Antonio Vidal-Puig	Adipose tissue expandability, lipotoxicity and the metabolic syndrome	University of Cambridge Metabolic Research Laboratories Wellcome Trust-MRC Institute of Metabolic Science
14	20180628	Jun Qin	Proteomics and Precision Medicine	Beijing Proteome Research Center
15	20180716	Yong Liu	The ER Stress Response Signaling in Metabolic Inflammation	Wuhan University
16	20180914	Xin Li	A new function of an old metabolite:succinate connects hyperglycemia and periodontitis	New York University
17	20180919	Longsheng Song	Calpain-mediated cleavage transforms junctophilin-2 from a structural protein to transcriptional regulator	University of Iowa Carver College of Medicine
18	20180920	Zeping Hu	Understanding Metabolic Reprogramming in Diseases Using Metabolomics and Metabolic Flux Analysis	Tsinghua University
19	20181009	Kang Chen	New insights of B lymphocyte function in immunological and reproductive health	Wayne State University
20	20181029	Biao Wang	BAT altruism: the hidden life of brown adipose tissue	Cardiovascular Research Institute, UCSF
21	20181102	Gregory Emery	Coordinating propulsive and contractile forces during collective cell migration	University of Montreal
22	20181105	Wei Yu	DNA damage, DNA repair and B cell development	Department of Immunology and department of genomes & genetics Institut Pasteur
23	20181105	Xiang Qin	Myosin oscillation: The production, transmission and regulation of intracellular biological force	University of Electronic Science and technology of China

24	20181105	Yun-Bo Shi	Epigenetic modifications in the regulation of developmental timing and rate by thyroid hormone receptor	National Institutes of Health
25	20181115	Aibin He	Long-term live imaging reconstructs in toto cell decisions for mouse heart morphogenesis	Peking-Tsinghua Center for Life Sciences
26	20181127	Ling Qi	Defining the role of ER-associated protein degradation in health and disease	University of Michigan
27	20181129	Changlin Tian	dynamic interactions between GPCR and G proteins illustrated using fluorescent lifetime spectroscopy	University of Science and Technology of China
28	20181210	Nan Cao	Chemical engineering of cardiac cell fate	Sun Yat-sen University

Courses and Teachers

The MARC, as an institute of the University of Nanjing, is home to approximately 206 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 2018, the following lecture series and courses were given by MARC staff at Nanjing University (unless indicated otherwise).

Information genomics:

Zhenji Gan

Cell Biology and Molecular Biology

Shuai Chen

Guoqiang Wan

Chaojun Li

Cell signaling

Geng Liu

Jianghuai Liu

Jun Yan

Chaojun Li

Zhongzhou Yang

Genetics

Qing Zhang

Jinzhong Qin

Di Chen

Xin Lou

Doctoral qualification exam I&II

All PI in MARC

Life, Evolution and Health

Xiang Gao

Zhaoyu Lin

Frontier of Cell Biology

Shiqing Cai (Institute of neuroscience, Chinese academy of sciences)

Yue Qin (Suzhou University)

Zilong Qiu (Institute of neuroscience, Chinese academy of sciences)

Jinke Cheng (Shanghai Jiao Tong University)

Ye Tian (Institute of genetics and developmental biology, Chinese academy of sciences)

Jun Qin (Beijing proteome research center)

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

PhD Theses

MARC students successfully defended the following PhD thsese in 2018

PhD Theses:

Group Xiang Gao
Dayuan Zou
Commensal bacterial expressing BlcE84 induces disruption of host epithelial barrier and local inflammation

Qin Chen
GSDMB promotes non-canonical pyroptosis by enhancing Caspase-4 activity

Group Zhongzhou Yang
Mingyang Jiang
RHAU couples mRNA translation and stability to sustain heart function and regeneration

Hengwei Jin
The SWI/SNF complex subunit BAF155 is essential for normal outflow tract cushion formation and OFT septation, as well as modulating mitochondrial dynamics and oxidative phosphorylation in the OFT septation.SWI/SNF.

