Annual REPORT

NBRI

2017

Model Animal Research Center of Nanjing University
Moe Key Laboratory of Model Animal for Disease Study
Nanjing Biomedical Research Institute of Nanjing University
National Resource Center for Mutant Mice

Director's Words

As expected, China made significant achievements in many fields in 2017, particularly in science and technology . These achievements encourage Chinese people make further progress with a great enthusiasm.

MARC has also made her progress in the past year. As highlighted in this annual report, Dr. Liu's lab revealed the physiological response of activated P53 to mild stress of the cells, which can lead to significant understanding interplay of stress, cell fate and tumorigenesis; Dr. Shen's lab found a regulatory scenario of tumor-associated macrophage in tumor progress and proposed a potential therapeutic strategy for tumor; Dr. Yang's lab established a reliable method tracking the regenerated cardiac cells; Dr. Chen Shuai proposed a myogenic mechanism for hyperlipidemia and hepatosteatosis; Dr. Zhu's lab revealed a mechanism for hyperresponsiveness of asthmatic airway smooth muscle. Many other labs have also explored their interests and made their achievements. However, we expect more creative achievements and breakthrough progresses.

Looking back at MARC's achievements throughout the year has always been one of my favorite moments. It reminds me of the reasons why I chose MARC to further my academic career, and how MARC has never let me down. MARC possesses a first-class of animal center with abundant gene-editing technology and mice resources that fuel our goals of making first-class achievements in developmental biology, cell biology, genetics and gene editing. She preserves our curiosity and boldness to make innovative and exciting explorations. We may forget our painstaking efforts, but we dare not forget our beginning ambition. I wish everyone of MARC will not forget this beginning ambition and make further progress.

In 2018, we have several new projects involving metabolic regulation of development biology, cell migration and debates diseases.

Remember what inspired us at the beginning of our missions.

Min-Sheng Zhu

Director

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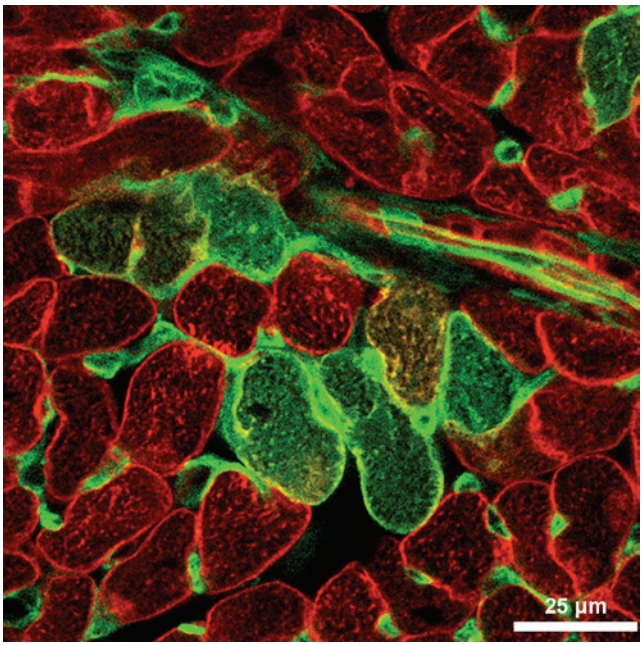
Group Zhongzhou Yang & Geng Liu

p53^{+} cells drive in vivo cardiomyocyte expansion

Qi Xiao, Guoxin Zhang, Huijuan Wang, Lai Chen, Shuangshuang Lu, Dejing Pan, Geng Liu, Zhongzhou Yang

The mammalian heart has a limited capacity to regenerate. Under certain conditions, however, cardiomyocyte proliferation has been observed, for example in resected neonatal hearts and in response to certain cytokine treatments. Nevertheless, the extent to which cardiomyocyte proliferation occurs both in steady state and after injury in the postnatal mouse is hotly debated, as studies are limited by a lack of reliable genetic tracing tools. Groups of Zhongzhou Yang and Geng Liu use a p53-based genetic tracing system to investigate postnatal cardiomyocyte proliferation and heart regeneration through neonatal, adolescent and adult stages. The authors observed clonal expansion of p53+cardiomyocytes in the neonatal heart, as well as in pre-adolescent and adult hearts. Interestingly, some of the labeled cardiomyocytes also formed larger clusters if given a longer tracing time, suggestive of a selectively long-lasting proliferative potential. The authors also investigated cardiomyocyte proliferation after cryo-injury and showed that p53+ cardiomyocytes exhibit cytomembrane localization of the sarcomeric protein cTnT during heart regeneration, consistent with previous studies. Finally, the authors demonstrated that the p53 genetic labeling system reliably traced proliferating cardiomyocytes following not only in cryo-injury, but also in two additional types of cardiac injury models in neonatal mice. This study reveals the specific lineage contribution to mammalian cardiac repair and provides evidence for the heterogeneity of cardiomyocytes in mammalian heart (from the journal of Development).

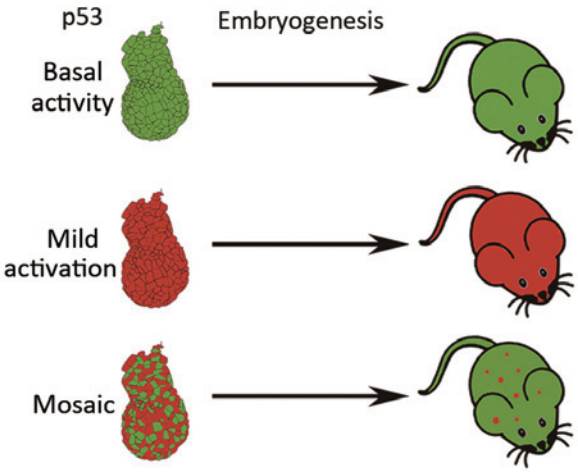


Group Geng Liu

p53 pathway linking stress response to cell competition

Guoxin Zhang, Yinyin Xie, Ying Zhou, Cong Xiang, Lai Chen, Chenxi Zhang, Xiaoshuang Hou, Jiong Chen, Hui Zong and Geng Liu

In response to a variety of stress signals, tumor suppressor p53 is activated to influence the cellular outcome. Various stresses result in different levels of p53 activation. Acute stresses such as DNA damage are able to trigger a high level of p53 activity, leading to cell cycle arrest or apoptosis. In contrast, the cellular response of mild p53 activity induced by low-level stress in vivo remains largely unexplored. Using a strategy of haploinsufficiency of Mdm2 and Mdm4 to induce mild p53 activation in vivo, Geng Liu group demonstrated that during mouse development, uniform activation of p53 at a moderate level was well tolerated in the embryos. In contrast, mild activation of p53 in individual cells led to a growth disadvantage and their out-competition when they were present together with non-activated cells. This also occurred in the fast turnover tissues of the adult mice. These results suggest that p53 may coordinate at a multi-cellular level for optimal stress response and fitness of the organism. With the emerging roles of cell competition in tissue homeostasis, aging, and cancer, this study suggested a close link between stress response and cell competition and underscored the delicate control of cell fate by p53 (from the journal of PNAS, 2017).



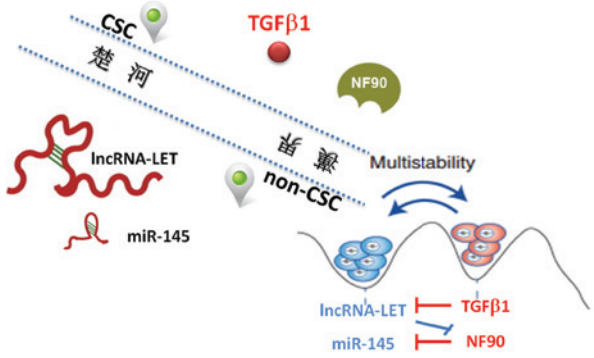
A model illustrating the distinct fate of cells with mild p53 activation in different multi-cellular contexts.

Group Jun Yan

TGFβ1 promotes gemcitabine resistance through regulating the lncRNA-LET/NF90/miR-145 signaling axis in bladder cancer

Junlong Zhuang#, Lan Shen#, Lin Yang , Xiaojing Huang, Qun Lu, Yangyan Cui , Xi Zheng, Xiaozhi Zhao, Dianzheng Zhang, Ruimin Huang, Hongqian Guo*, Jun Yan*

High tumor recurrence is frequently observed in patients with urinary bladder cancers (UBCs), with the need for biomarkers of prognosis and drug response. Chemoresistance and subsequent recurrence of cancers are driven by a subpopulation of tumor initiating cells, namely cancer stem-like cells (CSCs). However, the underlying molecular mechanism in chemotherapy-induced CSCs enrichment remains largely unclear. In this study, we found that lncRNA-Low Expression in Tumor (lncRNA-LET) was downregulated in chemoresistant UBC, accompanied with the enrichment of CSC population. Its low expression, which is directly repressed by TGFβ/SMAD signaling, is essential and necessary for UBC cell stemness and gemcitabine-induced tumor recurrence. Consequently, reduced lncRNA-LET increased the NF90 protein stability, which in turn repressed biogenesis of miR-145 and subsequently resulted in accumulation of CSCs. Notably, a clinically relevant specific inhibitor of TGFβRI -- LY2157299 sensitized gemcitabine resistant xenografts to gemcitabine and significantly reduced tumorigenecity. Moreover, overexpression of TGFβ1, combined with decreased levels of lncRNA-LET and miR-145 predicted poor prognosis in UBC patients. Collectively, we proved that the dysregulated lncRNA-LET/NF90/miR-145 axis by gemcitabine-induced TGFβ1 promotes UBC chemoresistance through enhancing cancer stemness. The combined changes in TGFβ1/lncRNA-LET/miR-145 provide novel molecular prognostic markers in UBC outcome. Therefore, targeting this axis could be a promising therapeutic approach in treating UBC patients.



Working model of TGFβ1/lncRNA-LET/NF90/miR-145 axis in chemoresistance.

Group Pingping Sheng

Caspase-1 cleaves PPARγ for potentiating the pro-tumor action of TAMs

Zhiyuan Niu[1] , Qian Shi[2], Wenlong Zhang[1] , Yuxin Shu[1] , Nanfei Yang[1] , Bing Chen[3], Qingsong Wang[4], Xuyang Zhao[4], Jiajia Chen[1] , Nan Cheng[1] , Xiujing Feng[1] , Zichun Hua[5], Jianguo Ji[4] & Pingping Shen[1]

1. State Key Laboratory of Pharmaceutical Biotechnology and MOE Key Laboratory of Model Animal for Disease Study, Model Animal Research Center, Nanjing University, Nanjing 210023, China.

2. Department of Cellular and Integrative Physiology, The University of Texas Health Science Center at San Antonio, STRF-Greehey North Campus, 8403 Floyd Curl Drive, San Antonio, Texas 78229-3904, USA.

3. Department of Hematology, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing 210008, China.

4. The State Key Laboratory of Protein and Plant Gene Research, College of Life Sciences, Peking University, Beijing 100871, China. 5 State Key Laboratory of Pharmaceutical Biotechnology School of Life Sciences, Nanjing University, Nanjing 210023, China.

Abstract

Tumor-associated macrophages are increasingly viewed as a target of great relevance in the tumor microenvironment, because of their important role in cancer progression and metastasis. However, the endogenous regulatory mechanisms underlying tumor-associated macrophage differentiation remain largely unknown. Here, we report that caspase-1 promotes tumor-associated macrophage differentiation by cleaving peroxisome proliferator-activatedreceptor gamma (PPARγ) at Asp64, thus generating a 41 kDa fragment. This truncated PPARγ translocates to mitochondria, where it directly interacts with medium-chain acyl-CoA dehydrogenase (MCAD). This binding event at-tenuates MCAD activity and inhibitsfatty acid oxidation, thereby leading to the accumulation of lipid droplets and promotingtumor-associated macrophage differentiation. Furthermore, the ad-ministration of caspase-1inhibitors or the infusion of bone marrow-derived macrophages genet-ically engineered to overexpress murine MCAD markedly suppresses tumor growth. Therefore, targeting the caspase-1/PPARγ/MCAD pathway might be a promising therapeutic approach to prevent tumor progression

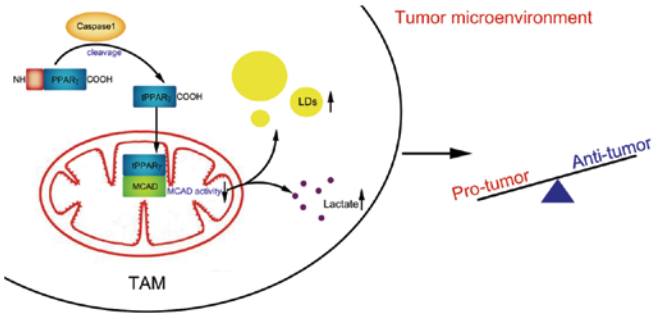


Figure legend: In the current study, we provided novel insights into the mechanisms underlying TAM differentiation and demonstrated that caspase-1 activity increases during TAM differentiation, and that caspase-1 cleaves PPARγ at Asp64 and generates a 41 kDa fragment, this truncated PPARγ translocates to mitochondria and inactivates MCAD. MCAD inhibition causes lipid droplets (LDs) accumulation in TAMs and enhanced lactate secretion, MCAD activity and metabolic reprogramming are highly correlated with pro-tumor action of TAMs.

Group Minsheng Zhu

Inflammatory mediators mediate airway smooth muscle contraction through a G protein-coupled receptor–transmembrane protein 16A–voltage-dependent Ca^{2+} channel axis and contribute to bronchial hyperresponsiveness in asthma

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#

Background:

Allergic inflammation has long been implicated in asthmatic hyperresponsiveness of airway smooth muscle (ASM), but its underlying mechanism remains incompletely understood. Serving as G protein-coupled receptor agonists, several inflammatory mediators can induce membrane depolarization, contract ASM, and augment cholinergic contractile response. We hypothesized that the signal cascade integrating on membrane depolarization by the mediators might involve asthmatic hyperresponsiveness.

Objective:

We sought to investigate the signaling transduction of inflammatory mediators in ASM contraction and assess its contribution in the genesis of hyperresponsiveness.

Methods:

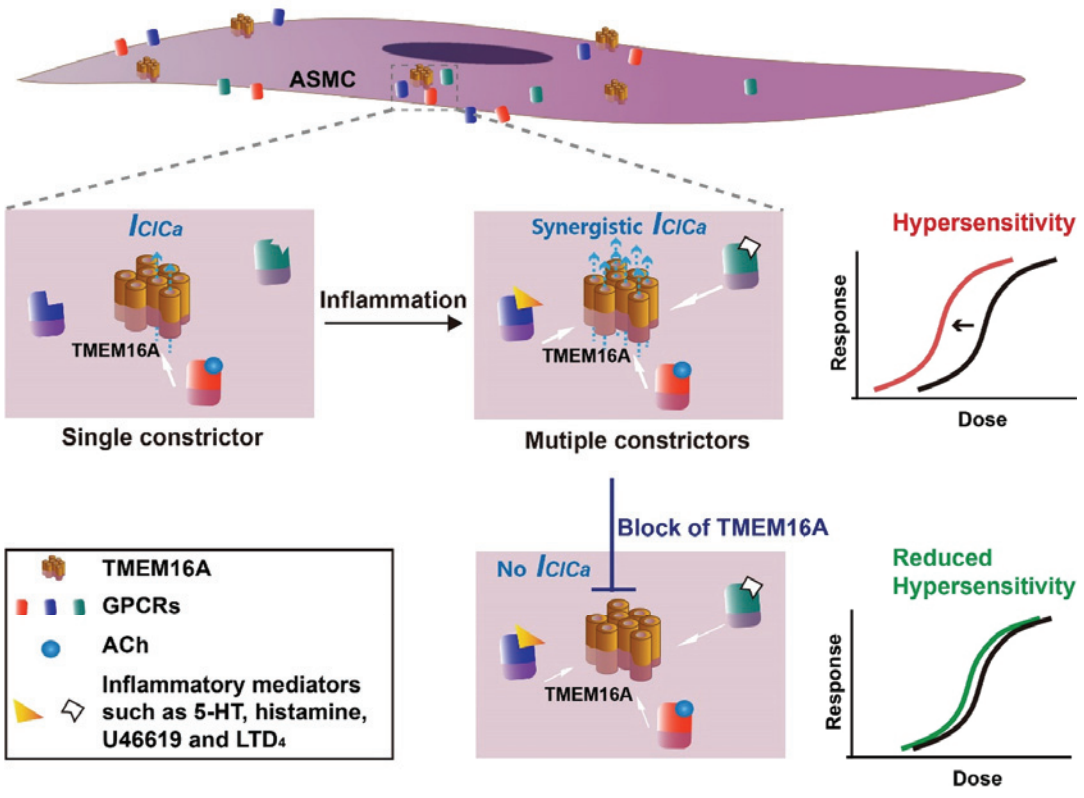
We assessed the capacity of inflammatory mediators to induce depolarization currents by electrophysiological analysis. We analyzed the phenotypes of transmembrane protein 16A (TMEM16A) knockout mice, applied pharmacological reagents, and measured the Ca^{2+} signal during ASM contraction. To study the role of the depolarization signaling in asthmatic hyperresponsiveness, we measured the synergistic contraction by methacholine and inflammatory mediators both ex vivo and in an ovalbumin-induced mouse model.

Results:

Inflammatory mediators, such as 5-hydroxytryptamin, histamine, U46619, and leukotriene D_4, are capable of inducing Ca^{2+}-activated Cl^- currents in ASM cells, and these currents are mediated by TMEM16A. A combination of multiple analysis revealed that a G protein-coupled receptor–TMEM16A–voltage-dependent Ca^{2+} channel signaling axis was required for ASM contraction induced by inflammatory mediators. Block of TMEM16A activity may significantly inhibit the synergistic contraction of acetylcholine and the mediators and hence reduces hypersensitivity.

Conclusions:

A G protein-coupled receptor–TMEM16A–voltage-dependent Ca^{2+} channel axis contributes to inflammatory mediator-induced ASM contraction and synergistically activated TMEM16A by allergic inflammatory mediators with cholinergic stimuli.



5-HT: 5-hydroxytryptamine; ACh: Acetylcholine; ASMC: Airway smooth muscle cell; GPCRs: G-protein-coupled receptors; I_{ClCa}: Ca^{2+} -activated Cl^- current; LTD_4: Leukotriene D_4

Pei Wang

Pei Wang received his Bachelor's degree of Biological Science in 2010 from School of Life Sciences, Nanjing University. He joined Dr. Min-Sheng Zhu's lab at the year of 2009 to study the molecular mechanisms of smooth muscle contraction and relaxation.

For the past seven years, his work focused on the function of a Ca^{2+} activated-Cl^- channel, TMEM16A, in smooth muscle contraction. Last year, he and colleagues reported that a RyR-TMEM16A-VDCC-MLCK axis underlies the molecular basis of internal anal sphincter spontaneous tone generation. Recently, he and colleagues found GPCR-ITMEM16A-VDCC axis plays an important role in inflammatory mediator-triggered sensitized contraction in airway smooth muscle, and this pathway participates in the formation of bronchial hyperresponsiveness. These discoveries are important for future drug development, for which indicated novel targets.

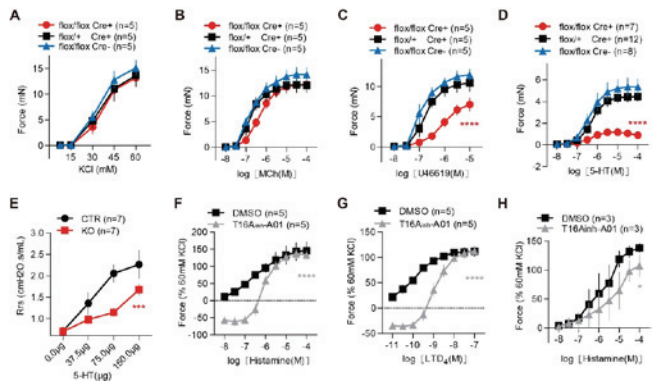
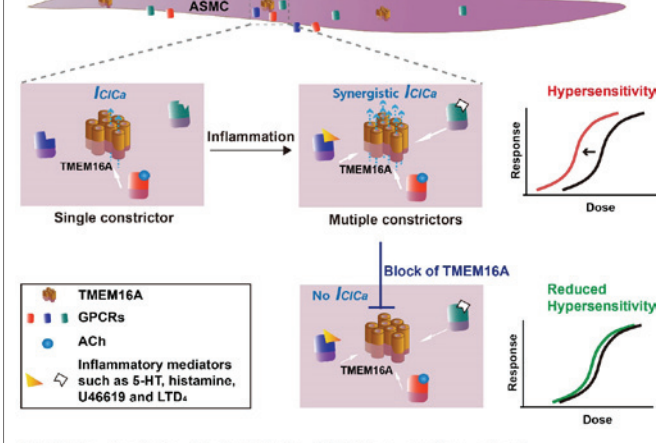


Figure1. TMEM16A is required by inflammatory mediators induced airway smooth muscle contraction

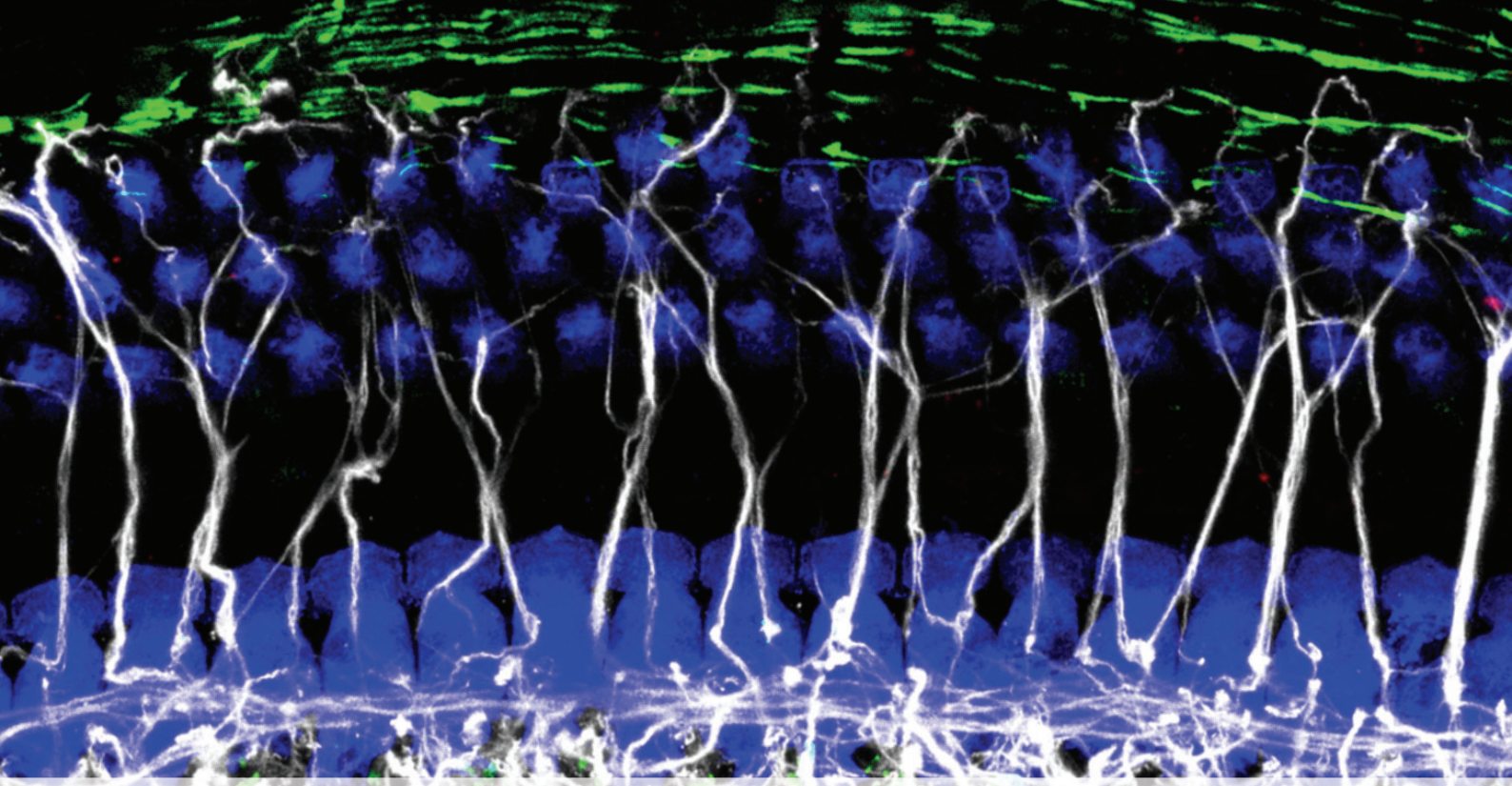


5-HT: 5-hydroxytryptamine; ACh: Acetylcholine; ASMC: Airway smooth muscle cell; GPCRs: G-protein-coupled receptors; I_{ClCa}: Ca^{2+} -activated Cl^- current; LTD_4: Leukotriene D_4

Figure2. TMEM16A contribute to bronchial hyperresponsiveness in asthma

Selected publications

1. Wang P*, Zhao W*, Sun J, Tao T, Chen X, Zheng YY, et al. Inflammatory mediators mediate airway smooth muscle contraction through a G protein-coupled receptor-transmembrane protein 16A-voltage-dependent Ca^{2+} channel axis and contribute to bronchial hyperresponsiveness in asthma. The Journal of allergy and clinical immunology, (2017).
2. Zhang CH*, Wang P*, Liu DH, Chen CP, Zhao W, Chen X, et al. The molecular basis of the genesis of basal tone in internal anal sphincter. Nature communications 7, 11358 (2016).



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.

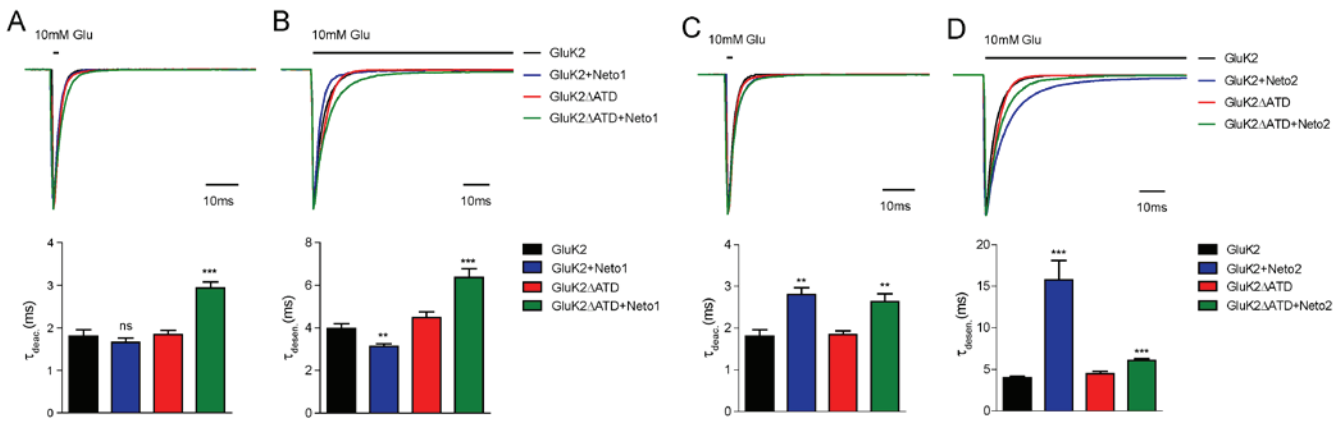


Figure 1. GluK2 ATD is critical for Neto regulation of KAR function.

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.

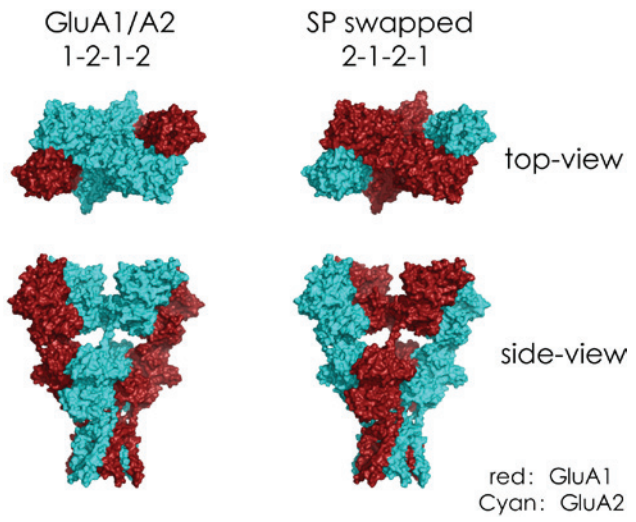


Figure 2. Signal peptides determine the spatial assembly of heteromeric AMPA receptors.

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.

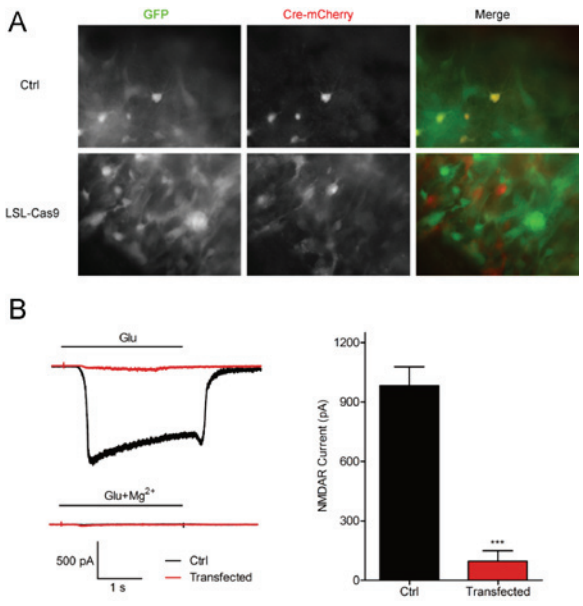


Figure 3. Targeted gene deletion in neurons using a Cas9 transgenic mouse.

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. 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. 7:517.
2. 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. pii: e20991.
3. 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:E5645-54.
4. 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.
5. 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.
6. 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
7. Herring B*, Shi Y*, Suh YH, Zheng CY, Schmid SM, Roche KW, Nicoll RA. (2013) Cornichon proteins determine the subunit composition of synaptic AMPA receptors. Neuron. 77(6),1083-96
8. Granger AJ, Shi Y, Lu W, Cerpas M, Nicoll RA. (2013) LTP requires an extrasynaptic pool of glutamate receptors independent of subunit type. Nature. 493(7433), 495-500

Group members

Graduate students

Yanjun Li
Change Ye
Dan Wu
Jiahui Sun
Jiang Chen
Pengshi Xiao
Guifang Duan
Chaohua Jiang
Han Du
Qingqing Li

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 the γ–secretase complex regulates neuronal survival and/or death. His lab uses a combination of mouse genetics, molecular biology, cellular and behavioral neuroscience techniques to address this question. Elucidation of molecular mechanisms for age-related neurodegeneration may help identify novel therapeutic targets for the prevention and the treatment of neurodegenerative diseases.

Contact Information

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

Molecular and cellular mechanisms for neurodevelopmental and neurodegenerative diseases

Decreased expression but increased activity of PDK1 has been reported in neurodegenerative disease. Since PDK1$^{-/-}$ mice die at embryonic day 9.5 (E9.5), viable forebrain specific PDK1 conditional knockout (cKO) mice were generated to study its role in neuron survival. First, Nissl staining revealed remarkable reduction on the size of the cortex in PDK1 cKO mice aged at 3 weeks or 3 months (Fig.1A). Second, the thickness of the cortex in PDK1 cKO mice was decreased by about 50% as compared to controls (Fig.1A). Third, stereological cell counting results showed that the cortical volume was dramatically decreased in PDK1 cKO mice (Fig.1B). The number of mature neurons was measured by counting total NeuN positive (+) cells. There was significant reduction in PDK1 cKO mice (Fig.1C). Consistently, Western results confirmed reduced NeuN levels (Fig.1D).

To investigate whether neuron loss occurred via apoptosis, TUNEL assay was conducted. Cell counting results indicated that the averaged number of TUNEL+ cells per section in PDK1 cKO mice was significantly larger than that in controls (Fig.2A), suggesting enhanced apoptotic cell death. To identify which cell type underwent apoptosis, double-staining for TUNEL/NeuN, TUNEL/Tuj1 or TUNEL/GFAP was performed. TUNEL+/NeuN+ and TUNEL+/Tuj1+ but not TUNEL+/GFAP+ cells were observed in PDK1 cKO mice, as compared to controls (Fig.2B). To study whether dendrites were affected in PDK1 cKO mice, double-staining of MAP2/Ctip2 was performed. The averaged dendritic length was significantly decreased in PDK1 cKO mice at 3 weeks or 3 months (Fig.3A-B). For neurons in cortical layer V, there was more than 80% of reduction on the averaged dendritic length in PDK1 mutants. For pyramidal neurons in hippocampal CA1, the averaged dendritic length was also dramatically reduced in PDK1 cKO mice.

To study whether there were changes on glial cells in PDK1 cKO mice, IHC on GFAP was performed. Increased number of GFAP+ cells was observed in PDK1 cKO mice (Fig.3C). Since the Cre recombinase is not only expressed in excitatory neurons but also in astrocytes in Emx1-Cre mice, double-staining for GFAP/PDK1 was conducted. GFAP+/PDK1+ cells were hardly detected and GFAP+ cells were largely PDK1 negative in PDK1 cKO mice (Fig.3D). Overall, the above data highlight critical roles of PDK1 in neuronal survival and astrogliogenesis.

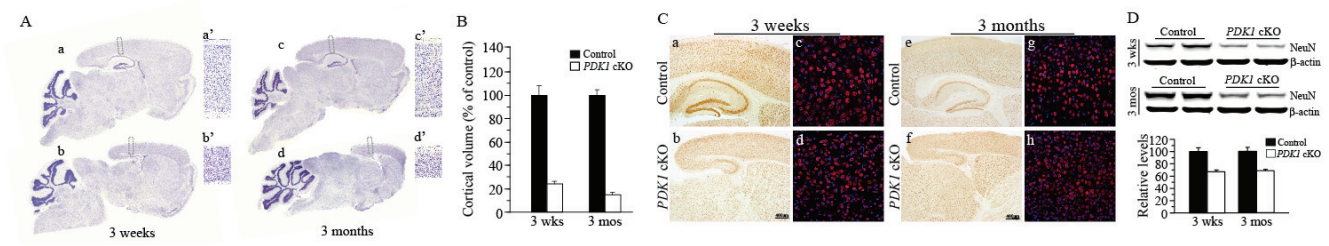


Figure 1. Microcephaly and neuron loss in PDK1 cKO mice. A. NIssl staining. B. Cortical volume. C. NeuN immunostaining results. C. Western blotting on NeuN.

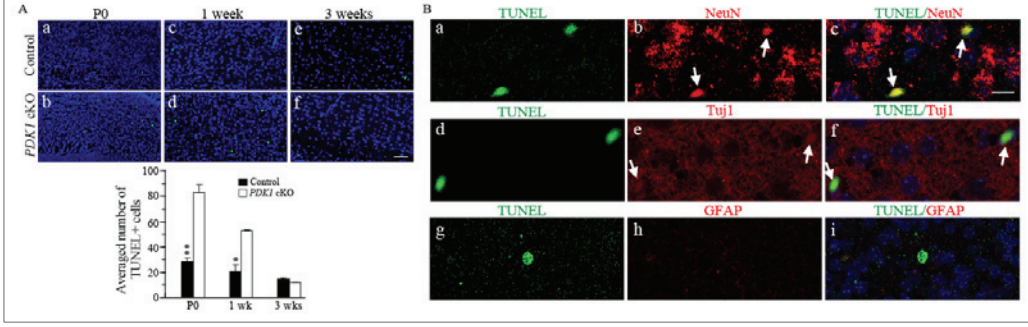


Figure 2. Apoptotic cell death in PDK1 cKO mice. A. TUNEL staining and the averaged number of TUNEL+ cells on brain sections at P0, 1 week and 3 weeks. B. Double-staining for TUNEL/NeuN (a-c), TUNEL/Tuj1(d-f) or TUNEL/GFAP (g-i).

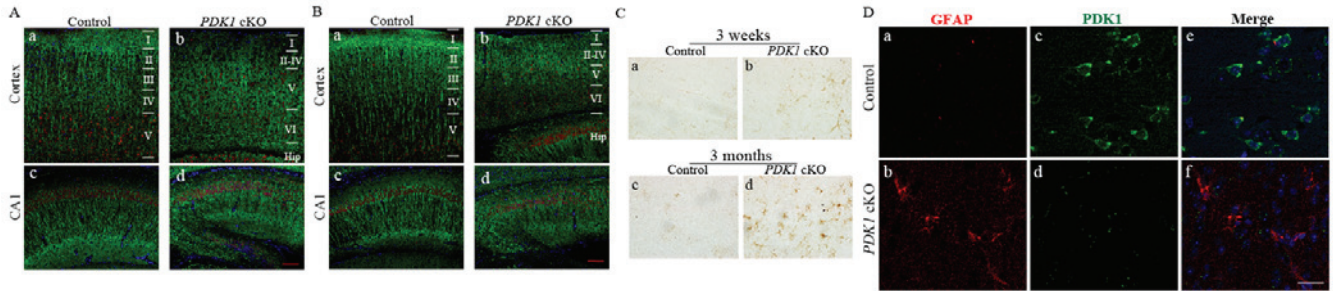


Figure 3. Decreased dendritic length but increased astrocytosis in PDK1 cKO mice. A-B. MAP2 staining on brain sections at 3 weeks and 3 months. C. Immunohistochemistry of GFAP. D. Double-staining of GFAP/PDK1.

Recent publications (*, Corresponding author)

1. 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, in press.
2. 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.
3. 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. Front. Cell. Neurosci., 2017; DOI: 10.3389/fncel.2017.00330.
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5. Wang H, Zhang B, Zhang T, Wang L, Zou X, Xu Y, Chen L, Chen G*. Impaired spatial learning is associated with disrupted integrity of the white matter in Akt3 knockout mice. CNS Neuroscience & Therapeutics, 2017; 23: 99–102.
6. He X, Li Y, Kalyanaraman C, Qiu L, Chen C, Xiao Q, Liu W, Zhang W, Yang J, Chen G, Jacobson MP, Shi Y. GluA1 signal peptide determines the spatial assembly of heteromeric AMPA receptors. Proc Natl Acad Sci USA, 2016;113: E5645-5654.
7. Hou J, Cheng S, Chen L, Wang Q, Wang H, Shi Y, Xu Y, Yin Z, Chen G*. Astroglial activation and tau hyperphosphorylation precede to neuron loss in a neurodegenerative mouse model. CNS Neuroscience & Therapeutics, 2016; 22: 244–247.
8. Wang L, Cheng S, Yin Z, Xu C, Hou J, Lu S, Yu T, Zou X, Peng Y, Xu Y*, Yang Z*, Chen G*. Conditional inactivation of Akt three isoforms causes tau hyperphosphorylation in the brain. Molecular Neurodegeneration, 2015; DOI☒10.1186/s13024-015-0030.
9. Cheng S, Hou J, Zhang C, Xu C, Wang L, Zou X, Yu, H, Shi Y, Yin Z*, Chen G*. Minocycline reduces neuroinflammation but does not ameliorate neuron loss in a mouse model of neurodegeneration. Scientific Reports, 2015; 5,10535☒DOI:10.1038.
10. Cheng S, Zhang C, Xu C, Wang L, Zou X, Chen G*. Age-dependent neuron loss is associated with impaired adult neurogenesis in forebrain neuron-specific Dicer conditional knockout mice. The International Journal of Biochemistry & Cell Biology, 2014; 57:186-96.

Group members

Group leader

Guiquan Chen

Graduate students

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

Graduate student from collaborator's lab

Xiaolian Ye

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.

We previously reported that extracellular high-mobility group box 1 protein (HMGB1), non-histone chromatin-associated nuclear protein, mediated chronic microglial activation through acting on Mac1 receptor. Nuclear HMGB1 regulates gene transcription genome stability. Interestingly, HMGB1 can be actively secreted by activated immunocompetent cells or passively released from necrotic cells into the extracellular milieu. Once released, HMGB1 can act as a DAMP (damage associated molecular pattern molecule) or a pro-inflammatory cytokine. Unlike the secretion of most cytokines, HMGB1 is released through endoplasmic reticulum (ER)- and Golgi-independent unconventional protein secretion pathways due to lacking classical secretion signal peptides. Posttranslational modifications including phosphorylation and acetylation participate in the nuclear-to-cytoplasmic translocation and extracellular secretion of HMGB1. However, it is unclear how these specific modifications of HMGB1 are regulated. We recently have found sirtuin 1 (SIRT1), a NAD+-dependent class III histone deacetylase, regulated HMGB1 acetylation state and its secretion in brain microglia. Resveratrol, an activator of SIRT1 blocked LPS-elicited acetylation and secretion of HMGB1 in microglia. In contrast, sirtinol, a specific NAD+-dependent class III histone deacetylase sirtuin inhibitor, increased the acetylation and secretion of HMGB1 in primary microglia (Figure 1A). Anti-HMGB1 antibody immunoprecipitated HMGB1 from the cell lysates after microglia were treated with LPS, resveratrol and sirtinol. Separated immunoprecipitates were immunoblotted and probed for acetyl-lysine to detect acetyl-HMGB1. The activator of SIRT1 blocked the acetylation of HMGB1 caused by LPS stimulation, and sirtinol increased the level of acetylation of HMGB1. Thus, HMGB1 is a direct deacetylation target of SIRT1 (Figure 1B). SIRT1-/- microglia secreted more HMGB1 than wildtype microglia after LPS stimulation (Figure 1C-E).

Deficiency in microglial SIRT1 made neurons more sensitive to multiple PD-relevant toxins including LPS, pesticide rotenone, and neurotoxin MPP+, as shown by more reduction in DA uptake and in the number of TH-IR neurons as well as the increased dendrite degeneration in neuron-glia cultures (Figure 2A-C) or re-constituted cultures (Figure 2D) containing SIRT1-/- microglia than cultures containing wildtype microglia. Moreover, anti-HMGB1 neutralizing antibody attenuated LPS-elicited dopaminergic neurodegeneration in both wildtype and SIRT1-/- cultures and blunted the differential neurodegeneration between the two genotypes (Figure 3). In conclusion, SIRT1 deacetylated HMGB1 and suppressed HMGB1 secretion from microglia thereby dampening neuroinflammatory response and dopaminergic neurodegeneration.

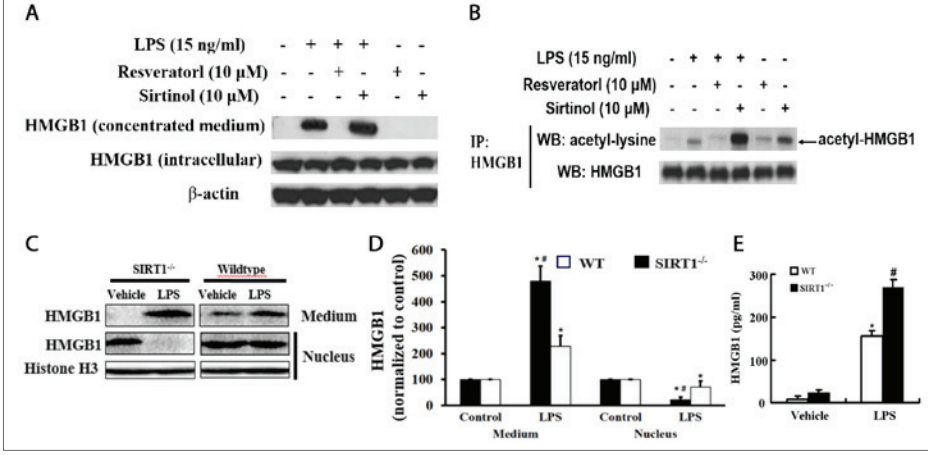

Figure 1. Deacetylation by SIRT1 inhibited HMGB1 secretion from primary microglia.

(A) SIRT1 activation by resveratrol attenuated HGMB1 secretion and SIRT1 inhibition by sirtinol increased HGMB1 secretion from primary microglia after LPS stimulation. (B) Immunoprecipitation (IP) experiments indicated that HMGB1 was a direct deacetylation target of SIRT1. (C-E) SIRT1-/- microglia secreted more HMGB1 than wildtype microglia after LPS stimulation detected by western blot (C, D) and ELISA (E).

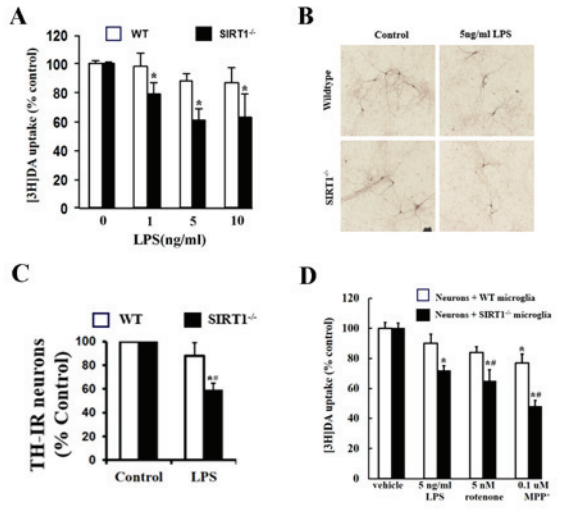

Figure 2. SIRT1-deficient microglia rendered dopamine neurons more sensitive to LPS-mediated neuroinflammation.

Neuron-glia cultures (A-C) or re-constituted cultures (D) containing SIRT1-/- microglia revealed more reduction in dopamine uptake and the number of dopamine neurons as well as increased dendrite degeneration than cultures containing wildtype microglia.

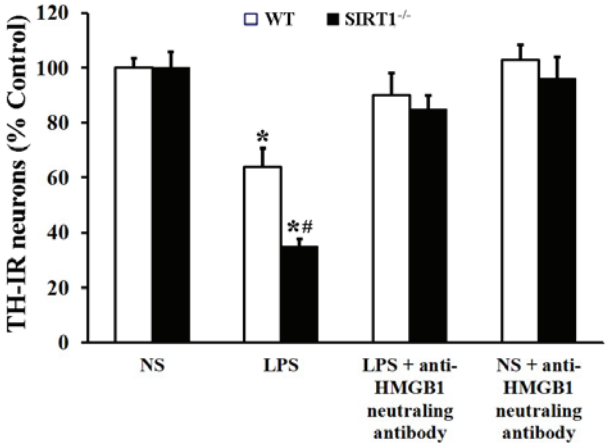

Figure 3. Anti-HMGB1 neutralizing antibody blunted the differential dopaminergic neurodegeneration between SIRT1-/- and wildtype cultures after LPS treatment.