Group Ying Xu
Pancheng Xie
Explore genetic determinants for clock amplitude and entrainment

Yang An
The affection of Cis-element, phosphorylation and codon usage on regulating circadian clock

Zhihui Zhang
A role of suprachiasmatic nucleus in response to time-restricted feeding

Group Qingshun Zhao
Meijing Liu
A genetic locus linked mapk15 is essential for the survival of zebrafish embryos via regulating vps28 expression

Group Jun Yan
Lan Shen
A genetic locus linked mapk15 is essential for the survival of zebrafish embryos via regulating vps28 expression

Group Zhenji Gan
Xijun Liang
Delineate the molecular mechanisms controlling skeletal muscle fitness relevant to exercise

Group Shuai Chen
Chao Quan
Functional study of the novel PKB substrate SPEG in heart

Group Ying Cao
Anhua Lei
Study on the transdifferentiation of tumor cells into neuron-like cells and its underlying mechanism

Group Jiong Chen
Dou Wang
Platelet-derived growth factor/VEGF receptor Signaling Controls Spatially Restricted Rab11 Activation to Promote Polarized Trafficking during Collective Migration

Xianping Wang
Study of Ecdysone Induced Nuclear Receptor E75 Regulate Border Cell Migration

Group Yun Shi
Jiang Chen
Cas9-based Conditional Knockout Strategy and Its Application in Neurons

Dan Wu
The Study of a Novel Hypoosmotic Stress Activated Ion Channel TMEM63B

Group Chaojun Li
Jia Liu
Geranylgeranyl diphosphate synthase (GGPPS) regulates hepatocellular carcinogenesis by targeting glucose metabolism

Group Qing Zhang
Xia Yao
Vap positively regulates Hippo signaling through Four-jointed

Group Jinzhong Qin
Wukui Zhao
Dissecting non-canonical PRC1 Functions in Embryonic Stem Cells.

Group Di Chen
Jianfeng Lan
Molecular mechanisms of the super longevity produced by inhibiting both IGF-1 and TOR pathways in C. elegans

Group Xin Lou
Linlin Zhang
The effect of CRISPR/Cas9-mediated SHP-1 or PD-1 editing on the anti-tumor activity of CAR T cells

2018 Laboratory Open Day

To popularize scientific knowledge, initiated by China Association for Science and Technology and the Chinese Society for Cell Biology, Laboratory Open Day on the theme "A dream-Heredity and Development" organized by MARC, NBRI, State Key Laboratory of Pharmaceutical Biotechnology, Jiangsu Society for Cell and Developmental Biology, Chinese Society for Cell Metabolism and Society for Cell Differentiation and Development, CSCB, was held on 26th May, 2018. More than 260 persons including pupil and their parents, junior student, senior students and other biological enthusiasts attended the activities to get close to cute little animals, laboratory work, opening the door for scientific mysteries exploration.

Prof. Zhongzhou Yang, the president of Society for Cell Differentiation and Development of CSCB, gave an introduction about the history and development of MARC, chatted with participants and then interviewed by Nanjing Radio and Television Station.

Besides four chapters: exhibiting four model animals, distributing scientific booklets, playing popular science video, as well as showing posters about MARC research work, we added six vivid and interesting presentations of model animals this years. We showed the participants how these animals such as mice, rats, African claw frogs, zebrafish, fruit flies, and nematodes become the favorite of scientists and the contribution of animals in scientific research. Otherwise, plenty of interesting lab experiments were available for people to be involved in.

Laboratory Open Day aimed to make science no longer mysterious, and inspire participants motivation in science. This activity was reported by medias.

The CSCB Training Course on Mouse Genetics and Phenotyping Sept 17-21, 2018 Model Animal Research Center, Nanjing University, China

Genetic mouse models are vital to understand the functions of genes and genetic networks in health and disease. Organized by Chinese Society for Cell Biology (CSCB), Model Animal Research Center (MARC) of Nanjing University, Nanjing Biomedical Research Institute (NBRI) of Nanjing University and Jiangsu Society for Cell and Developmental Biology, the inaugural CSCB Training Course on Mouse Genetics and Phenotyping was held from Sept 17-21, 2018 at MARC. The course aims to help participants understand the versatility of mouse as an important model for studying gene function and human diseases, to improve the technical skills of participants in mouse phenotyping and histological analyses, to promote application and standardization of mouse genetics in international research community and to facilitate the collaboration and innovation in biomedical advances using mouse models, particularly in the "one belt one road" initiative countries.

Twenty participants from Pakistan, Sri Lanka, Thailand, Philippines and Singapore attended the course. Dr Shuai Chen, director of MARC and Dr Xiaoyan Ding, vice-president of CSCB delivered the opening speeches. Dr Chen introduced the research and resources at MARC and highlighted the mission of this training course for promoting biomedical research and collaboration in "one belt one road" initiative countries. Dr Ding introduced the mission and vision of CSCB and its efforts in helping young scientists succeed in biomedical research, encouraged the participants to embrace the training course and provide valuable feedback and suggestions for continuous success of the course.