Selected publications(* Corresponding author)

1. 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
2. Chu CH, Wang S, Chen SH, Wang Q, Lu RB, Gao H-M*, and Hong JS (2016) Neurons and astroglia govern microglial endotoxin tolerance through macrophage colony-stimulating factor receptor-mediated ERK1/2 signals. Brain Behavior and Immunity 55: 260–272
3. Chu CH, Chen SH, Wang Q, Langenbach R, Li H, Zeldin D, Chen SL, Wang S, Gao H-M*, Lu RB*, Hong JS (2015) PGE2 Inhibits IL-10 Production via EP2-Mediated β-Arrestin Signaling in Neuroinflammatory Condition. Molecular Neurobiology. 52(1):587-600.
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5. Gao H-M*, Zhou H, Hong J-S (2012) NADPH oxidases: novel therapeutic targets for neurodegenerative diseases. Trends in Pharmacological Sciences 33(6): 295-303 (SCI citations: 40)
6. 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
7. 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)
8. 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
9. 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
10. 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)
11. 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

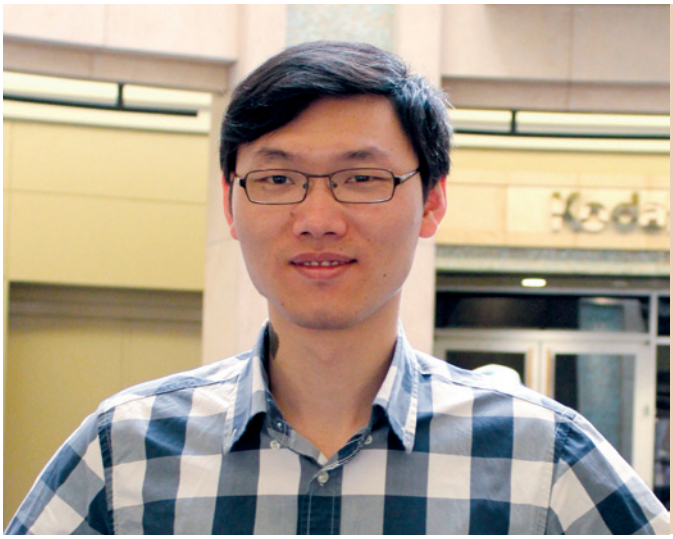
De-Zhen Tu

Yue Liu

Qiyao Liu

Tian Guan

Ru Yang



Guoqiang Wan, Ph.D.

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

Contact Information

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

Regeneration of auditory cells and synapses for hearing restoration

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

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

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

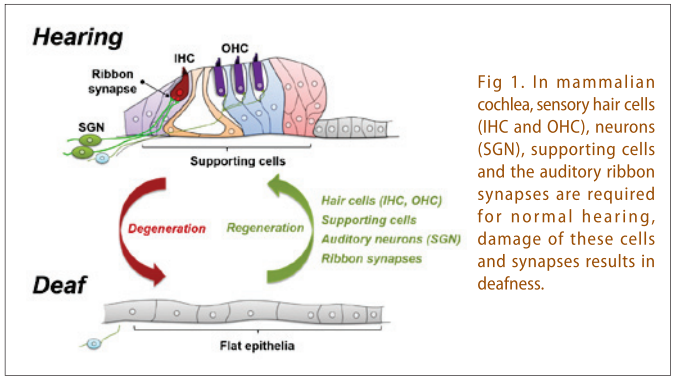


Fig 1. In mammalian cochlea, sensory hair cells (IHC and OHC), neurons (SGN), supporting cells and the auditory ribbon synapses are required for normal hearing, damage of these cells and synapses results in deafness.

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

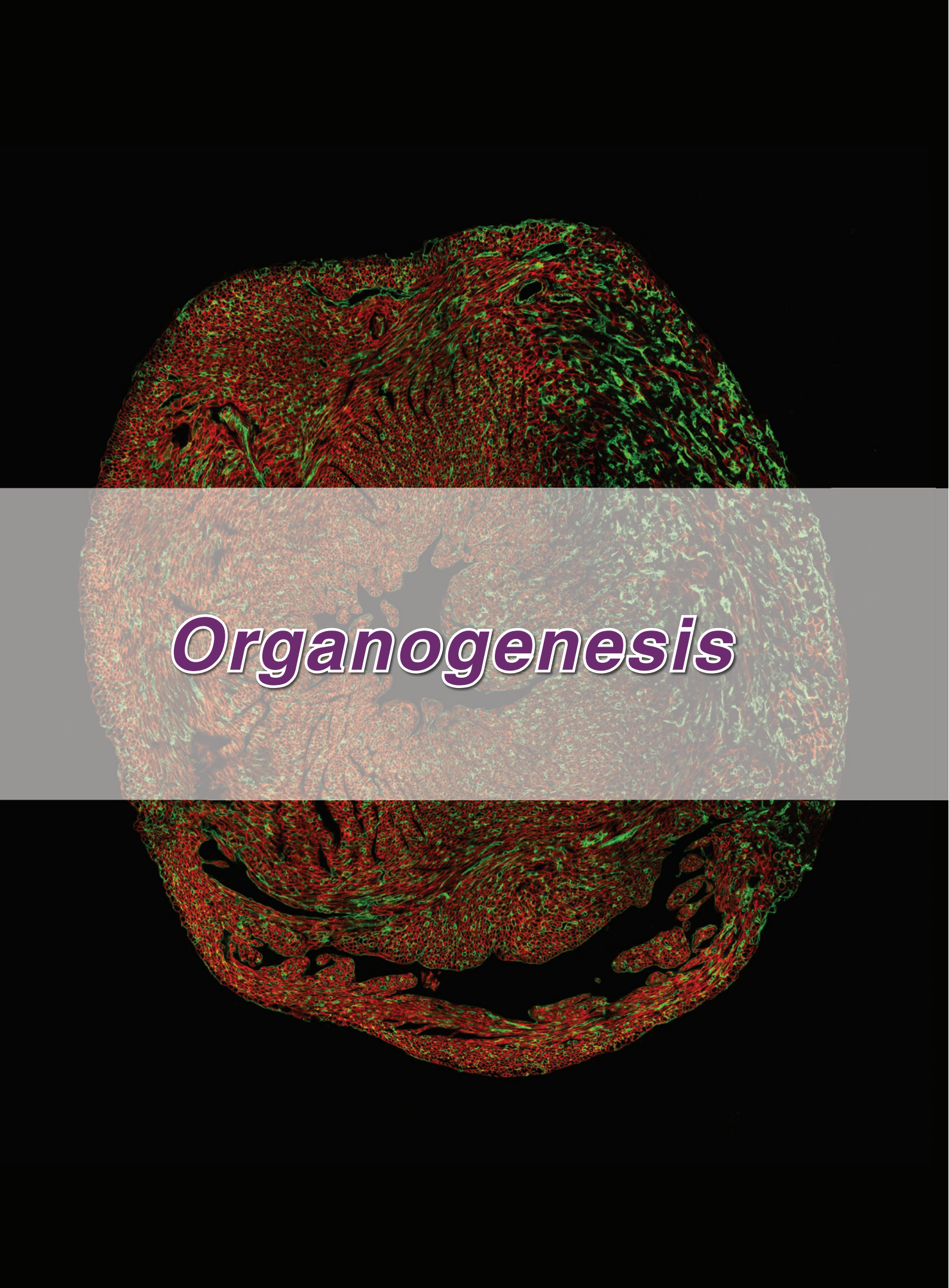
More than 50% of prelingual deafness is genetic, most often autosomal recessive and nonsyndromic. Hearing impairment is genetically heterogeneous that may be caused by mutations in more than 100 genes. Our lab aims to establish a research platform to identify novel genes and mutations involved in auditory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

From left to right:

Chaorong Yu (16' graduate student)
Guoqiang Wan, PhD (PI)
Gabriel Corfas, PhD (visitor)
Zhen Chen (17' graduate student)
Wen Wei (17' graduate student, rotation)
Qing Liu (16' graduate student)
Sihao Gong (17' graduate student)

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

1. Wan, G.# and Corfas, G.# (2017). Transient auditory nerve demyelination as a new mechanism for hidden hearing loss. Nature Communications, 8:14487
2. 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.
3. Mellado Lagarde, M.M.*, Wan, G.*, Zhang, L., Gigliello, A.R., McInnis, J.J., Zhang, Y., Bergles, D.E., Zuo, J.# & Corfas, G.# (2014). Spontaneous regeneration of cochlear supporting cells after neonatal ablation ensures hearing in the adult mouse. Proceedings of the National Academy of Sciences of the United States of America, 111(47), 16919-24. Editor's Choice: Kiberstis, P.A. (2014). Science, 346(6214), 1197.
4. Wan, G., Gómez-Casati, M.E., Gigliello, A.R., Liberman, M.C. & Corfas, G. (2014). Neurotrophin-3 regulates ribbon synapse density in the cochlea and induces synapse regeneration after acoustic trauma. eLife, 3, e03564. Comment in: Cunningham, L.L. & Tucci, D.L. (2015). New England Journal of Medicine, 372(2), 181-182
5. 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 behind morphogenesis

My lab is mainly interested in how morphogenetic processes such as cell migration and epithelial morphogenesis are regulated during development. My lab has employed a mainly genetic approach, using the model animal Drosophila melanogaster and cell biological techniques to conduct most of the experiments. And there are two model systems that we mainly use in the lab: the migrating border cells and the follicle epithelia in the Drosophila ovary.

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

The single layer of developing follicle epithelium (during oogenesis) is a system that we used to probe the mechanism of apical-basal polarity generation and maintenance. We are currently studying the mechanism underlying apical polarity formation and maintenance using this system. Lastly, we have also collaborated with other mouse labs in MARC to study the role of actin disassembly regulators in two different morphogenetic processes in the neonatal mice, namely gonocyte migration in the testis and myofibre assembly in the heart.

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

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

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

3.Generation of distinct cell polarities during collective migration of border cells.

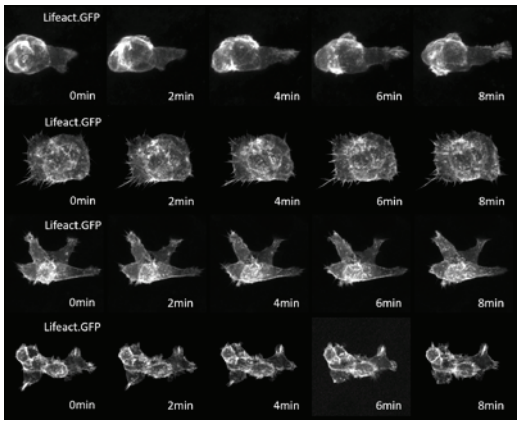


Figure 1. Time-lapsed series showing clusters of 8 border cells (WT, top row; mutants, bottom 3 rows) extending dynamic actin-rich protrusions during collective migration.

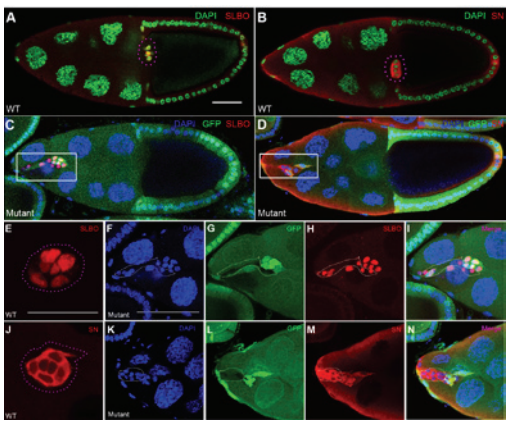


Figure 2. Wildtype border cell clusters (A,B) reached the final destination while mutant mosaic border cell clusters (C,D) displayed delayed migration within the egg chambers.

Publications and Manuscripts in 2017 (*corresponding author)

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, under revision in Development
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, under revision in Developmental Cell
3. Zhu, K., Liu, M., Fu, Z., Zhou, Z., Kong, Y., Liang, H., Lin, Z., Luo, J., Zheng, H., Wan, P., Zhang, J., Zen, K., Chen, J.*, Hu, F.*, Zhang, C.*, Ren, J.* and Xi Chen*. Plant microRNAs in larval food regulate honeybee caste development. PLoS Genetics, 13(8), p.e1006946. (2017)
4. Xiao, Y., Ma, H., Wan, P., Qin, D., Wang, X., Zhang, X., Xiang, Y., Liu, W., Chen, J.*, Yi, Z.*, Li, L*. Trp-Asp (WD) repeat domain 1 is Essential for Mouse Peri-implantation Development and Regulates Cofilin Phosphorylation. Journal of Biological Chemistry, 292(4), pp.1438-1448 (2017)

Group members

Technical staff

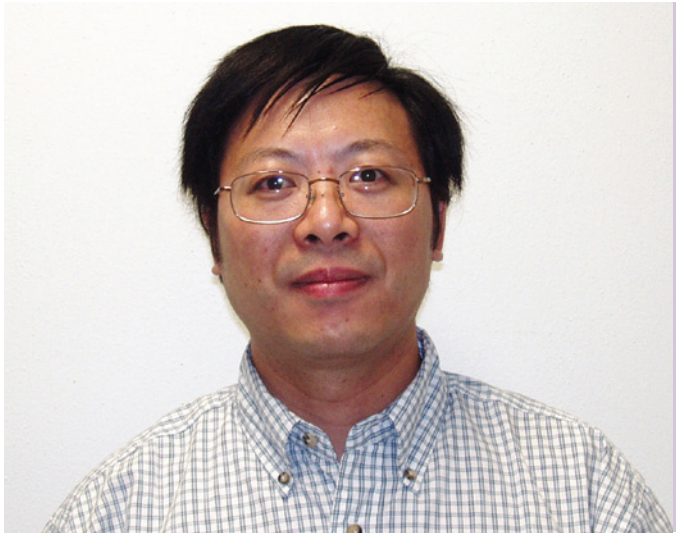
Zhou Ping

Graduate students

Guo Xuan
Kang Di
Wang Heng
Wang Dou
Wang Xianping
Wu Mengqi
Xu Zehao
Xu Jianbing
Qiu Zhiqian

Former graduate students

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



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

1.Regulation of hedgehog signaling
2.The mechanism of mitochondrial homeostasis

Research in my lab is mainly focused on two fields: one is the regulation of Hedgehog signaling, the other is the mechanism of mitochondrial homeostasis.

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

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

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

1.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.

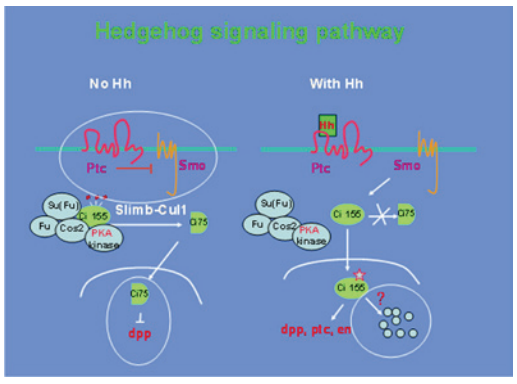


Figure 1. Hedgehog signaling pathway in Drosophila.

2. Capping enzyme mRNA-cap/RNGTT regulates Hedgehog pathway activity by antagonizing protein kinase A

Hedgehog (Hh) signaling plays a pivotal role in animal development and its deregulation in humans causes birth defects and several types of cancer. Protein Kinase A (PKA) modulates Hh signaling activity through phosphorylating the transcription factor Cubitus interruptus (Ci) and G protein coupled receptor (GPCR) family protein Smoothened (Smo) in Drosophila, but how PKA activity is the capping-enzyme mRNA-cap, which positively regulates Hh signaling activity through modulating PKA activity. We provide genetic and biochemical evidence that mRNA-cap inhibits PKA kinase activity to promote Hh signaling. Interestingly, regulation of Hh signaling by mRNA-cap depends on its cytoplasmic capping-enzyme activity. In addition, we show that the mammalian homolog of mRNA-cap, RNGTT, can replace mRNA-cap to play the same function in the Drosophila Hh pathway and that knockdown of Rngtt in cultured mammalian cells compromised Shh pathway activity, suggesting that RNGTT is functionally conserved. Our study makes an unexpected link between the mRNA capping machinery and the Hh signaling pathway, unveils a new facet of Hh signaling regulation, and reveals a potential drug target for modulating Hh signaling activity.

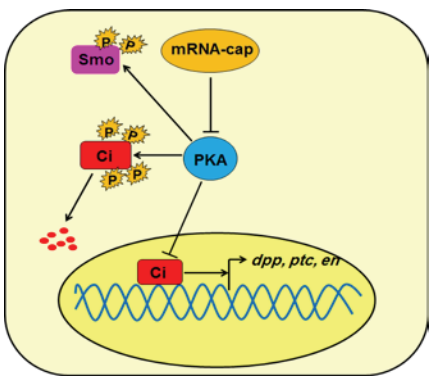


Figure 2. Regulation of Hh signaling through the mRNA-cap-PKA axis. mRNA-cap inhibits PKA activity to modulate the stability of Ci and Smo, thus affecting Hh pathway output.

Selected Publications

1. Zhou Z#, Yao X#, Pang S, Chen P, Jiang W, Shan Z, Zhang Q*. (2017) The deubiquitinase UCHL5/UCH37 positively regulates Hedgehog signaling by deubiquitinating Smoothened. J Mol Cell Biol. doi: 10.1093/jmcb/mjx036.
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5. Zhou Z, Xu C, Chen P, Liu C, Pang S, Yao X, Zhang Q*. (2015) Stability of HIB-Cul3 E3 ligase adaptor HIB Is Regulated by Self-degradation and Availability of Its Substrates. Sci Rep. 12;5:12709. doi: 10.1038/srep12709.
6. An J, Ren S, Murphy S, Dalangood S, Chang C, Pang X, Cui Y, Wang L, Pan Y, Zhang X, Zhu Y, Wang C, Halling G, Cheng L, Sukov W, Karnes R, Vasmatzis G, Zhang Q, Zhang J, Cheville J, Yan J, Sun Y, Huang H. (2015) Truncated ERG Oncoproteins from TMPRSS2-ERG Fusions Are Resistant to SPOP-Mediated Proteasome Degradation. Mol Cell. 59:1-13.
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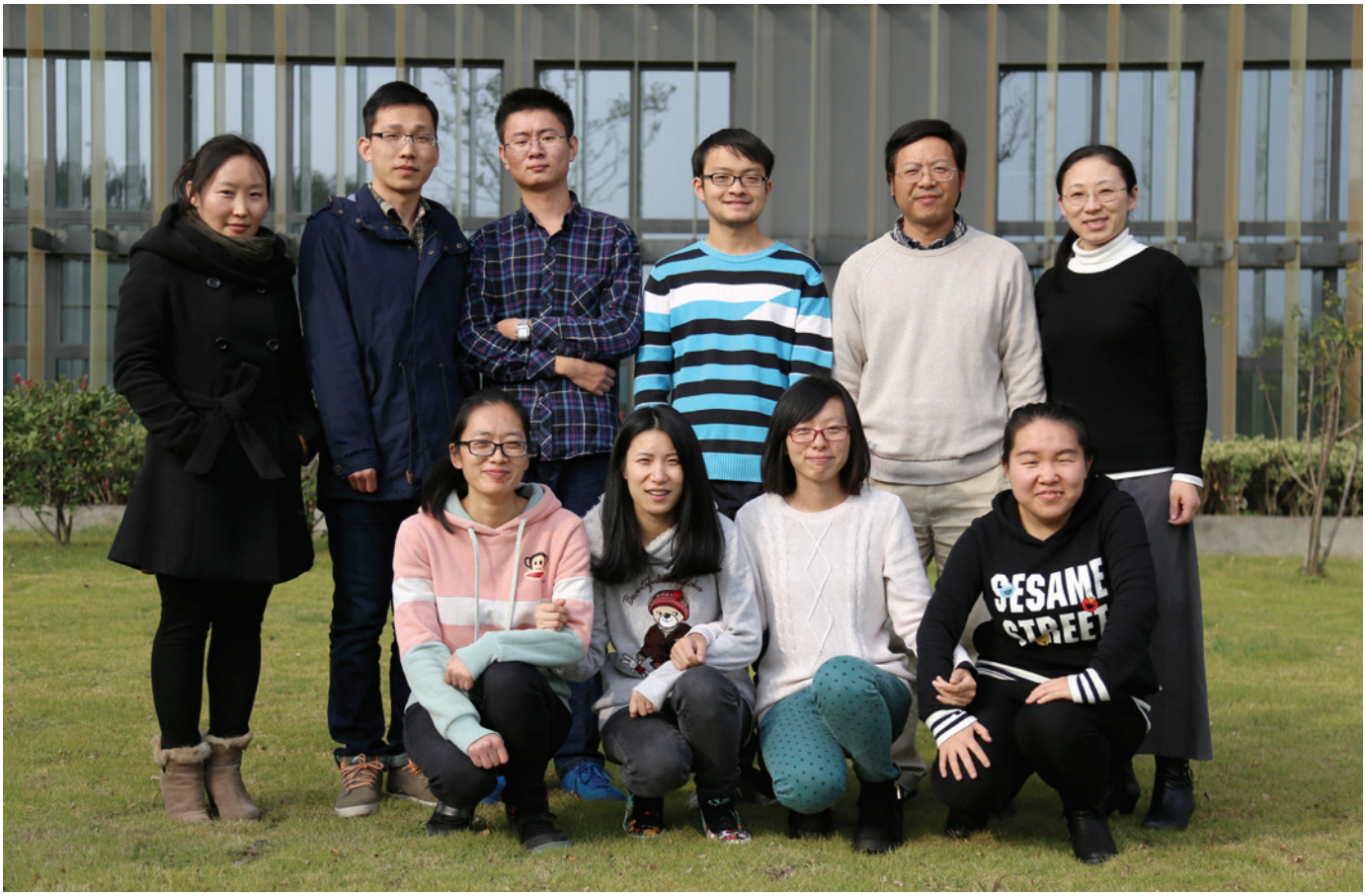
9. Zhang L, Ren F, Zhang Q, Chen Y, Wang B, Jiang J. (2008) The TEAD/TEF Family of Transcription Factor Scalloped Mediates Hippo Signaling in Organ Size Control. Dev Cell. 14, 377-387.
10. Zhang Q#, Zhang L#, Wang B, Ou CY, Chien CT, Jiang J. (2006) A Hedgehog-induced BTB protein modulates Hedgehog signaling responses by degrading Ci/Gli transcription factor. Dev Cell. 10, 719-729.
11. 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
Ping Chen
Miya Su
Xia Yao
Shu Pang
Weirong Jiang
Zhaoliang Shan

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 Xenopus developmental biology and tumor biology.

Contact Information

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

Towards the understanding of a unified principle of tumorigenesis.

Cancer cells are immature cells resulting from cellular reprogramming by gene misregulation, and re-differentiation is expected to reduce malignancy. It is unclear, however, whether cancer cells can undergo terminal differentiation. We show that, inhibition of a few epigenetic modification enzymes, which all promote cancer development and progression, leads to postmitotic neuron-like differentiation with loss of malignant features in cell lines of different solid cancer types. The regulatory effect of these enzymes in neuronal differentiation resided in their intrinsic activity in embryonic neural precursor/progenitor cells. We further found that most pan-cancer promoting genes and the signal transducers of the pan-cancer promoting signaling pathways, including the 'EMT' mesenchymal marker genes, display neural-specific expression during embryonic neurulation. In contrast, many tumor suppressor genes, including the 'EMT' epithelial marker gene CDH1, exhibited non-neural or no expression (Figure 1). Moreover, cancer cells also express a broad range of neural specific genes in addition to those cancer-promoting genes. This correlation indicated that cancer cells and embryonic neural cells share a regulatory network, mediating both tumorigenesis and neural development (Figure 2). This result suggests that tumorigenesis represents a process of loss of cell lineage identity and acquirement of characteristics of embryonic neural cells (Figure 3). This is in contrast to the idea that cancer initiation and development is the result of losing epithelial and acquiring mesenchymal properties of cells. This elucidates a previously unrecognized nature of tumorigenesis and sets up a conceptual paradigm for cancer research.

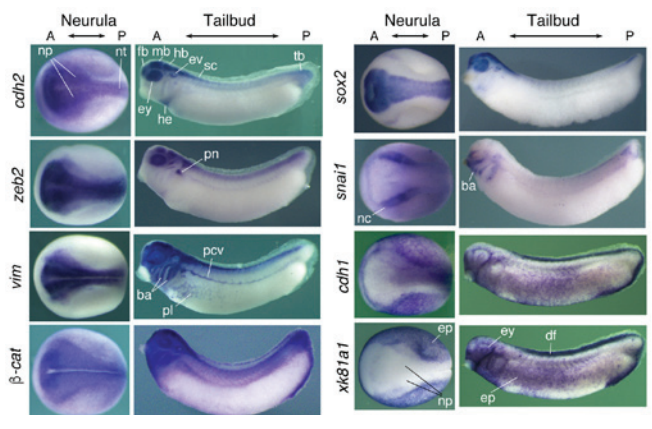


Figure 1. Cancer 'EMT' marker expression indicates that 'EMT' is not an epithelial to mesenchymal transition, but a non-neural to neural transition.