This training course includes both comprehensive lectures and hands-on workshops on mouse genetics and phenotyping, involving metabolic homeostasis and disease models, immune system function and homeostasis, organ development and regeneration and neurodevelopment and disease models. In addition, the course also provided opportunities for each participant to present and share their research projects, with feedbacks from both fellow participants and senior researchers at MARC. Through this interactive training course, the participants not only gained knowledge and techniques on the applications of mouse models in development and disease, but also established new connections and potential collaborations for research advances.

On Sept 20, the training course was joined by senior scientists and leaders from the Faculty of Pharmaceutical Sciences (FPS), Chulalongkorn University of Thailand. Eight delegates from Chulalongkorn University and over 15 scientists at MARC and NBRI attended the round-table discussion and proposed academic and research collaborations including student and faculty exchanges, joint research projects, and joint proposal for research funding supports. Dr Pornchai Rojsitthisak, the vice-dean of FPS and Dr Shuai Chen, the director of MARC signed the memorandum of understanding (MOU) between the FPS of Chulalongkorn University and MARC of Nanjing University.

The Seventh Sino-Japan Summer Course of Genetic Mouse Models
July 23-25, 2018
Model Animal Research Center, Nanjing University, China

The Sino-Japan Summer Course of Genetic Mouse Models aims to provide essential trainings and cultivate innovation for the next generation scientists on the development and applications of genetic mouse models for human diseases; to encourage communication and collaboration among scientists from biomedical sciences, developmental biology and related fields. In the past six years, the summer course has been successfully organized yearly by research centers from China, Japan and South Korea. The seventh summer course was held from July 23-25, 2018 at MARC. This course is co-organized by Model Animal Research Center (MARC) of Nanjing University, RIKEN BioResource Research Center and the Chinese Society for Cell Biology (Developmental Biology Chapter), supported by Nanjing Biomedical Research Institute (NBRI) of Nanjing University, State Key Laboratory of Pharmaceutical Biotechnology, Chinese Society for Cell Biology (Cell Metabolism Chapter) and Jiangsu Society for Cell and Developmental Biology.

The summer course consists of two sessions, including lectures and practical training courses. List of lecturers includes renowned scientists on humanized mouse models, scientists and researchers from RIKEN, senior scientists from research institutes in China as well as scientists at MARC. A total of 66 participants from various universities, institutes and hospitals around the country were selected to attend the course. The comprehensive course covered research topics including mouse genetics and genome manipulations, mouse resources for biomedical research, disease models and phenotyping and humanized mouse models, last of which is the theme of the course this year. The participants actively engaged in both lecture and practical sessions and gained valuable experiences in application of mouse models for biomedical research. The course also provides an essential platform for continuous exchanges of ideas and sharing of resources among participants and scientists at MARC.

2018 Annual Conference of MARC

The 2018 Student of MARC election convention was held on December 3th, Tingting Fu from Dr. Zhenji Gan's laboratory and Chao Quan from Dr. Shuai Cheng were awarded the 2018 Student of MARC for their excellent research. Dayuan Zou from Dr. Xiang Gao's laboratory was nominated.

The 2018 MARC Annual Conference was held in Science hall of Gulou Hospital On December 4th. This conference was organized by Dr. Xiang Gao . More than 200 scientists and students from Nanjing University attended the conference.

Dr. Xiang Gao, director of MARC, made welcome remarks at the opening ceremony, in the following sessions, PIs presented latest research progresses and scientific ideas from their laboratories, followed by lively discussions between the speakers and the audiences. More than 100 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, eleven students received 2018 Outstanding Poster Prize.

2018 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 9th Summer Camp from July 18 to 22 this summer. 47 excellent undergraduates were selected from a pool of 199 applicants .

Wonderful programs have been organized in order to increase the interaction between undergraduate students and our faculty members/graduate students. 5 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 ,C.elegans and Zebrafish.

We respectively held academic salons, dedicates PI with summer camp students communicate with each other at three nights .Moreover, 2 of our outstanding graduate students, Pei Wang and Lin Liu, 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.

2018 Students Union

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

In the year of 2018, outdoor activities will not only spice up our life, but also more importantly inspire passion and enthusiasm for physical exercises, which is more than necessary for scientific students like us. Additionally, many students also participated in organizing academic activities. 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.

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