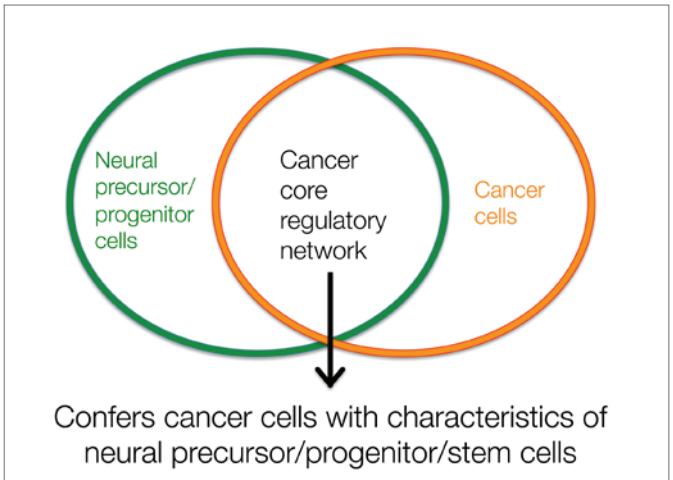


Figure 2. Cancer cells and neural precursor/progenitor/stem cells share a set of regulatory networks, which confer cancer cells the properties of neural progenitor cells.

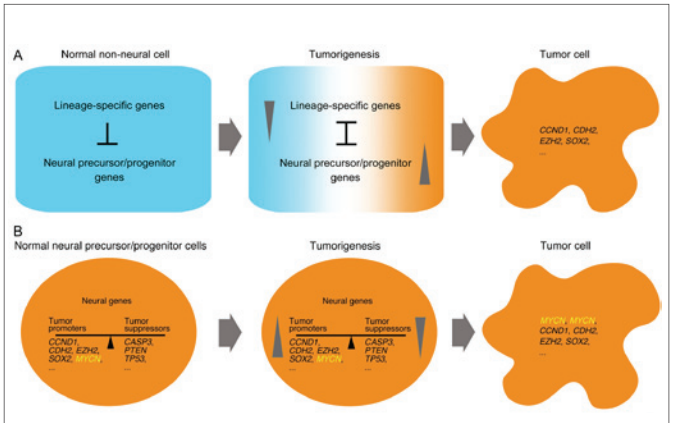


Figure 3. Tumorigenesis is a process of gradual loss of original cell identity and gain of properties of neural precursor/progenitor cells. (A) Tumorigenesis in non-neural cells. (B) Tumorigenesis in the nervous system. Tumors in the nervous system originate from neural progenitor cells, which harbor both promoting and inhibitory signals for proliferation, migration, differentiation, etc., so as to keep normal neural development in a balanced fashion. When the balance is broken, for example, by amplification of MYCN, tumorigenesis may occur.

Selected publications (*Correspondence author)

1. 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)
2. 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.
3. 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
4. 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.
5. 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.
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7. Cao Y*. 2013. Regulation of germ layer formation by pluripotency factors during embryogenesis. Cell Biosci. 3(1):15.
8. 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.

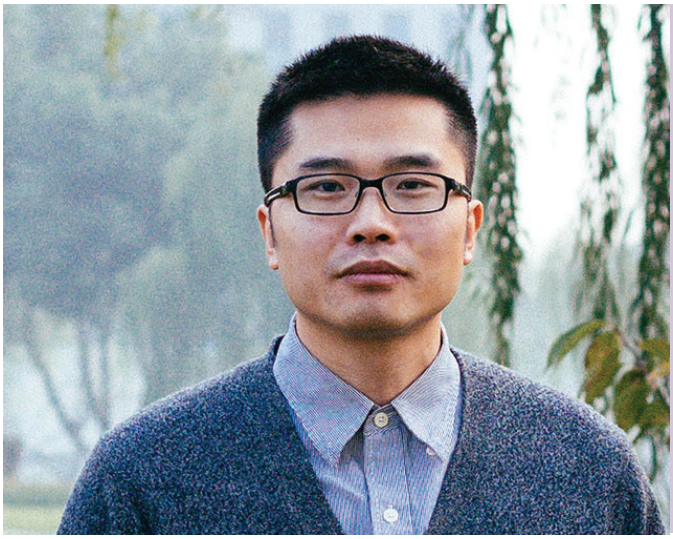
Group members

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

Technicians
Ma Haihua
Yan Yuelou



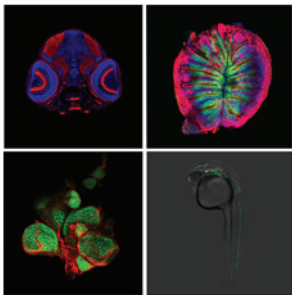
Xin Lou, Ph.D.

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. Hou N, Yang Y, Scott IC, Lou X. The Sec domain protein Scfd1 facilitates trafficking of ECM components during chondrogenesis.Developmenal Biology. 2017 Jan 1;421(1):8-15.1
2. 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.
3. Lou X*, Burrows JT, Scott IC*. Med14 cooperates with brg1 in the differentiation of skeletogenic neural crest. BMC Developmental Biology 2015 Nov 15:41 (co-corresponding author)
4. Lou X, Deshwar AR, Crump JG, Scott IC.Smarcd3b and Gata5 promote a cardiac progenitor fate in the zebrafish embryo.Development. 2011 Aug;138(15):3113-23.

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 (Figure 1) by acting upstream of pou5f3 and sall4 (Dong et al., 2017).

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 (Yue et al., 2017; Under revision. Figure 2).

Engineered endonuclease (EENs) including ZFN, TALEN and CRISPR/Cas9 are powerful tools to create genome edited animals without species limitation. Using the knock out tools of ZFN and TALEN, we produced heritable targeted inactivation of myostatin genes in yellow catfish, the first endogenous gene knockout in aquaculture fish (Dong et al., 2011, Dong et al., 2014). To increase the efficiency of germline transmission of induced mutations and particularly knockin alleles created by CRISPR/Cas9, we co-microinjected yfp-nanos3 mRNA with Cas9 mRNA, sgRNA and ssDNA donor. In comparison with the common practice of selecting founders by genotyping fin clips, our new strategy of selecting founders with tentatively fluorescent-labeled PGCs significantly increases the ease and speed of generating heritable knocking and knockout animals with CRISPR/Cas9 (Dong et al., 2014). Collaborating with the groups of Professors Zhou and Zhu, we developed an alternative novel tool for DNA editing (SGN: structure-guided nuclease) without target sequence limitation (Xu et al., 2016).

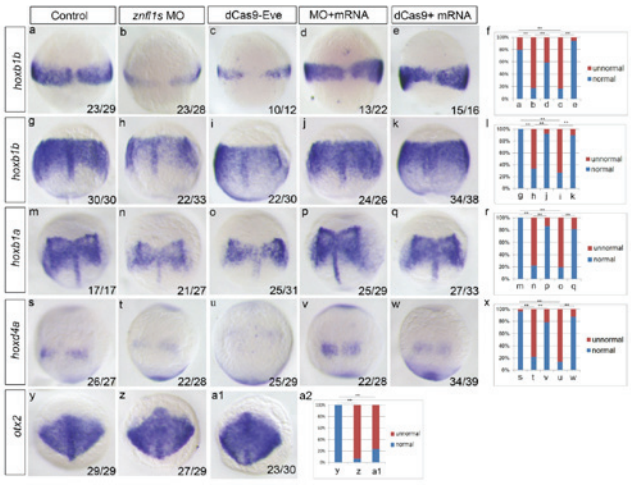


Figure 1. Knocking down znfl1s affects the posterior neuroectoderm development through reducing the expressions of hoxb1b, hoxb1a and hoxd4a in zebrafish gastrula.

Expressions of hoxb1b, hoxb1a, hoxd4a and otx2 were examined in 8 hpf (a-e) and/or 10 hpf (g-k, m-q, s-w, y-z, a1) embryos microinjected with control MO (a, g, m, s, y), znfl1s MO (b, h, n, t, z), dCas9-Eve mRNA plus sgRNAs (c, i, o, u, a1), znfl1s MO plus znfl1 mRNA (d, j, p, v), , and dCas9-Eve mRNA plus sgRNAs and znfl1 mRNA (e, k, q, w), respectively. The statistical analyses of the data derived from panel a-e, g-k, m-q, s-w, and y-a1 were shown in diagrams f, l, r, x and a2, respectively. All embryos except y-z-a1 were positioned in dorsal view. Embryos y, z and a1 were positioned in top view. **P<0.01.

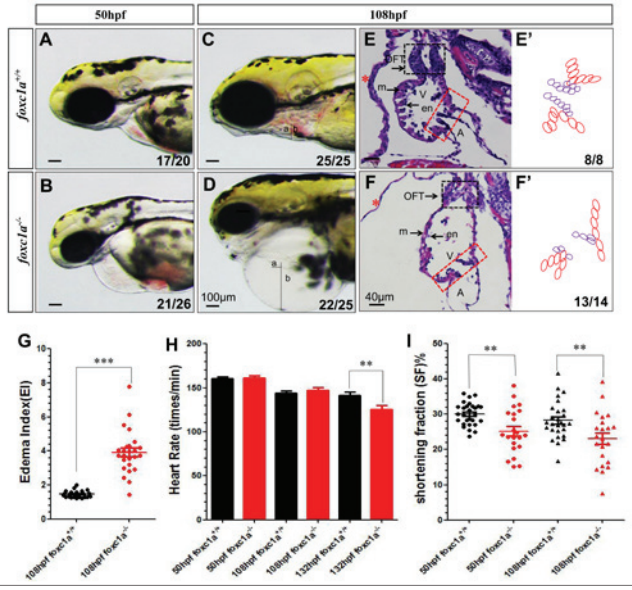


Figure 2. Foxc1a knockout zebrafish embryos exhibit severe cardiovascular defects.

A-D, Lateral views of wild type siblings (A, C) and foxc1a knockout mutants (B, D) at 50 hpf (A-B) and 108 hpf (C-D); a, the semi-diameter of ventricle; b, the semi-diameter of the pericardial cavity. E-F, Section of heart of wild type (E) and mutants (F) at 108 hpf. E'-F', Schematic of AVC region in wild type (E') and mutants (F') at 108 hpf. The red cells in the outside are myocardium and the purple cells in the inner are endocardium. Red asterisk denotes pericardial membrane; A, atrium; V, ventricle; my, myocardium; en, endocardium; OFT, out flow tract. Rectangle outlined with red dots, valve leaflets; Rectangle outlined with black dots: OFT region. G, The scatter plot showing the value of edema index (EI) in wild type and mutants at 108 hpf. H, The histogram showing heart rates in wild type (black histogram) and mutants (red histogram) at 50 hpf, 108 hpf and 132 hpf, respectively. I, The scatter plot showing the value of ventricle minor axis shortening fraction (SF) in wild type and mutants at 50 hpf and 108 hpf.

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

1. 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.
2. Jie Gong, Xin Wang, Chenwen Zhu, Xiaohua Dong, Qinxin Zhang, Xiaoning Wang, Xuchu Duan, Fuping Qian, Yunwei Shi, Yu Gao, Qingshun Zhao**, Renjie Chai**, Dong Liu*. 2017. Insm1a Regulates Motor Neuron Development in Zebrafish. Frontiers in Molecualr Neuroscience. 10: 274.
3. 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.
4. 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)
5. 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.
6. 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.
7. Dong Liang, Wenshuang Jia, Jingyun Li, Kui Li, Qingshun Zhao*. 2012. Retinoic acid signalling plays a restrictive role in zebrafish primitive myelopoiesis. PLoS ONE, 7(2): e30865.
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.
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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
Yunyun Yue
Meijing Liu
Qinxin Zhang
Luqingqing He
Nan Li
Zhaojunjie Zhang

Graduate students from collaborators' labs

Siyan Chen

Technician staff

Xiaochun Yu

Old lab members

Fang Xu, PhD
Ping Hu, PhD
Jie Bao, PhD
Dong Liang, PhD
Kui Li, PhD
Jingyun Li, PhD
Junbo Li, PhD
Zhangji Dong, PhD
Wenshuang Jia, PhD
Xiaohua Dong, PhD
Shasha Cao, PhD
Zhiying Zou, MS
Lu Sun, MS
Wei Song, MS
Xiaolin Wang, MS
Mei Zhang, MS

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 septaldefect (VSD) and hypoplastic pulmonary artery (PA) or PA stenosis (Fig. 2).

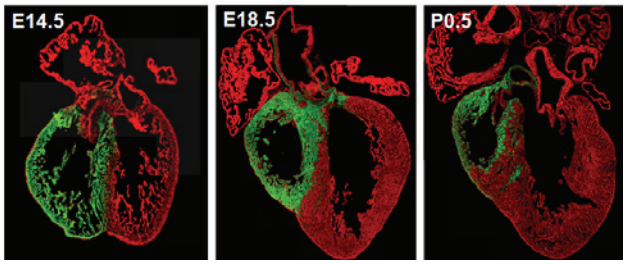


Fig. 1. Mef2c-AHF-Cre; Rosa26-mTmG lineage tracing of mouse heart. The green cells are Mef2c-AHF $^{+}$.

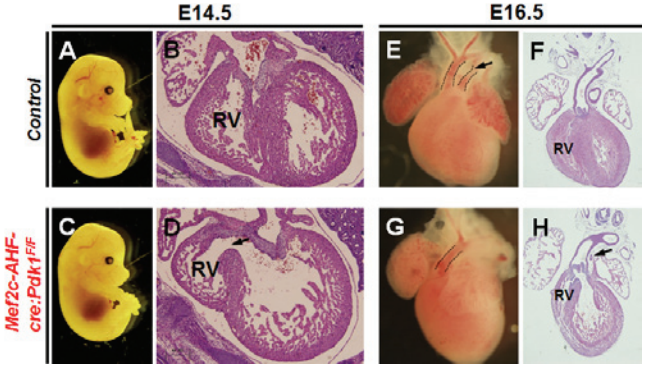


Fig.2. Deletion of PDK1-encoding gene (Pdk1) gives rise to a hypoplastic right ventricle syndrome (HRHS) mouse model including a small RV, severe interventricular septal defect (IVS) and hypoplastic pulmonary artery (PA) or PA stenosis.

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. Yangyang Liu, Mengjiao Hu, Dongjiao Luo, Ming Yue, Shuai Wang, Xiaoyan Chen, Yangfan Zhou, Yi Wang, YanchunCai, Xiaolan Hu, YuehaiKe, Zhongzhou Yang*, Hu Hu*. (2017)Class III PI3K Positively Regulates Platelet Activation and Thrombosis via PI(3) P-Directed Function of NADPH Oxidase. Arterioscler Thromb Vasc Biol. 37:2075-2086. (*Co-corresponding author)
3. JunweiNie, Mingyang Jiang, Xiaotian Zhang, Hao Tang, HengweiJin, Xinyi Huang, Baiyin Yuan, Chenxi Zhang, Janice Ching Lai, YoshikuniNagamine, 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)
4. Wen Luo, Xia Zhao, HengweiJin, 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.
5. Xia Zhao,Shuangshuang Lu,JunweiNie,Xiaoshan Hu,Wen Luo,Xiangqi Wu,Hailang Liu,Qiuting Feng,Zai Chang,Yaoqiu Liu,Yunshan Cao,Haixiang Sun,Xinli Li,Yali Hu,Zhongzhou Yang.(2014)Phosphoinositide-Dependent Kinase 1 and mTORC2 SynergisticallyMaintain Postnatal Heart Growth and Heart Function in Mice. Mol. Cell. Biol.34(11):1966-75. (Spotlight article/cover)
6. Baiyin Yuan,Ping Wan,Dandan Chu,JunweiNie,Yunshan Cao,Wen Luo,Shuangshuang Lu,Jiong Chen* and Zhongzhou Yang*. (2014) A Cardiomyocyte-Specific Wdr1 Knockout Demonstrates Essential Functional Roles for Actin Disassembly during Myocardial Growth and Maintenance in Mice.Am J Pathol. 184(7):1967-80 (*Co-corresponding author)
7. Xiangqi Wu, Yunshan Cao, JunweiNie, Hailang Liu, Shuangshuang Lu, Xiaoshan Hu, Jingai Zhu, Xia Zhao, Jiandong Chen, Xiaohu Chen, Zhongzhou Yang* and Xinli Li*. (2013) Genetic and Pharmacological Inhibition of Rheb1-mTORC1 Signaling Exerts Cardioprotection against Adverse Cardiac Remodeling in Mice. Am. J. Pathol. 182: 2005-2014.
8. Ruomin Di, Xiangqi Wu, Zai Chang, Xia Zhao, Qiuting Feng, Shuangshuang Lu, Qing Luan, Brian A. Hemmings, Xinli Li and Zhongzhou Yang. (2012) S6K inhibition renders cardiac protection against myocardial infarction through PDK1 phosphorylation of Akt. Biochem. J. 441:199-207.
9. Shuangshuang Lu, JunweiNie, Qing Luan, Qiuting Feng, Qi Xiao, Zai Chang, Congjia Shan, Daniel Hess, Brian A. Hemmings and Zhongzhou Yang*. (2011) Phosphorylation of the Twist1-family basic helix-loop-helix transcription factors is involved in pathological cardiac remodeling. PLoS ONE 6: e19251.
10. Zai Chang, Qin Zhang, Qiuting Feng, Jie Xu, TengTeng, Qing Luan, Congjia Shan, Yali Hu, Brian A Hemmings, Xiang Gao and Zhongzhou Yang. (2010) Deletion of Akt1 causes heart defects and abnormal cardiomyocyte proliferation. Dev. Biol.347: 384-391.
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)

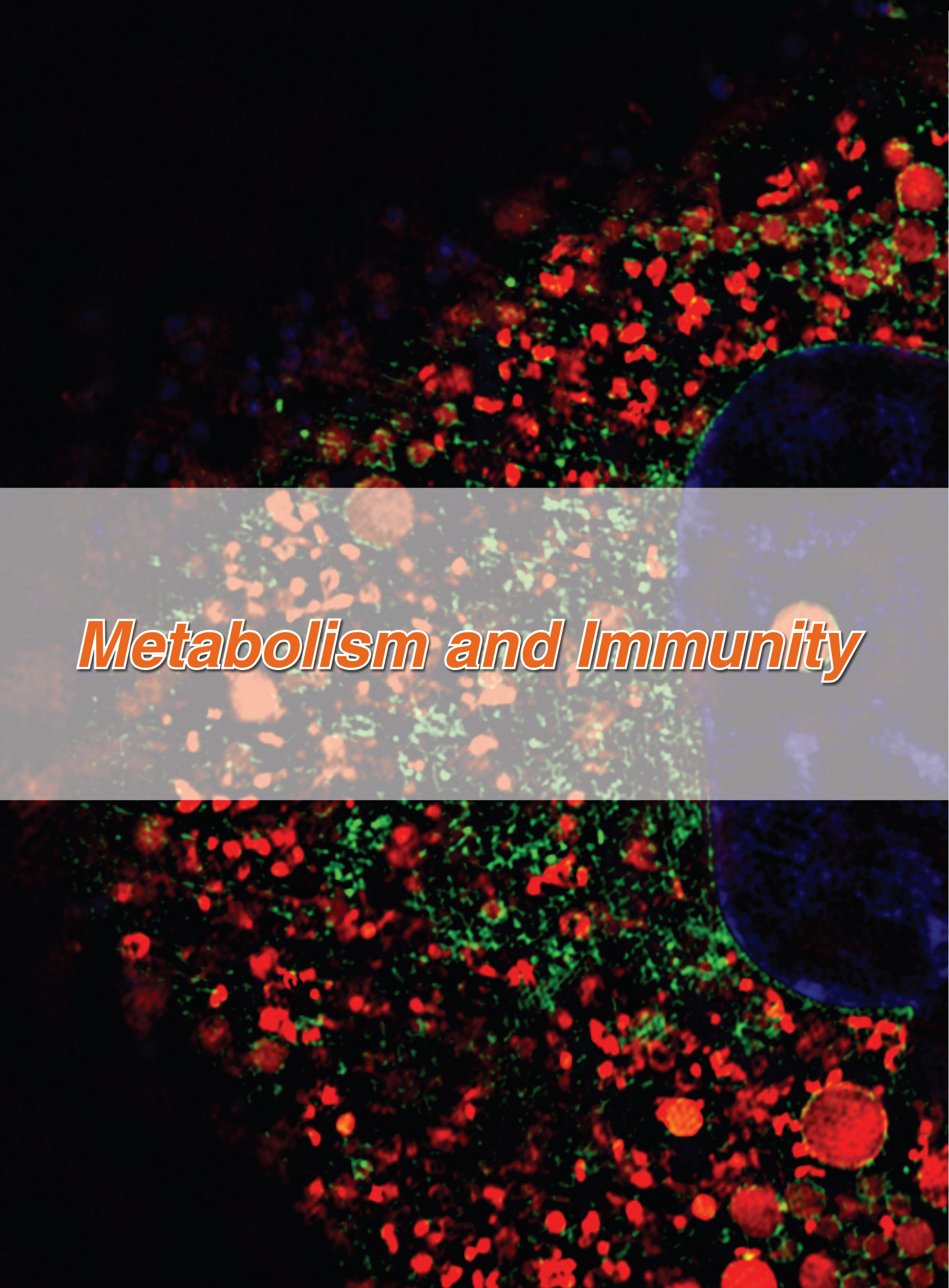
Lab members

Group leader
Zhongzhou Yang

Secretary
Wenjing Fan

Technical staff
Congjia Shan

Graduate students
Mingyang Jiang
HengweiJin
Meng Xia
Hongmei Luo
Xinyi Huang
Yingcao Shi
Huijuan Wang
Mingjie Xu
Ke Zhao
Jie Li


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, and then trained as postdoctoral fellow at the Jackson Laboratory. In 2000, he came back to Nanjing University, and served as the founding director for MARC at 2001. 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@nicemice.cn

Physiological regulation and metabolic homeostasis

The advance of modern technologies, especially the NGS and gene editing, are 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 this exciting time and identify the previous unsolvable biological questions. For 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 Mollmmun).

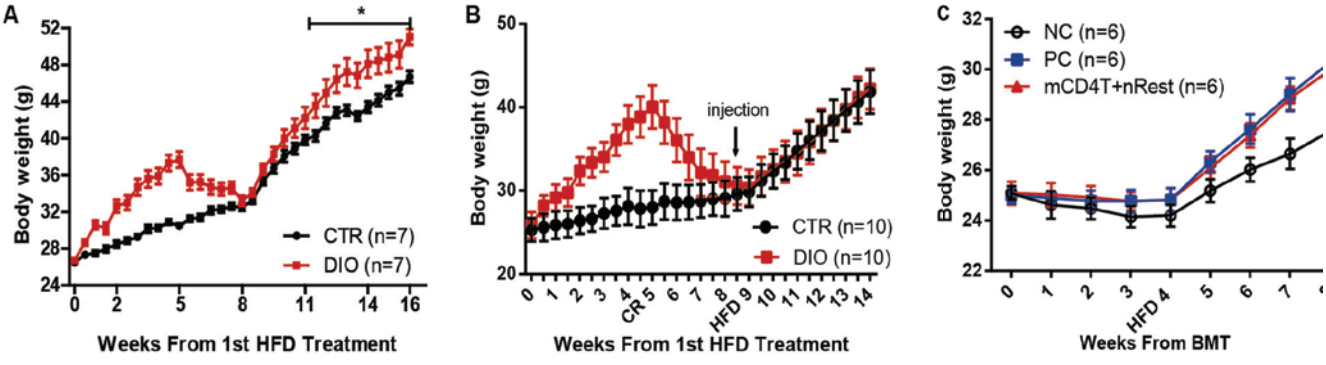


Figure 1. Obesity memory is stored and carried by CD4+ T lymphocytes.

(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. Understanding the role neuronal JMJD3 in Kisspeptin–Estrogen feedback loop and reproductive function (Figure 2)

The hypothalamic-pituitary-gonadal axis controls development, reproduction, and metabolism. Although most studies have focused on the hierarchy from the brain to the gonad, many questions remain unresolved concerning the feedback from the gonad to the central nervous system, especially regarding the potential epigenetic modifications in hypothalamic neurons. In the present report, we generated genetically modified mice lacking histone H3 lysine 27 (H3K27) demethylaseJumonji domain-containing 3 (JMJD3) in hypothalamic rat-insulin-promoter-expressing neurons (RIP-Cre neurons). The female mutant mice displayed late-onset obesity owing to reduced loco- motor activity and decreased energy expenditure. JMJD3 deficiency also results in delayed pubertal onset, an irregular estrous cycle, impaired fertility, and accelerated ovarian failure in female mice owing to the dysregulation of the hypothalamic–ovarian axis. We found that JMJD3 directly regulates Kiss1 gene expression by binding to the Kiss1 promoter and triggering H3K27me3 demethylation in the anteroventral periventricular nucleus. Further study confirmed that the aberrations arose from impaired kisspeptin signaling and subsequent estrogen deficiency. Estrogen replacement therapy can reverse obesity in mutant mice. Moreover, we demonstrated that Jmjd3 is an estrogen target gene in the hypothalamus. These results provide direct genetic and molecular evidence that JMJD3 is a key mediator for the kisspeptin–estrogen feedback loop (Song et al, Endocrinol).

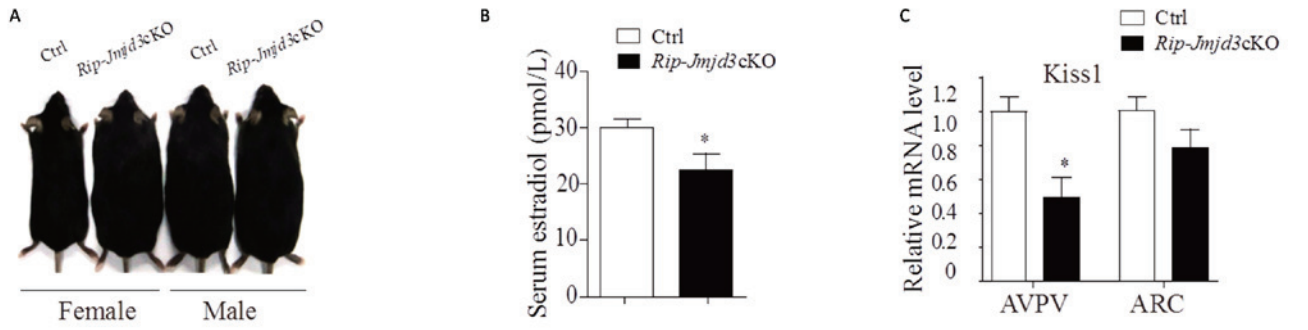


Figure 2. Female-specific obesity in Rip-Jmjd3cKO mice.

(A) Control and *Rip-Jmjd*3cKO female mice at 7 months old.

(B) Serum estradiol levels in control and *Rip-Jmjd*3cKO mice at 4 months old

(C) Relative mRNA levels of Kiss1 in AVPV and ARC hypothalamic nucleus in control and Rip-Jmjd3cKO females at 4 weeks old.

3. Dissecting the molecular and cellular mechanism for Hnrnpuhaploinsufficiency (Figure 3)

Haploinsufficiency often associates with clinic syndromes, especially the neurological and metabolic dysfunctions. It was reported that patients with one copy chromosomal deletion in 1q44 had sleep disorders where is the location of Hnrnpugene.We found that deletion of one cope of heterogeneous nuclear ribonucleoprotein U (hnRNP U) in mice results in unconsolidated circadian rhythms, which manifested as fragmented locomotor activity and defective metabolism.Mechanistically, we showed hnRNP Uhaploinsufficiency causes the decreased expression of arginine vasopressin (Avp) and vasoactive intestinal polypeptide (Vip), the neuropeptides regulating communication of suprachiasmatic nucleus (SCN) neurons and daily physiological rhythms.Consistently, mutant mice were resistant to light pulse but sensitive to Avp and Vipsuprachiasmatic microinjection.Moreover, hnRNP U bound directly to the promoters of Avp and Vip with Bmal1: Clock heterodimers in phase-dependent manners. These findings demonstrate that hnRNP U confers accuracy and robustness to circadian rhythms by directly regulating Avp and Vip in the SCN (Lai et al, Am J Path).

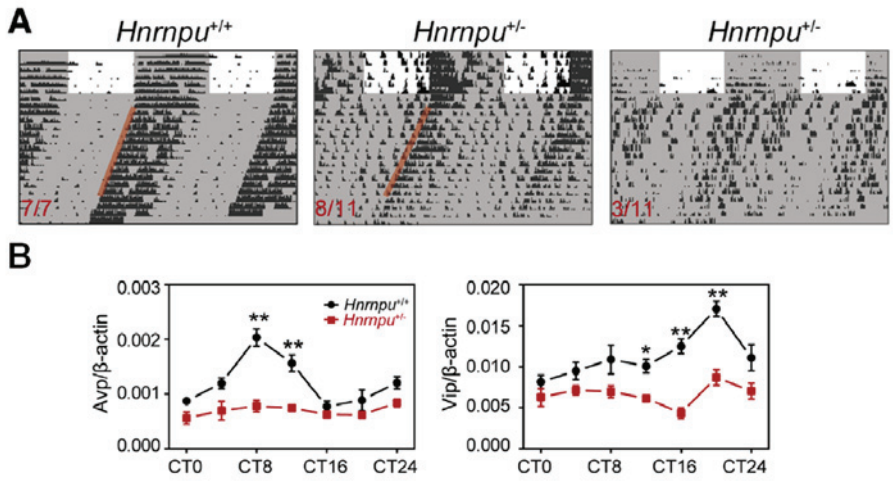


Figure 3. HnRNP U haploinsufficiency caused unconsolidated circadian rhythms by decreasing expression of Avp and Vip.

(A) Representative double-plotted actograms of wheel-running show disorganized activity rhythms in Hnrnpu$^{+/-}$ mice.

(B) Avp and Vip mRNA expression levels in the SCN were reduced in Hnrnpu$^{+/-}$ mice.

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).

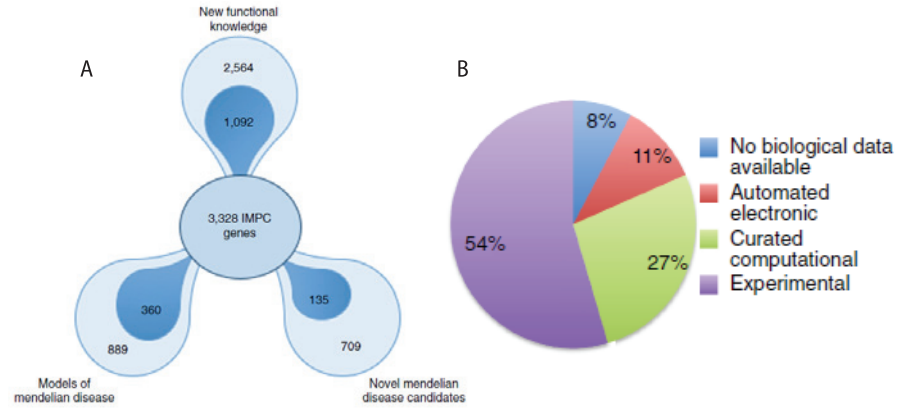


Figure 4. Novel mouse models of disease

(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).

Publications in 2017

1. Rozman et al (2017) Identification of novel genetic elements in metabolism by high-throughput mouse phenotyping. Nature Comm, in press
2. Lai et al (2017) Haploinsufficiency of hnRNP U changes activity pattern and metabolic rhythms. Am J Path, in press
3. Zou JH et al (2017) CD4+ T cells memorize obesity and promote weight regain. Cell Mol Immunol, in press
4. Meehan TF et al (2017) Disease model discovery from 3,328 gene knockouts by The International Mouse Phenotyping Consortium. Nat Genet. 49:1231-1238
5. Song AY et al (2017) JMJD3 is crucial for female AVPV RIP-Cre neuron controlled kisspeptin-estrogen feedback loop and reproductive function. Endocrinol, 158:1798-811 Editorial: A shot in the dark exposes more trees in the forest. By Kurian JR, Endocrinol, 158:1572-4
6. Li F et al (2017)The microbiota maintain homeostasis of liver-resident γδT-17 cells in a lipid antigen/CD1d-dependent manner. Nat Comm. 7:13839
7. Karp N et al (2017) Prevalence of sexual dimorphism in mammalian phenotypic traits. Nature Comm, 8:15475

Group members

Research associate

Zhaoyu Lin

Postdoc

Peiliang Shi

Graduate students

DayuanZou
YujieZou
Qin Chen
Xu Li
Yufang Wang
Jingwen Pei
Dingyu Wang
Qi Wang

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.

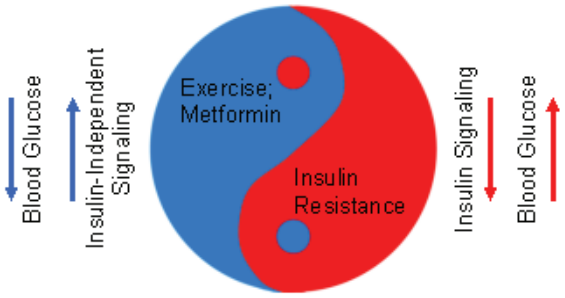


Figure 1. Insulin dependent and independent signaling in the pathogenesis of type II diabetes and its treatment.

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).

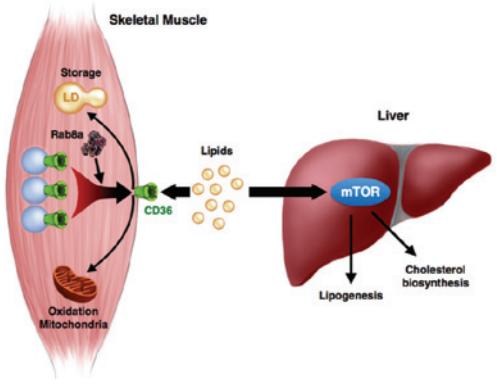


Figure 2. Lipid uptke and storage in skeletal muscle plays a critical role in the development of NAFLD.

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).

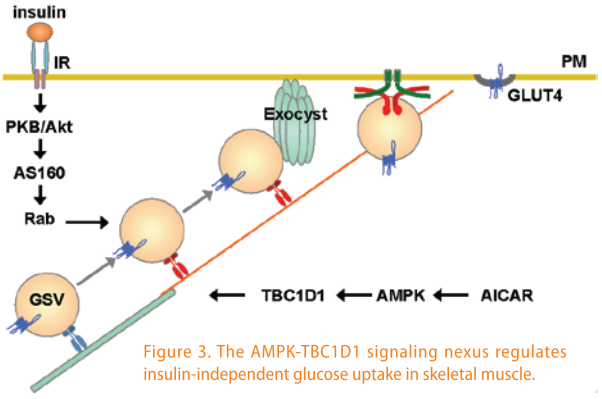


Figure 3. The AMPK-TBC1D1 signaling nexus regulates insulin-independent glucose uptake in skeletal muscle.

Selected Publications(* corresponding author)

1. 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)
2. 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)
3. 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)
4. 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)
5. 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)
6. 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
7. 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)
8. 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)
9. 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)
10. 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)
11. 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)
12. 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)
13. 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)
14. 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

Graduate students

Qiaoli Chen
Chao Quan
Min Li
Yang Sheng
Ping Rong
Qian Du
Sangsang Zhu
Wen Wei
Minjun Liu
Xinyu Yang

Post 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.

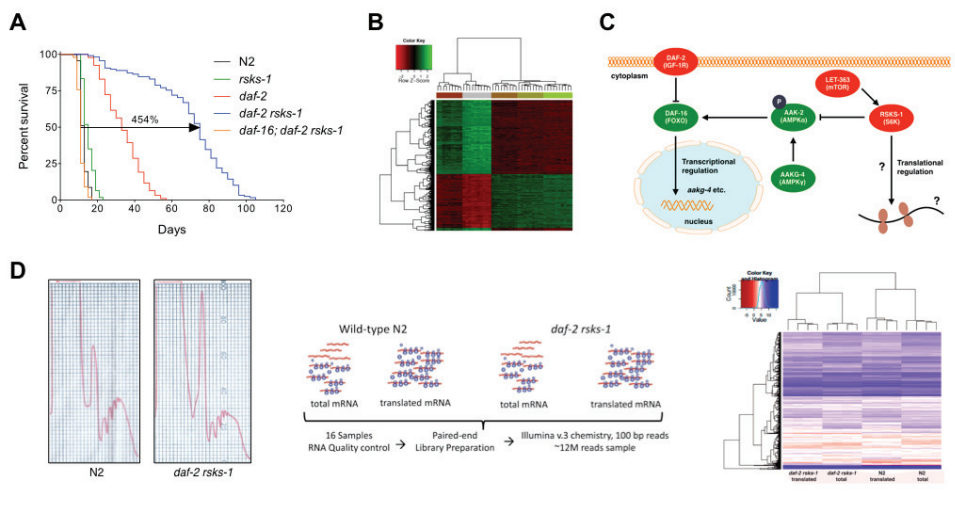


Figure 1. Functional genomics analysis of the super long-lived daf-2 rsks-1 mutant.

(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.

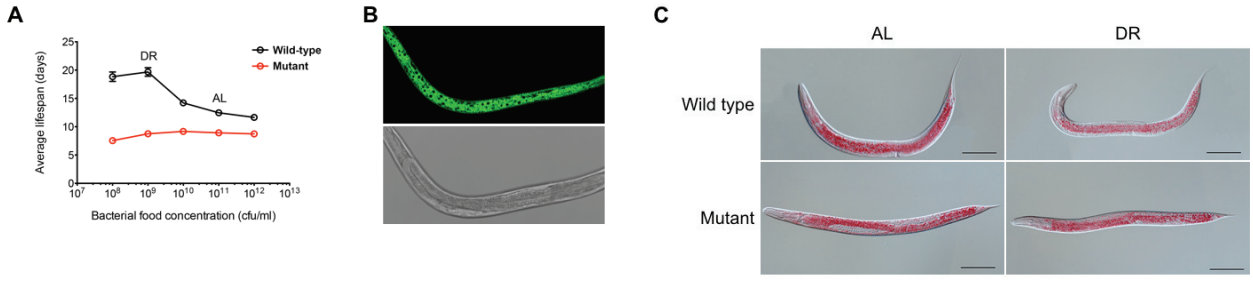


Figure 2. Characterization of lipid metabolism in dietary restriction-induced lifespan extension.

(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 (Fig. 1). 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 (Fig. 2).

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 plan to assess the contribution of micro RNAs in the pathology of centered nuclear myopathy (CNM), and aim to provide with a new therapeutic strategy for this disease.

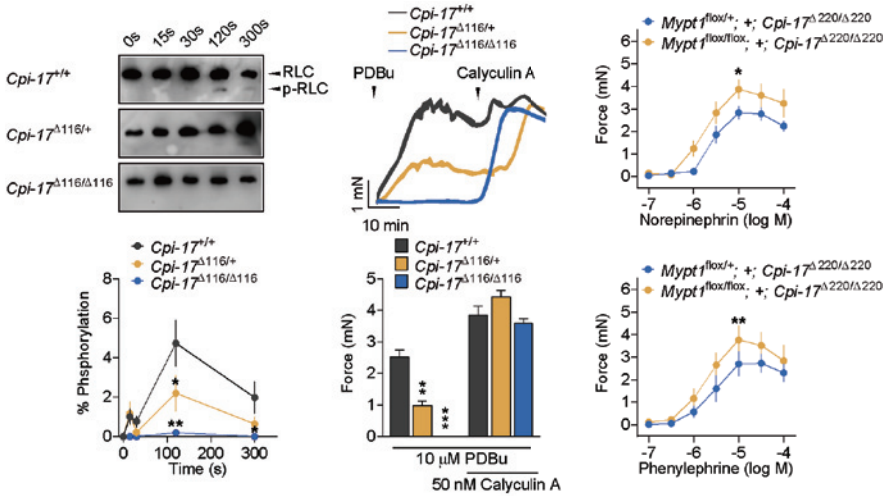


Figure1. Characterization of the contractile response of skinned mesenteric arteries of CPI-17 knockout mice.

The mesenteric artery segments form Cpi-17+/+, Cpi-17Δ116/+ and Cpi-17Δ116/Δ116 mice were permeable with α-toxin, and measured contraction force in response to calcium, PDBu and GTPγS. (A) Mutation of CPI-17 did not alter the maximal force induced by calcium. The skinned mesenteric arteries from Cpi-17+/+, Cpi-17Δ116/+ and Cpi-17Δ116/Δ116 mice were incubated with calcium at pCa 4.5 and then measured the maximal force. (B) pCa-force relationship in mesenteric arteries from Cpi-17+/+, Cpi-17Δ116/+ and Cpi-17Δ116/Δ116 mice was not altered. The relative forces represent the sustained force evoked by each dosage of calcium that were normalized to the sustained force of pCa 5.0 from the same tissues. (C) Potentiated contractile response induced by GTPγS. The skinned mesenteric artery segments were pre-contracted with pCa6.3 and then stimulated with 10 μM GTPγS. (E) Potentiated contractile response induced by PDBu. (E and F) The representative force recordings of D and F. Error bars, mean ± s.e.m. ** P < 0.01, *** P < 0.001, **** P < 0.0001, paired t-test.

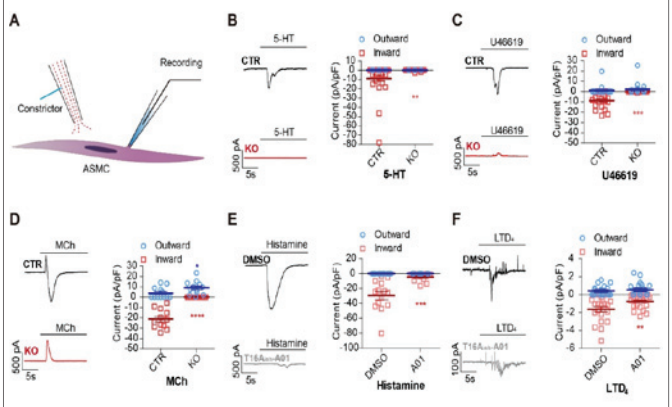


Figure 2 . TMEM16A mediates agonist-induced inward currents in ASMCs.

(A) Recording configuration. (B-D) Left: Representative traces of 5-HT- (B), U46619- (C) and MCh- (D) induced currents in ASMCs from CTR and TMEM16A KO mice. Right: Quantification of the results (5-HT: n=26 cells from 4 mice of each genotype; U46619: n=20 cells from 4 mice for CTR, n=22 cells from 4 mice for KO; MCh: n=12 cells from 3 mice per genotype). (E-F) Left: Representative traces of histamine- (E) and LTD4- (F) induced currents in DMSO and T16Ainh-A01 pre-incubated guinea pig ASMCs. Right: Quantification of the results (histamine: n=14 cells from 3 guinea pigs per group; LTD4: n=21 cells for the DMSO group and n=23 cells for the T16Ainh-A01 group from 4 guinea pigs each). The holding potential in all recordings was -60 mV. The blue blocks indicate the peak values of the outward currents, and the pink circles indicate the peak values of the inward currents. The bars represent the mean ± s.e.m., *P <0.05, **P <0.01, ***P <0.001, ****P <0.0001, two-tailed Student's t-test.

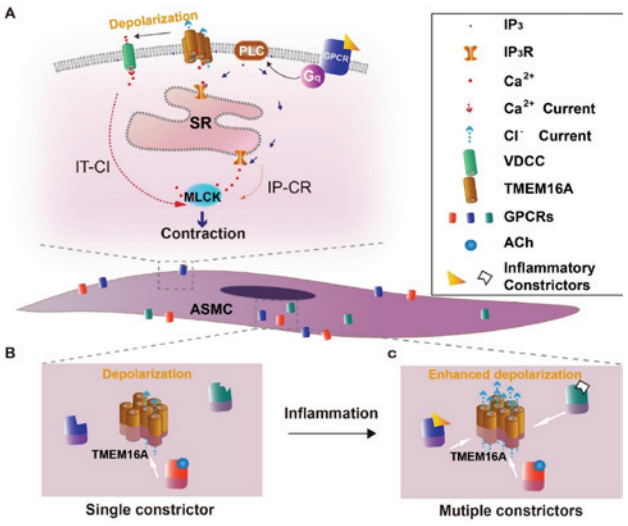


Figure 3. Schematic showing the hypothesized TMEM16A-based signalling model.

Inflammatory constrictors bind to their corresponding GPCRs and sequentially activate the Gq-PLC-IP3 axis. IP3 activates IP3Rs and induces Ca2+ release from the sarcoplasmic reticulum (SR). Ca2+ released from the SR can directly (IP-CR) or indirectly activate MLCK through the TMEM16A-VDCCs axis, mediate Ca2+ influx (IT-CI), amplify the cytosolic Ca2+ signal and activate more MLCK through this indirect pathway. Under simultaneous stimulation of inflammatory constrictors and ACh, ASMCs always show augmented contraction. This augmentation effect may be due to the interaction of the signalling network formed by different receptors. If the spatial distance between different GPCRs is small enough, the activation of these receptors may enhance local activation of IT-CI, hence augment contractile response. IP-CR: IP3-mediated calcium release from the SR; IT-CI: IP3/TMEM16A-mediated calcium influx.

Selected Publications

1. 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 2017 in press
2. Gao N, Tsai MH, Chang AN, He W, Chen CP, Zhu M, Kamm KE, Stull JT. Physiological vs. pharmacological signalling to myosin phosphorylation in airway smooth muscle. J Physiol. 2017 Oct 1;595(19):6231-6247.
3. Qin X, Liu S, Lu Q, Zhang M, Jiang X, Hu S, Li J, Zhang C, Gao J, Zhu MS, Feil R, Li H, Chen M, Weinstein LS, Zhang Y, Zhang W. Heterotrimeric G stimulatory protein alpha subunit is required for interstinal smooth muscle contraction in mice. Gastroenterology 2017;152(5):1114-1135

Group members

Group leader Min-Sheng Zhu

Graduate students

Tao Tao Jie Sun Fan She Yue-Qiong Li Li-Sha Wei Yan-Yan Zheng Wei Wang Yu-Wei Zhou

Postdoc Xin Chen Ph.D Pei Wang 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

Chaojun 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 lots of signaling proteins, including small G proteins. Geranylgeranyl diphosphate synthase (GGPPS) catalyzes the formation of geranylgeranyl diphosphate (GGPP) from farnesyl diphosphate (FPP), both of which are used to prenylate proteins with CAAX motif in their carboxyl termini. The prenylated proteins then are able to associate with membrane to initiate their function. We first identified GGPPS as a directly target gene of Egr-1, which can positively feedback to increase Egr-1 accumulation during chronic stress stimulation through enhance Ras prenylation and membrane association (Am J Path, 2011a, 2011b; J Biol Chem 2011; EMBO J, 2011). The prenylation includes two type modifications of protein: farnesylation and geranylgeranylation. Our hypothesis is that the balance of protein farnesylation and geranylgeranylation or FPP and GGPP inside the cell is critical to cell homeostasis by affecting signal transduction and protein functions. Thus, we have constructed GGPPS Floxed mice and conditionally deleted GGPPS gene in different tissues to examine its functions on cell homeostasis and its involvements in human diseases. We found that GGPPS regulated protein prenylation balance is involved in spermatogenesis and infertility (J Exp Med, 2013; Sci Rep, 2016; PLoS Genetics, 2017); hypertrophy and heart failure (J Path, 2015); insulin granule docked pool formation (J Path, 2016); lipid-induced muscle insulin resistance (J Biol Chem, 2015); pulmonary development (Am J Path, 2016). We 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. Protein prenylation promotes hepatic carcinogenic Warburg effect and regulate NAFLD/HCC progression (Bin Xue; Chao-Jun Li)

Patients with obesity exhibit high non-alcoholic fatty liver disease (NAFLD) prevalence and increased susceptibility to hepatocellular carcinoma (HCC) in parallel. Herein, we report that the high-fat-diet (HFD) augments glycolysis and accelerates NAFLD/HCC progression, which was mediated by geranylgeranyl diphosphate synthase (GGPPS), a critical enzyme in the mevalonate pathway. The down-expression of GGPPS predicts an advanced stage of NAFLD and recurrence of NAFLD-associated HCC. Long-term fat overloading decreases GGPPS expression in mice, which shifts the fuel preference toward glycolysis rather than fatty acid oxidation (FAO). Liver-specific Ggpps deficiency drives carcinogenic Warburg effect and then induces hepatic inflammation, thus exacerbating fibrosis/HCC development. Underlying mechanism study suggests that hyper-farnesylation of liver kinase B1 (LKB1) and subsequent AMP-activated protein kinase (AMPK) activation serve as a central regulator. We conclude that GGPPS-controlled protein prenylation is a susceptibility factor for NAFLD/HCC progression via the LKB1-glycolysis-inflammation axis, and its expression in NAFLD requires more stringent surveillance to ensure timely treatment.

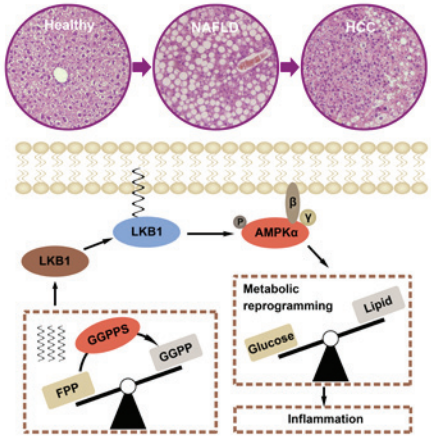


Figure 1. A working model that how ggpps controlled protein prenylation promotes hepatic carcinogenic Warburg effect and regulate NAFLD/HCC progression.

2. Liver first responses to nutrition overloading and remodels lipid deposition in white adipose tissue through hepatocytes-derived exosomal miRNAs let-7e-5p (Yue Zhao; Chao-Jun Li)

Nutrition overloading will induce lipid redistribution among metabolic organs, like liver, adipose, skeletal muscle, while their interplay that adjusts the adaptive lipid homeostasis remains unknown. Here, we show that it is liver who first responses to nutrition overloading, then send the signal to remodel triglyceride deposition in adipose. The signal is conveyed by hepatocyte-derived miRNAs that are increased in response to nutrition overloading, directly target to adipocyte carried by exosomes. Among these exosomal miRNA, let-7e-5p directly enhanced triglyceride deposition in adipocytes by down-regulating Pgc1α to increase adipogenesis and lipogenesis and inhibit lipid oxidation. Our results also show that the secretion of exosomes depends on Rab27A geranylgeranylation, which regulated by Geranylgeranyl diphosphate synthase (Ggpps), whose expression is highly enhanced in response to HFD. When Ggpps is liver-specifically deleted, exosome formation and secretion was largely inhibited and nutrition overloading fails to remodel adipose tissue lipid deposition. In sum, our findings indicate that the liver, not only a "sensor" of over nutrition but also a "commander", triggers the integrated response to modulate metabolic function of body by directly secreting miRNA-containing hepatocyte-derived exosomes.

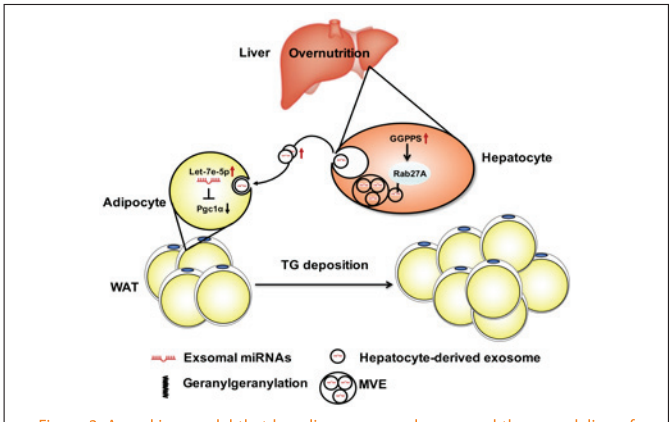


Figure 2. A working model that how liver sense and command the remodeling of adipose tissue lipid deposition under nutrition overloading.

3. GGPP regulates liver glucose and lipid metabolism by directly binging to FBP1 and changing FBP1 tetramer confirmation (Lei Fang; Chao-Jun Li)

Using a tandem affinity purification strategy and mass spectrometry, we depicted an interactive landscape of GGPP and its direct binding proteins in liver. When complemented with bioinformatics tools, this proteomic analysis provided a novel interconnection between GGPP and key metabolic enzymes. Collectively, we have provided a functional compartmentalization overview and an organizational framework of intracellular GGPP-Metabolic Enzyme Interaction which can serve as a resource for future investigations. Driven by TCGA and further validation experiments, we investigated hepatic GGPP direct binding proteins may uncover an essential role in controlling the onset and progression of NAFLD-HCC. Mechanistically, GGPP formed a complex with Fructose-1,6-bisphosphatase 1 (FBP 1), a rate-limiting enzyme in gluconeogenesis and a functional tumor suppressor especially in liver, to change its complex conformation and enhance its activity.

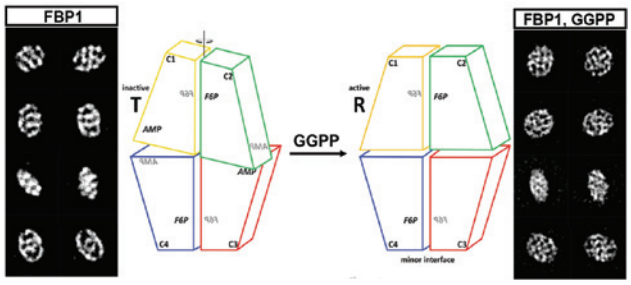


Figure 2. A primary observation that how GGPP can bind with FBP1 and change its confirmation.

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

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

Group members

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)

Former graduate students

Xiu-Xing Wang (2012):
Postdoc Fellow, 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.

Fan Diao (2015):

The Medical School of Massachusetts University, USA

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

Graduate students:

Zhong Chen	Yong-Juan Sang
Jing-Zi Zhang	Tong-Yu Zhang
Jia Liu	Wei-Wei Chen
Di Shen	Feng Zheng
Rui-Lou Zhu	Yue Hua
Jing Wu	Ruo-Yu Zhao
Dan-Yang Chong	Meng-Fei Zhao
Qiao-Li Tang	Qi Cheng
Tian-Xiang Feng	Kang Li
Qian Sun	

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.

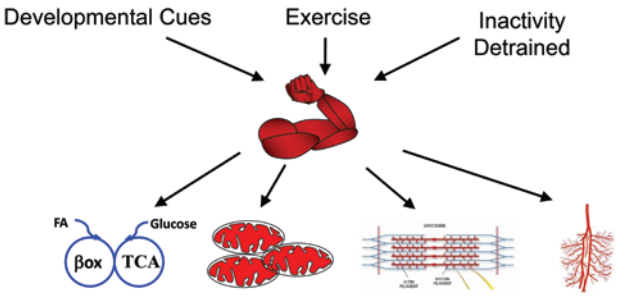


Fig 1. The muscle fitness is determined by developmental as well as physiological inputs. These inputs coordinately control the programs including fuel burning, mitochondrial ATP production, contraction, and angiogenesis.

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.

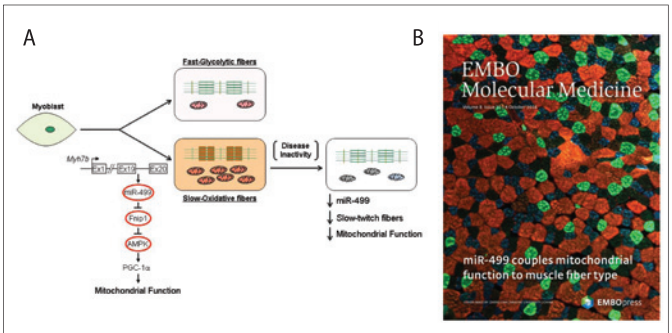


Fig 2. (A) The schematic depicts a proposed model for the miR-499/Fnip1/AMPK circuit that orchestrates mitochondrial function to match muscle contractile machinery during fiber type transition. (B) EMBO Mol Med. October 2016 Cover.

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).

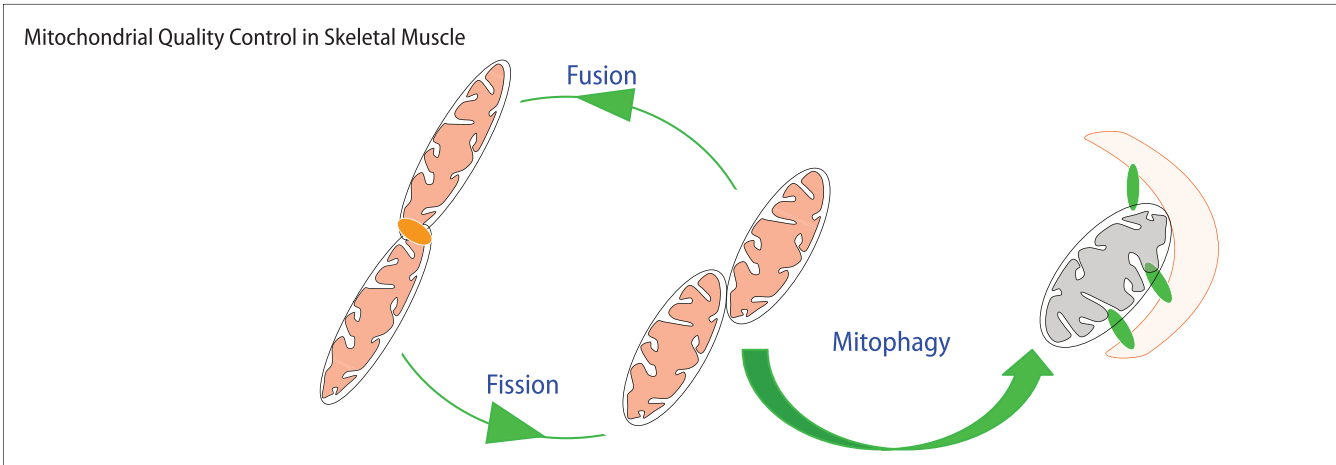


Fig 3. Skeletal muscle mitochondrial remodeling upon adaption to exercise and diseases.

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1. Liu J, Liang X, Zhou D, Lai L, Xiao L, Liu L, Fu T, Kong Y, Zhou Q, Vega R, Zhu MS, Kelly DP. Gao X. Gan Z*.Coupling of mitochondrial function and skeletal muscle fiber type by a miR-499/Fnip1/AMPK circuit. EMBO Mol Med. 2016; 8(10):1212-1228. Cover story.
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Group members

Group leader

Zhenji Gan

Postdoctoral fellow

Jing Liu

Graduate students

Xijun Liang
Tingting Fu
Lin Liu
Liwei Xiao
Zhisheng Xu
Yujing Yin
Zongchao Sun
Chenyun Ding

Former graduate students

Jing Liu (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.

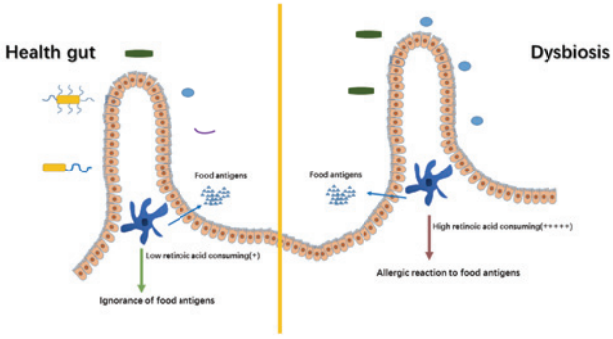

Figure 1. Microbiota repress food allergic challenge via modulating dendritic cells retinoic acid response

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.

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Hongyu 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.

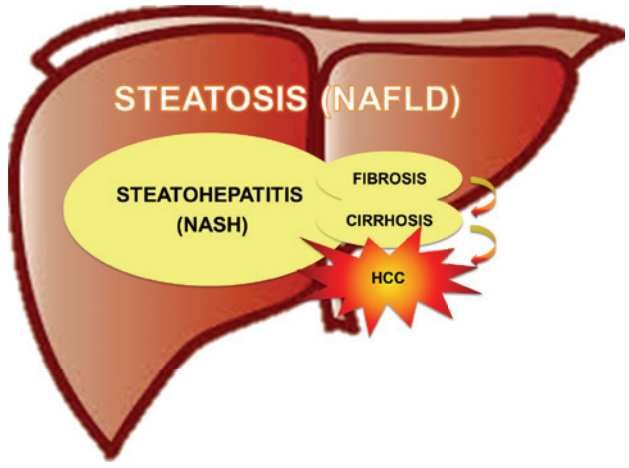

Figure 1 The progression of NAFLD/NASH.

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Group members

PI	Graduate students
Hong-Yu Wang	Qian Ouyang


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, USA 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

Cell behavior, metabolism and tumorigenesis

Our laboratory is interested in studying the determinants of cell behaviors and their close connections with 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, differentiation, cell competition 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, signaling and metabolism may allow us to fully understand the complex cell behaviors in many fundamental processes including development, ageing and tumorigenesis.

1. p53 signaling pathway influences cell behaviors and cell fate control

p53 is extremely important for stress response and tumor suppression as exemplified by its mutations found in over 50% of human cancers. 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.

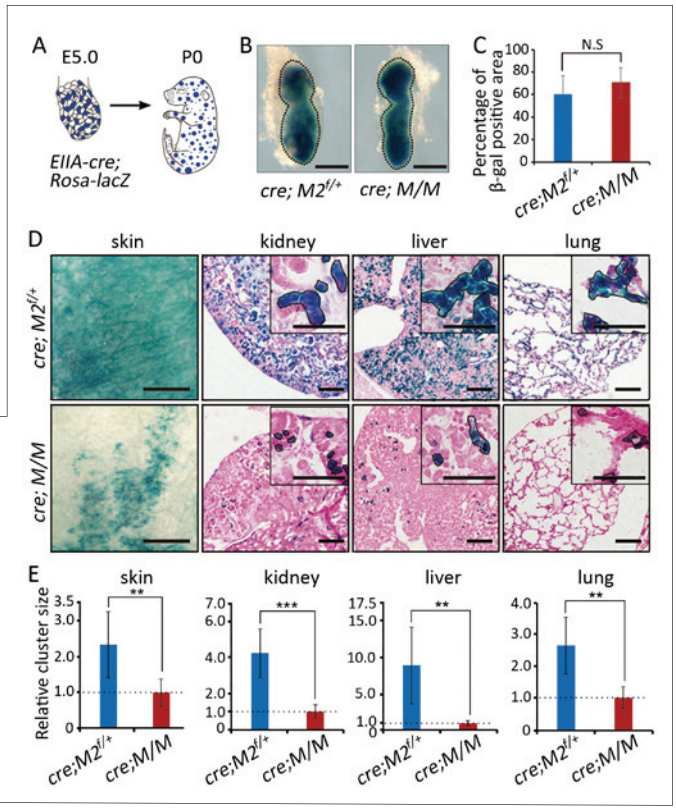

Figure 1. Cells with the Mdm2$^{+/-}$ Mdm4$^{+/-}$ genotype were out-competed in EIIA-cre induced mosaic embryos.

(A) A schematic presentation of the mosaic mice.

(B) Whole-mount X-gal staining of E6.5 EIIA-cre; Rosa-lacZ; Mdm2flox/+ (M2f/+) 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; Mdm2flox/+ (M2f/+) 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; Mdm2flox/+ (M2f/+) 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; Mdm2flox/+ (M2f/+) mice (n=4). Data are presented as Mean±SD. **p≤0.01, ***p≤0.001 (two-tailed t test).

2. Cellular metabolisms influence cell behaviors in vivo

To begin studying the influence of cellular metabolism on cell behaviors and function, we are currently in the process of addressing the following issues: 1) how different metabolic preferences affect cell behaviors 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 specifically manipulated in vivo. These new genetic tools will facilitate the analysis of metabolic advantage, cooperation, influence, switch and adaptation in various in vivo contexts; 2) how can the metabolic state of a cell be monitored in vivo? By analyzing a novel reporter for mitochondrial function, we have found interesting metabolic heterogeneity in cells and tissues that may correspond to the function and quality control mechanism of the cells (Fig.2). We will continue to study the metabolic heterogeneity within the tissues and their links to homeostasis and diseases.

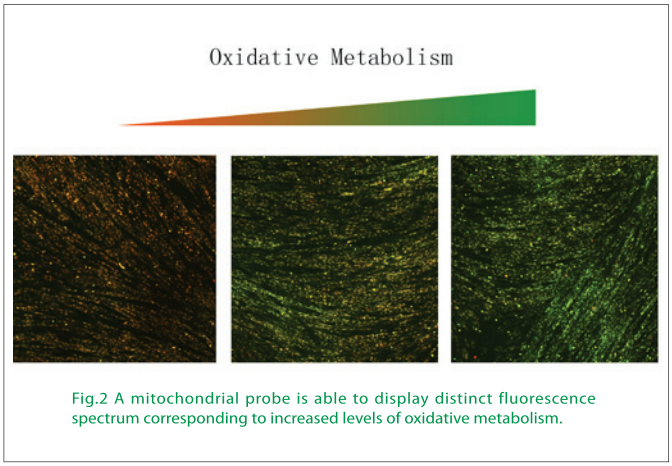

Fig.2 A mitochondrial probe is able to display distinct fluorescence spectrum corresponding to increased levels of oxidative metabolism.

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Group members

Former lab members

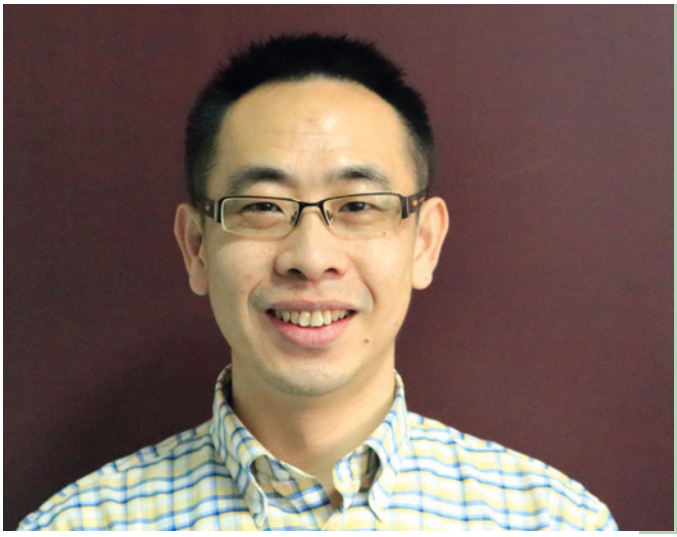
Zhang Qin
Chen Lai
He Xueyan
Zhang Chenxi
Zhang Guoxin
Zhou Ying

Graduate students

Xie Yinyin
Xiang Cong
Hou Xiaoshuang
Hou Weikang
Peng Yamei
Liu Lulu
Sun Aina
Zhang Yannan

Technical staff

Ren Yan



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 2017).

2.Castration resistant prostate cancer (CRPC) 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 Northwest A&F University, we characterized triptolide, one chemical derived from the Chinese herb thunder god vine (Tripterygium wilfordii Hook F.), possess anti-cancer effects against CRPC cells. Mechanistically, triptolide at nM level directly targets XPB, subsequently suppressing CDK7 activity on AR(S515). Reduced phosphorylation level of AR at S515 suppresses the transcriptional activities of both AR full length and truncated variant AR-V7, which is essential for the development of enzalutamide resistance (Fig. 2). Essentially, 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 other pathways (Xu P, et al, Nat Commun 2017). 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. Through PCR microarray and functional assay, we identified that TGFβ1/lncRNA-LET are dysregulated, accounting for such chemo-resistance. Treatment with a clinical trial TGFβRI inhibitor, LY2157299, significantly and strikingly delayed the chemoresistance to gemcitabine. At molecular level, reduced lncRNA-LET by the overactived TGFβ1 signaling pathway releases the tethering with NF90. The stabilized NF90 binds to and interferes the biogenesis of tumor suppressive miR-145, eventually leading to the increase of CSC population (Fig. 3). The correlation of TGFβ1, lncRNA-LET, NF90 and miR-145 reinforce the notion that such regulatory axis is essential for bladder cancer stemness and interruption of such axis may enhance chemoresistance to gemcitabine (Zhuang J, et al, Theranostics 2017).

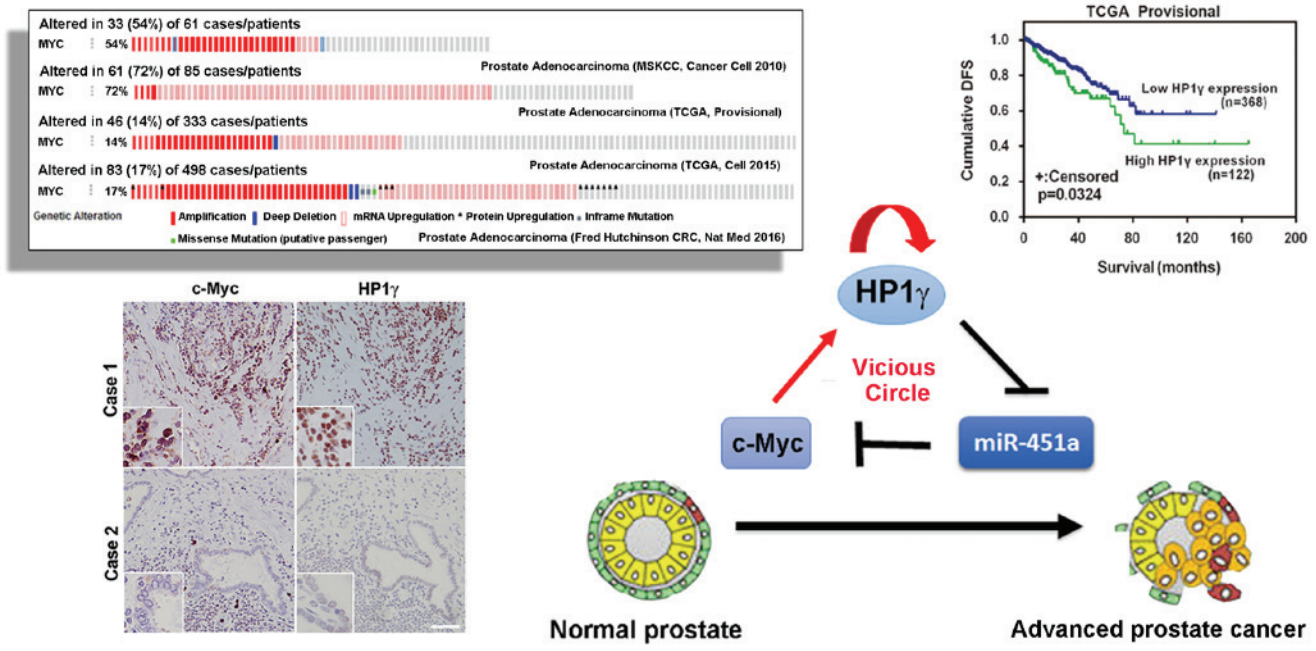


Fig 1. A vicious regulatory circuitry of c-Myc/HP1γ/miR-451a determines prostate cancer aggressiveness (Chang C, et al. Oncogene 2017).

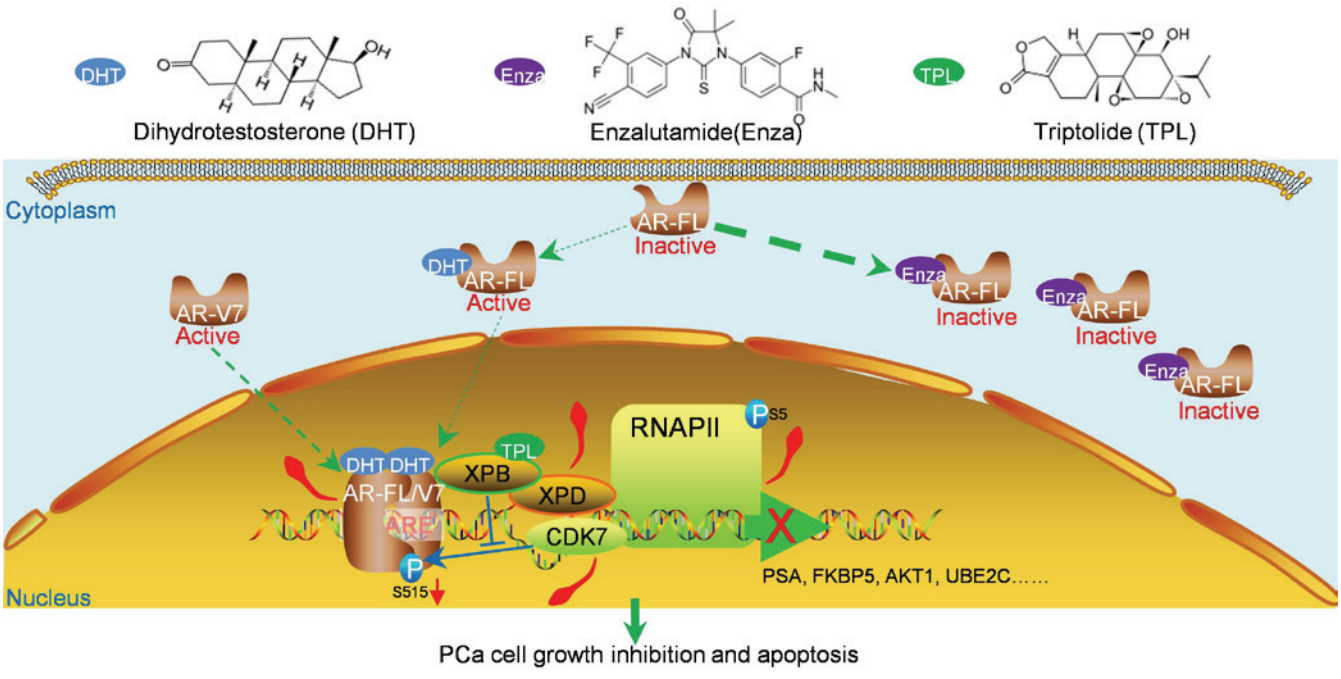


Fig 2. Triptolide suppresses enzalutamide resistant prostate cancer cell survival through targeting androgen receptor signaling (Han Y, et al. Theranostics 2017).

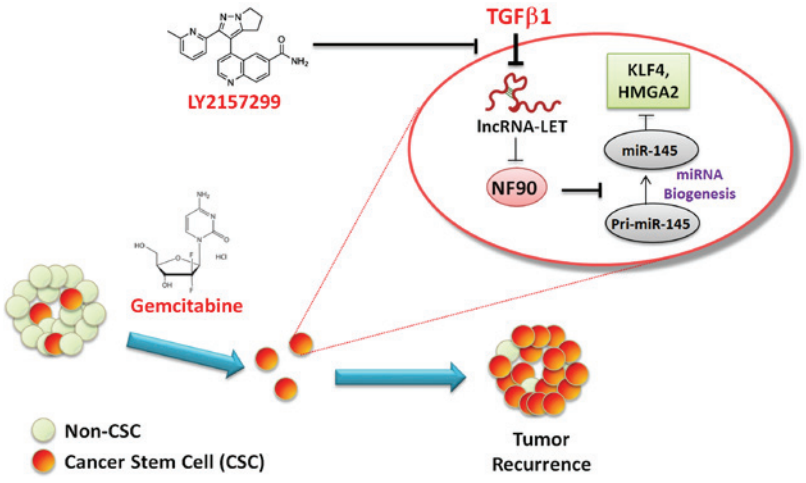


Fig 3. Suppression of bladder cancer stemness through targeting TGFβ1 signaling enhances chemotherapy to Gemcitabine (Zhuang J, et al. Theranostics 2017).

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Group members

Techinician Xiaohong Yu

Graduate students

Xiaojing Huang
Junzun Li
Yangyan Cui
Zemin Gao
Lan Shen
Shuai Yang
Jiakuan Liu
Meiqian Li

Trainees in collaboration

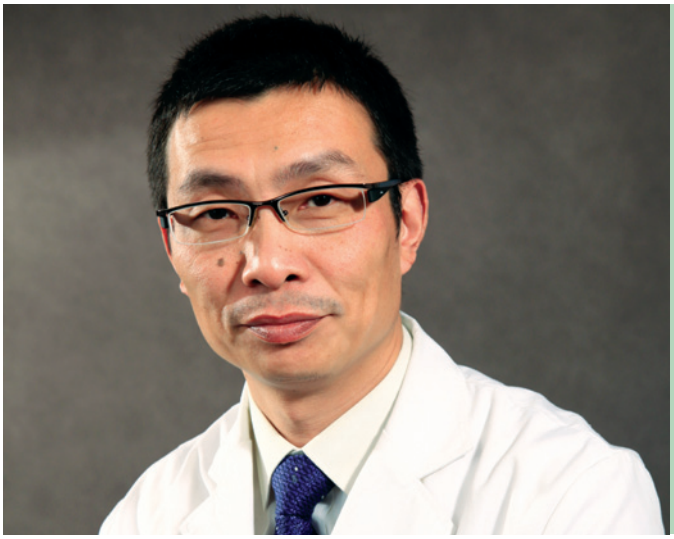
Lin Yang
Yanshi Xue
Yuting Song

Undergraduate student

Jiaqi Zhang

lab alumi

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



Qing Jiang, Ph.D., M.D.

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

Contact Information

Tel : +86-25-83107008
Fax: +86-25-83317016
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 toevaluate the effect of intraarticular injection of the small-molecule compound kartogenin (KGN) on the restoration of afull-thickness cartilage defect. To confirm that KGN can induce humanMSCs into chondrocytes, we compared the isolated SMSCsfrom the synovium tissue and the used pellets culture systemwith or without KGN. The average diameter of the KGNNP was 270 nm, identified by dynamic light scattering (DLS) (Figure 1), which was consistent with the result of SEMimaging. KGN nanoparticles was quickly formed through afree-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. The 3D digital models of samples with defects and corresponding healthy parts were obtained using high-resolution 3D scanning. The Boolean operation was used to achieve the shape of the defects, and then the target geometries were imported in a 3D bioprinter. Two kinds of photopolymerized hydrogels were synthesized as bioinks. Finally, the defects of bone and cartilage were restored perfectly in situ using 3D bioprinting(Figure 2) (Li 2017).The tissue bank for cartilage and ligament has been established and enlarged.

Rare skeletal diseases usually are misdiagnosed, so that patient cannot get theoptimal treatment. Genetic factors such as gene mutations play a considerable rolein etiology and pathogenesis of these rare and developing diseases of skeletalsystem. 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 forProgressive pseudorheumatoid dysplasia, 1 novel mutation in CHST3 that are responsible for Spondyloepiphyseal dysplasia with congenital joint dislocations,2novel mutations in HSPG2 for Schwartz-JampelSydrome 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 investigated the role of the energy sensor AMP-activated protein kinase (AMPK) in maintaining a healthy state of articular cartilage and in OA development. Using cartilage-specific, tamoxifen-inducible AMPKα1conditional knockout (AMPKα1 cKO), AMPKα2 conditional knockout (AMPKα2 cKO) and AMPKα1α2 conditional double knockout (AMPKαcDKO) mice, we found that compared with wild-type (WT) littermates, mutant mice displayed accelerated severity of surgically induced OA, especially AMPKα cDKO mice (Fig 1). Furthermore, male but not female AMPKαcDKO mice exhibited severely spontaneous ageing-associated OA lesions at 12 months of age. The chondrocytes isolated from AMPKαcDKO mice resulted in an enhanced interleukin-1β (IL-1β)-stimulated catabolic response. In addition, upregulated expression of matrix metalloproteinase-3 (MMP-3), MMP-13 and phospho-nuclear factor-κB (phospho-NF-κB) p65 and increased levels of apoptotic markers were detected in the cartilage of AMPKα cDKO mice compared with their WT littermates in vivo. Thus, our findings suggest that AMPK activity in chondrocytes is important in maintaining joint homeostasis and OA development (Zhou 2017).

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

Developmental dysplasia of the hip (DDH) is the mostfrequent inborn deformity of the locomotors apparatus.Genetic factors play a considerable role in pathogenesis of DDH. At current stage, we have performed GWAS study of DDH. In a previous association study of DDH in North Chinese population, we haddetected associations between DDH and single nucleotidepolymorphisms (SNPs) in GDF5, TBX4, and ASPN by case-controlstudies in Chinese Han population. The association betweenPAPPA2 and DDH should be evaluated by additional studies.

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

1. Theresearch 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 defectreconstruction using a hydrogel with sustained release of smallmolecule 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 withsustained small molecule BIO. (Excellent Young ScholarsNSFC 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 injuryNatural Science Foundation of Jiangsu Province, China (SBK2017040751)

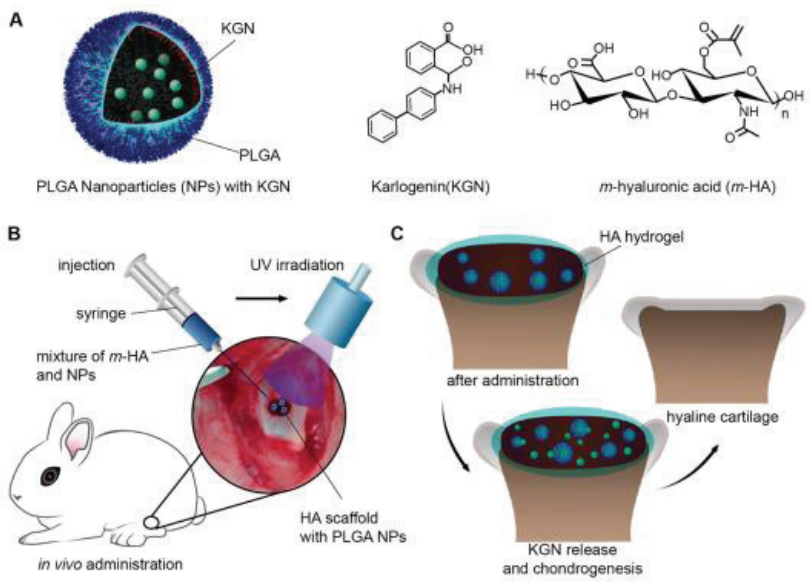


Figure 1.

(A) Schematic of KGN-loaded PLGA nanoparticles, molecule structures of KGN, and acrylated hyaluronic acid (m-HA).

(B)Schematic of the surgical procedure for the cartilage defects repair.

(C) Schematic of the hyaline cartilage chondrogenesis with photo-crosslinked HA scaffold encapsulated with KGN-loaded nanoparticles.

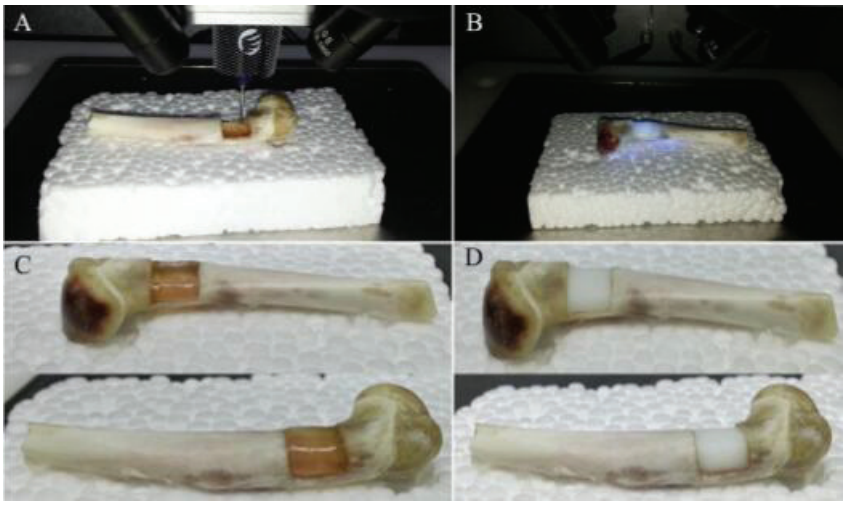


Figure 2.

Process of 3D bioprinting and photopolymerization on bone defect. Due to the influence of UV light of camera, the light was turned off when shooting video and photographs in one of the three printing process. Photopolymerization was taken at the end of printing.

(A) Repair of bone defect through in situ 3D bioprinting with alginate hydrogel.

(B) Exposure to UV light.

(C) Alginate hydrogel that was printed to repair the bone defect was transparent before photopolymerization.

(D) The color of alginate hydrogel turned milky white after being exposing to UV light in few seconds. The bone defect was restored perfectly.

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9. Li Q, Dai B, Yao Y, et al. (2017)Chronic Kidney Dysfunction Can Increase the Risk of Deep Vein Thrombosis after Total Hip and Knee Arthroplasty. BioMed research international. 2017;2017:8260487.(IF=2.476)
10. Song K, Rong Z, Yang X, et al.,Jiang Q*.(2016) Early Pulmonary Complications following Total Knee Arthroplasty under General Anesthesia: A Prospective Cohort Study Using CT Scan[J]. Biomed Res Int. 2016:1-5.(IF=2.134)

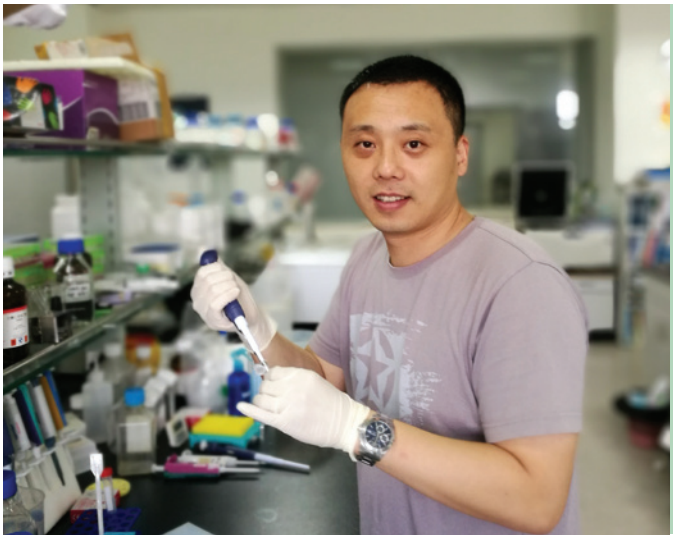
Group members

Technical staff

Huajian Teng
Dongyang Chen
Zhihong Xu
Dongquan Shi
Sheng Shen
Jin Dai
Jianghui Qin
Xingquan Xu
Kai Song
Lan Li
Wenjin Yan
Qiting Ge
Jing Jin
Rui Wu

Graduate students (2017)

Ke Lu
Sicong Min
Bingyang Dai
Liang Qiao



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

Macrophage biology and molecular immunology

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 that not only serve as the major sentinels of tissue integrity, but also represent key regulators and effectors of an immune response. Consistent with their vital roles in immunity, dysregulation within the macrophage compartment is known to underlie many disease states and often to impact therapeutic responses. Therefore, to study the complex role(s) by macrophages is fundamental for furthering our understandings to numerous diseases. We use mouse models to investigate the regulation of macrophages in inflammation and cancer. Ultimately, we hope that our bench discoveries can in some way impact management of human diseases.

1. The impact of type I IFN on differentiation of tumor-associated macrophages (TAMs):

Initially identified as a key anti-viral cytokine, the type I IFN has shown moderate antitumor activities in humans. Therefore, uncovering targets that modulate its anti-tumor activities has therapeutic implications. Using a syngenic tumor implantation model, we found that poly(I:C)-IFN treatment could significantly reduce the numbers of TAMs, associated with the suppressive effect on tumor growth (Fig. 1A, B). This was associated with a visible inhibitory activity by IFN on differentiation of monocytes toward macrophages (Fig. 1C). We further uncovered a mir-155-dependent mechanism that underlies such an activity (Fig. 1D, E, F, G). Surprisingly, type I IFN acting at this critical developmental window also drives a pro-repair program in macrophages, both in vitro (Fig. 1H) and in tumors (not shown). Importantly, poly(I:C) and an inhibitor of such a pro-repair program showed clear synergistic effects on reducing tumor growth in mice (Fig. 1I). Several projects have since evolved to provide more mechanistic insights to such unanticipated observations.

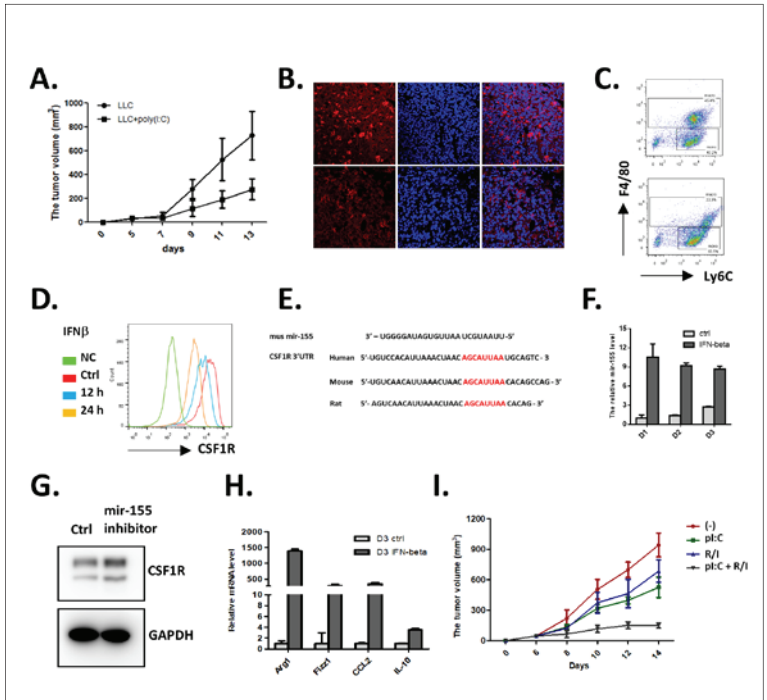


Figure 1: Multifaceted actions by type I IFN on tumor-associated macrophages (TAMs).

An IFN inducer (poly(I:C)) had moderate anti-tumor activities on lewis lung carcinoma (LLC) grafts. (B) Poly(I:C) treatment led to reduced density of TAMs. (C) IFN inhibited MCSF-dependent differentiation of mouse monocytes towards macrophages. (D, E, F, G) IFN elicited an mir-155-dependent inhibitory effect on CSF1R expression. (H) IFN rewired macrophage differentiation program toward a pro-repair phenotype. (I) Poly(I:C) and R/I showed synergistic effect on LLC tumor growth.

2. Metabolic regulation of macrophage plasticity:

We previously identified a glycolysis-mediated immunometabolic axis for regulating the efferocytosis activity by macrophages (Jiang H. et al, 2016). A key player involved is the glycolysis-activating enzyme PFKFB3. Since efferocytosis mainly serves as an immunosuppressive mechanism, we hypothesized that PFKFB3-glycolysis might also impact the polarization of macrophages towards the immunosuppressive, M2 state (Fig. 2A). Indeed, macrophage lacking PFKFB3 showed blunted polarization toward the M2, but not the M1 state (Fig. 2B, C). Taking advantage of the Pfkfb3-deficient mouse model, we are currently analyzing the role of glycolytic metabolism in differentiation and functional polarization of macrophages in vivo.

3. 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.

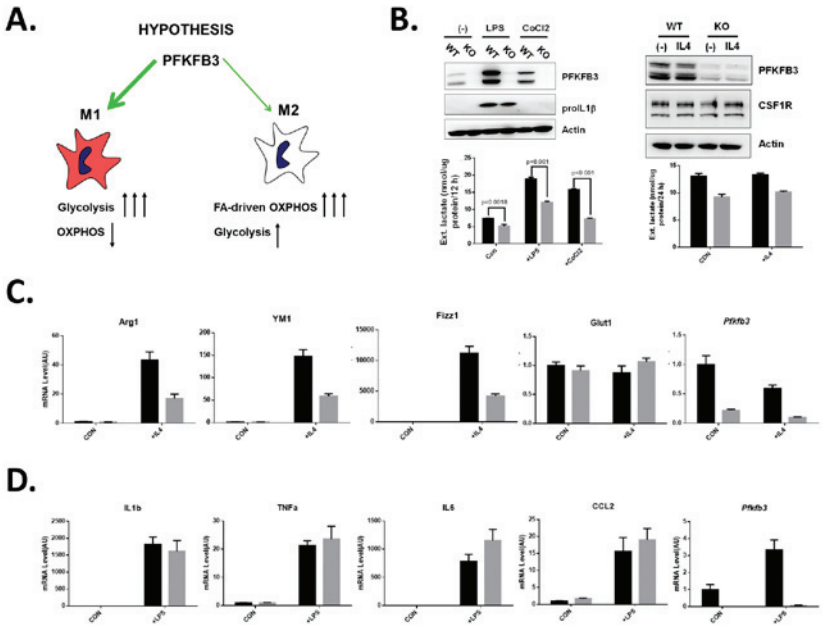


Figure 2: Regulation of macrophage polarization by PFKFB3.

(A) An initial hypothesis that glycolytic activator may impact the M2 polarization, in addition to a long-perceived role on M1 polarization. (B) Effective depletion of PFKFB3 in M1 and M2 macrophages using floxed mice. (C and D) PFKFB3 deficiency in macrophages significantly impaired M2 (C), but not M1 (D) polarization.

Selected publications (*corresponding author)

1. Jiang H, Shi H, Sun M, Wang Y, Meng Q, Guo P, Cao Y, Chen J, Gao X, Li E* and Liu J*. PFKFB3-driven macrophage glycolytic metabolism is a crucial component of innate antiviral defense. J Immunol (2016), 197(7), 2080-90.
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3. Zhang Y, Wang Y, Zhang C, Wang J, Pan D, Liu J* and Feng F*. Targeted Gene Delivery to Macrophages by Biodegradable Star-Shaped Polymers. ACS Appl Mater Inter (2016), 8(6):3719-24.
4. Du Y, Meng Q, Zhang J, Sun M, Shen B, Jiang H, Kang Y, Gao J, Huang X* and Liu J*. Functional annotation of cis-regulatory elements in human cells by dCas9/sgRNA. Cell Res (2015), 25(7):877-80.
5. Tong, Y., Li, F., Lu, Y., Cao, Y., Gao, J.* and Liu, J.*, Rapamycin-sensitive mTORC1 signaling is involved in physiological primordial follicle activation in mouse ovary. Mol. Reprod. Dev. (2013), 80: 1018–1034.
6. 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.

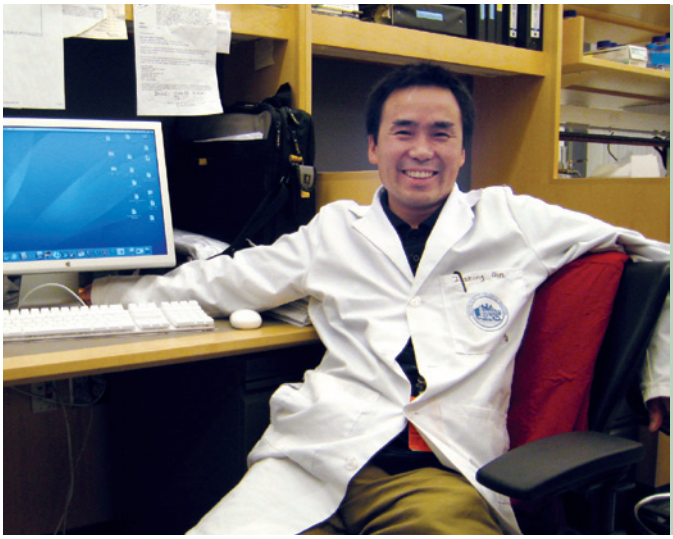
Group members

Technical assistant:

Yan-lan Cao

Graduate students:

Yuan-yuan Tong
Qing-zhou Meng
Pan-pan Guo
Ya-feng Wang
Li-min Yang
Meng-fan Zhang
Gui-quan Zhang



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.

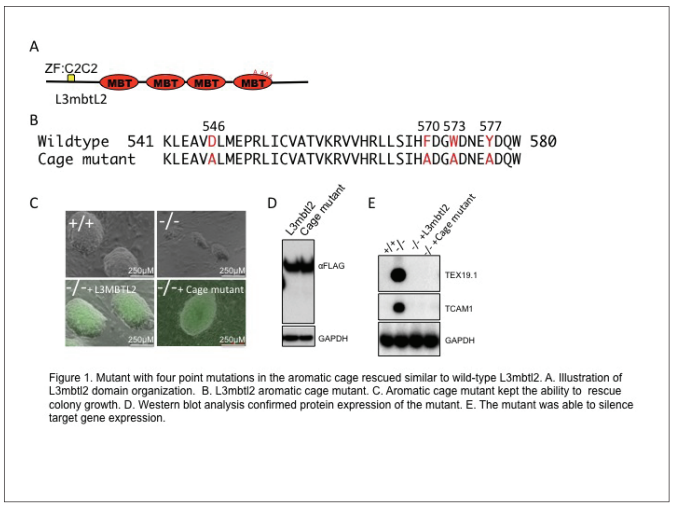


Figure 1. Mutant with four point mutations in the aromatic cage rescued similar to wild-type L3mbtl2. A. Illustration of L3mbtl2 domain organization. B. L3mbtl2 aromatic cage mutant. C. Aromatic cage mutant kept the ability to rescue colony growth. D. Western blot analysis confirmed protein expression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

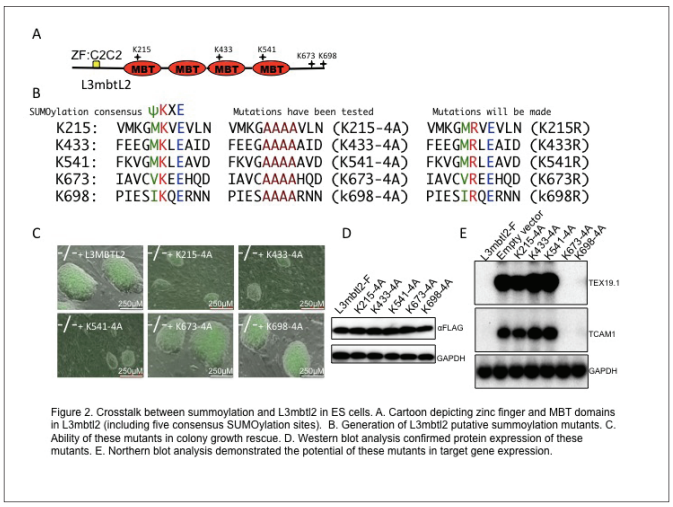


Figure 2. Crosstalk between summoylation and L3mbtl2 in ES cells. A. Cartoon depicting zinc finger and MBT domains in L3mbtl2 (including five consensus SUMOylation sites). B. Generation of L3mbtl2 putative summoylation mutants. C. Ability of these mutants in colony growth rescue. D. Western blot analysis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

Selected publications

1. A. Foudi, D. Kramer, J. Qin, D. Ye, A. Behlich, S. Mordecai, F. Preffer, K. Hochedlinger, S.H. Orkin, and H. Hock. 2014. Distinct, strict requirements for Gfi-1b in bone marrow red cell and platelet generation. J Exp Med 211:909-27.
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Group members

Group leader
Jinzhong Qin

Technical assistants
Huan Tong

Graduate students
Wukui Zhao
Saisai Wang
Yun Yan

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

Cancer related inflammation

Tumor associated macrophage (TAM) are increasingly viewed as a target of great relevance in the tumor microenvironment, because of their important role in cancer progression and metastasis. However, the endogenous regulatory mechanisms underlying the interactions between TAMs and cancer cells remain largely unknown. With this as our goal, we are currently studying the actions and related regulatory mechanisms of certain soluble factors secreted by TAMs as tumors grow, which have been identified in our previous study. We have found that PDGF derived from TAMs can trigger the activation of CDK5 in breast cancer cells. CDK5 activation induces the phosphorylation of PPARγ at Ser273 which is correlated with tumor malignant progression. PPARγ phosphorylation are detectable in patients with triple-negative breast cancer. The formation of CDK5/PPARγ axis in breast cancer cells modifies the ubiquitin ligase activity of PPARγ and then regulates the exosome expression pattern, promoting cancer progression. Blockade of CDK5/PPARγ axis via CDK5 inhibitor, or PPARγ ligand suppresses tumor growth significantly. And, targeting the PDGF blocks the communications between TAMs and cancer cells.

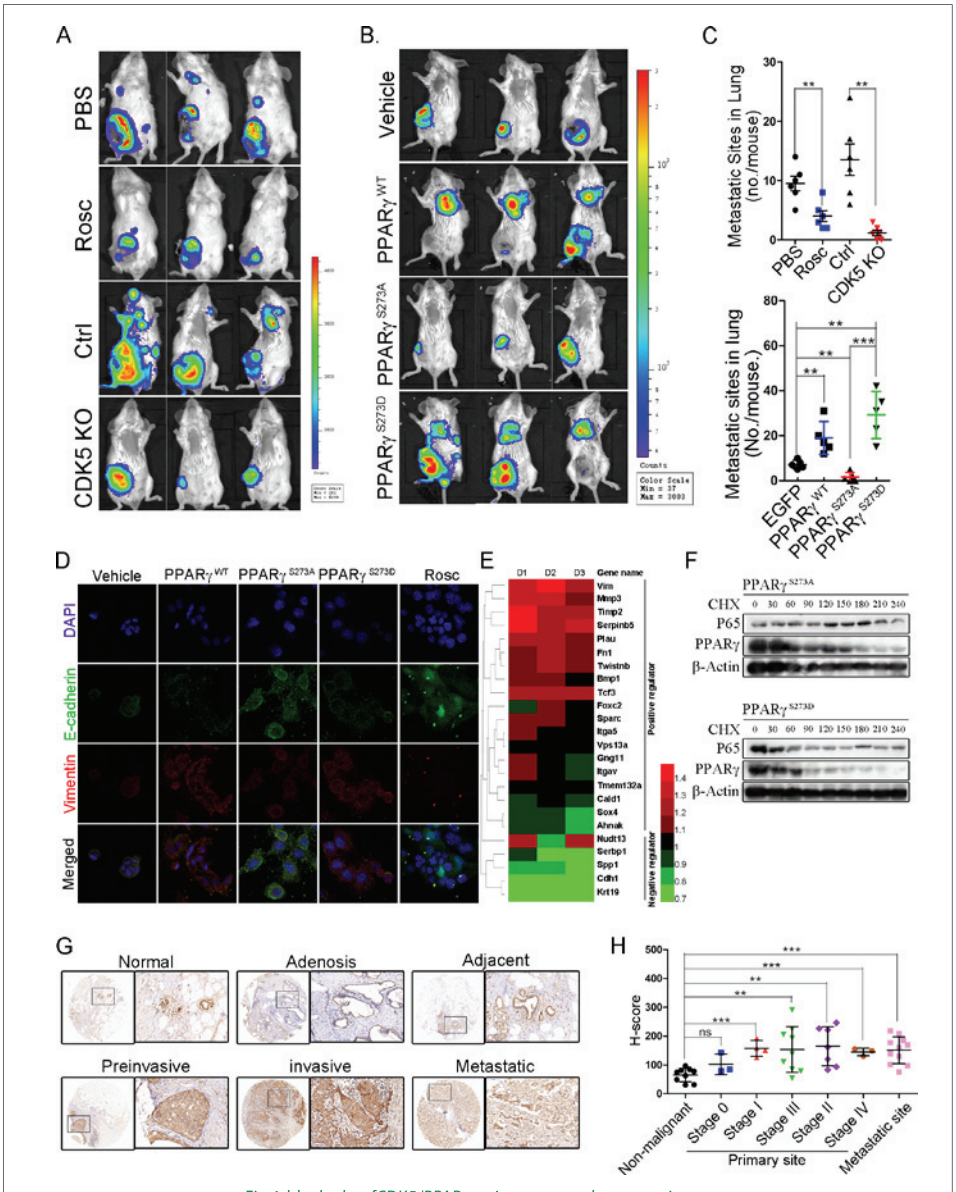

Fig.1 blockade ofCDK5/PPARg axis attenuated metastatic progression of breast cancer via inhibition of EMT

AML therapy

Acute monocytic leukemia (AMoL) is considered a type of acute myeloid leukemia. This type of cancer is usually characterized with clonal disorder of hemopoietic progenitor cells, which lose the ability to differentiate normally and to respond to normal regulators of proliferation. We figured out that MSC and a chemical SW22 treatment alone or synergistically all could promote the differentiation of acute monocytic leukemia cells and hereby suppress the progression of AMoL significantly, while, the efficacy of combined regiment was more significant than the treatment with SW22 or MSC alone (fig.2). Thus, SW22 and MSC might be promising candidates for AML therapy.

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

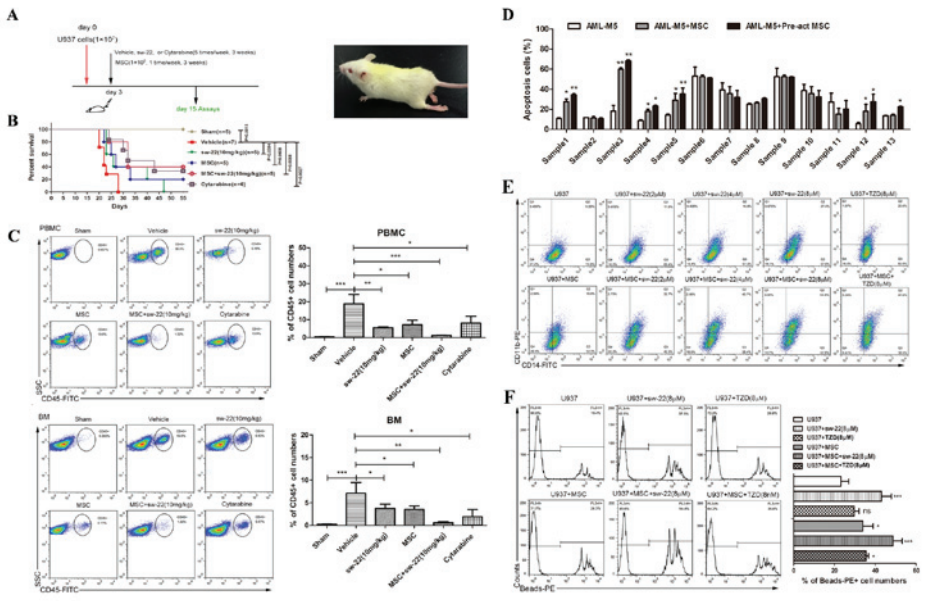

Fig2. MSCs induce monocyte differentiation

Clinical diagnostic techniques

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

Selected publications and awards

1. Niu Z.Y., Shi Q., Zhang W.L., Chen B., Wang Q.S., Zhao X.Y., Chen J.J., Shu Y.X., Cheng N., Feng X.J., Ji J.G., Shen P.P.*, Caspase-1 cleaves PPARγ for potentiating the pro-tumor action of TAMs, Nature Communications, 2017,8:766.doi: 10.1038/s41467-017-00523-6.
2. Feng X.J., Yu W., Li X.D., Zhou F.F., Zhang W.L., Shen Q., Li J.X., Zhang C., Shen P.P.*, Apigenin, a modulator of PPARγ, attenuates HFD-induced NAFLD by regulating hepatocyte lipid metabolism and oxidative stress via Nrf2 activation, Biochemical Pharmacology, 2017, 136:136-149.
3. Niu Z.Y., Huang Y.H., Chen Y.J., Chen B., Wu Y.Y., Zhang C., Shen P.P.*, Caspase-1 promotes monocyte/macrophage differentiation by repressing PPARγ, FEBS Journal,2017,284:568–585.
4. Sun T.Z.*, Li X.D., Shen P.P.*, Modeling amplified p53 responses under DNA-PK inhibition in DNA damage response. Oncotarget, 2017,8(10):17105-17114.
5. Feng X.J., Weng D., Zhou F.F., Young D. Owen, Qin H.H., Zhao J.F., Yu W., Huang Y.H., Chen J.J., Fu H.J., Yang N.F., Chen D.H., Li J.X., Tan R.X., Shen P.P.*, Activation of PPARγ by a natural flavonoid modulator, apigenin ameliorates obesity-related inflammation via regulation of macrophage polarization, EbioMedicine 2016(9): 61–76
6. Yao Y.F., Shi Q., Chen B., Wang Q.S., Li X.D., Li L., Huang Y.H., Ji J.G., Shen P.P.*, Identification of Caspase-6 as a New Regulator of Alternatively Activated Macrophages, Journal of Biological Chemistry, 2016, 291(33):17450-17466
7. Shu Y.X. Lu Y., Pang X.J., Zheng W., Huang Y.H., Li J.H., Ji J.G., Zhang C., Shen P.P.*Phosphorylation of PPARγat Ser84 promotes glycolysis and cell proliferation in hepatocellular carcinoma by targeting PFKFB4, Oncotarget, 2016, DOI: 10.18632/oncotarget.12764
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9. Lu Y., Zhang Y., Li L., Feng X.J., Ding S., Zheng W., Li J.X., Shen P.P.*. TAB1: a target of triptolide in macrophages, Chemistry & Biology, 2014, 21(2):246-256.
10. Feng X.J., Qin H.H., Shi Q., Zhang Y., Zhou F.F., Wu H.C., Ding S., Niu Z.Y., Lu Y., Shen P.P.*. Chrysin Attenuates Inflammation by Regulating M1/M2 Status via Activating PPARgamma. Biochemical Pharmacology, 2014, 89: 503-514.

Group members

Group leader

Pingping Shen

Graduate students

Jun Cui
Ying Liu
Qian Shi
Ting Chen
Xiaofeng Bao
Yuanyuan Su
Yuanyuan Wu
Jiafa Xu
Yinnai Wei
Haocheng Wu
Weiwei Yang
Tingzhe Sun
Xiaojuan Pang

Students in lab

Wentong Fang
Yuncheng Bei
Nan Cheng
Bei Liu
Wenlong Zhang
Nanfei Yang
Yuxin Shu

Teachers

Yahong Huang
Yan Lu

Technician

Wei Zheng

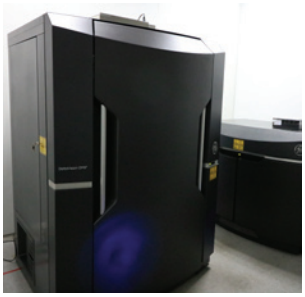
Core Facilities at Model Animal Research Center have been set up for over one year since it provided service last spring. The major mission of Core Facilities is to provide cutting edge resources to our research community, enabling them to make meaningful advance in biological and biomedical research.

Equipped with state-of-the-art facilities, our goal is to offer the highest quality of scientific technology in rapid turn-round time, while operating in a cost-effective manner. To achieve such aims, Core Facilities provides expertise in applications, technology testing and development, educational workshops, and training and consultation to the MARC's research community. Instruments browsing and booking are based on online system which requires registration. Only trained and authorized investigators in MARC have right to use instruments in Core Facilities. Routine group trainings are held 2-3 times per semester and individual personal training can also be provided for special requirement. Core Facilities also set group chat in Wechat to promptly response to users' questions and solve problem. Core also provide services to external investigators at both academic institutions and commercial enterprises, and so far we served over 10 external collaborators.

The Core Facilities of MARC provide 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



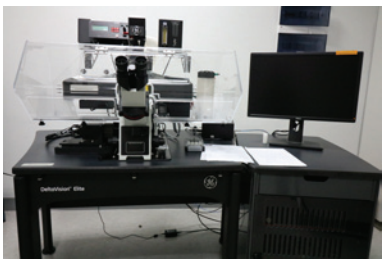
DeltaVision OMX platform offers super resolution imaging using 3D structured illumination (3D-SIM), which allows precise visualization and measurement of features that are below the diffraction limit. 3D-SIM projects a structured light pattern onto the sample. The illumination pattern interacts with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

Feature highlights

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

► GE DeltaVision Elite

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



Feature highlights

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

► Zeiss LSM 880

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

Feature highlights

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

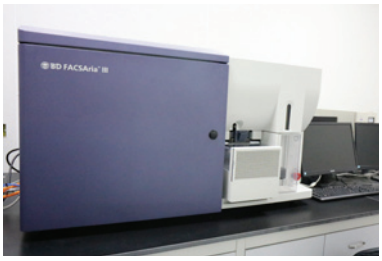
Flow cytometry core

► BD FACS LSRFortessa

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

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

► BD FACSAaria III



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

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

Proteomics core and metabonomics core

► Agilent 6550 iFunnel Q-TOF

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

- METLIN Personal Metabolite Database Software

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

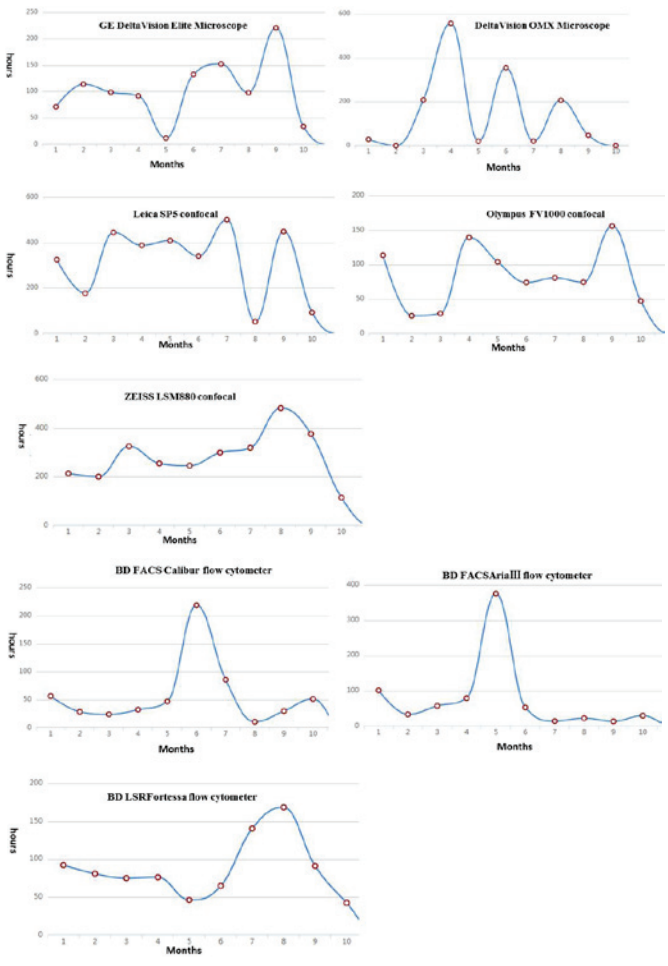
- Spectrum Mill

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

- Mass Profiler Professional Software

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

The use of most popular and busy facilities is summarized in figures. Most of equipments are fully used and could satisfy users' demand. We'll try our best to provide better support in technologies and experimental design to the investigators within or outside MARC. It will enhance effective usage of resources and maximize the use of expensive instruments required for these advanced technology platforms. It is expected to further promote and facilitate multi-disciplinary research studies and collaboration among the research community.

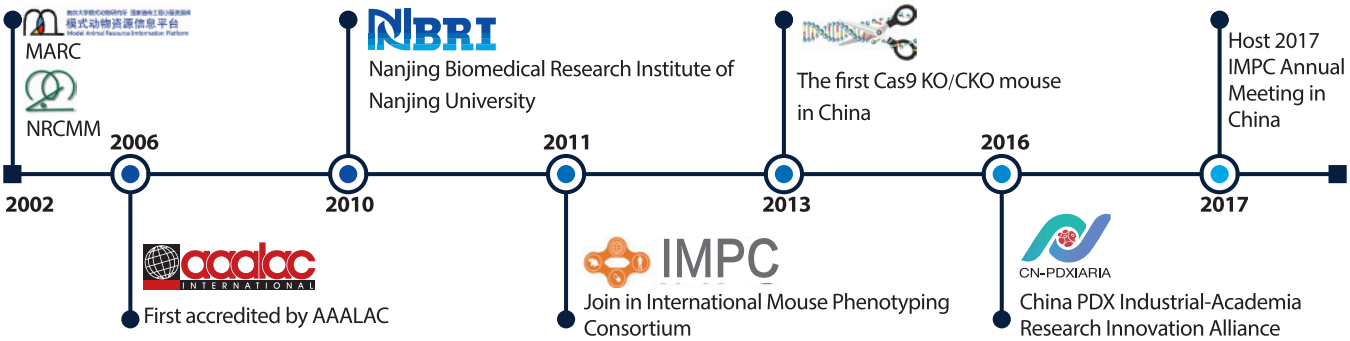


Nanjing Biomedical Research Institute of Nanjing University

Nanjing Biomedical Research Institute of Nanjing University (NBRI) is a strategical emerging industrial platform established by Nanjing University and Nanjing High-tech Area. NBRI focuses on "expanding independent resources through creation and innovation, fostering emerging platforms through talent aggregation, driving industrial development with high and new technologies and guiding resource services with social demand", and is opening a new world of laboratory animal science in China.

NBRI hosts one of the best animal facilities for mouse models in the world, this facility has been accredited by AAALAC International since 2006 and has the 150m^2 Diagnostic Lab with PEP Certification by ICLAS.

NBRI is identified as the support unit of "National Genetic Engineering Mouse Resources Library" by the Ministry of Science and Technology. The Independent researched immunodeficient mice NCG is the first experimental model animal of China moving forward to international market, we have cooperated with the biggest model animal supplier in the USA Charles River, to carry out marketing in Europe and America.



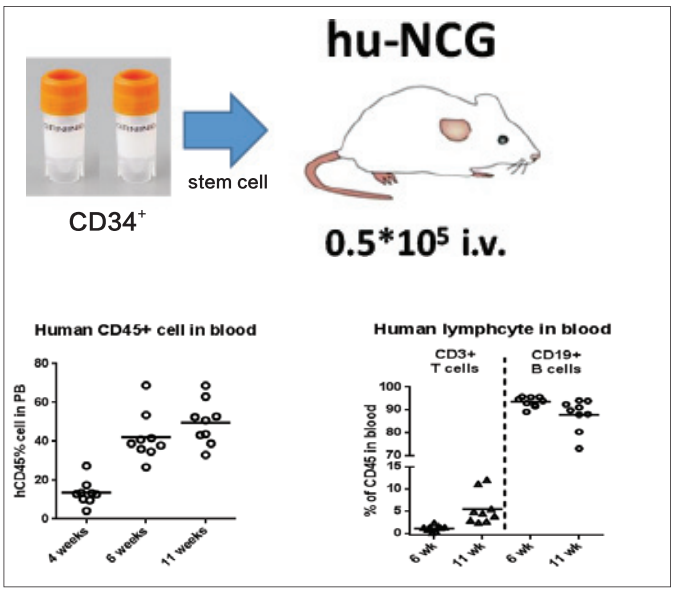
2017 IMPC ANNUAL MEETING & DISEASE MODEL RESOURCE APPLICATION FORUM

2017 Highlights

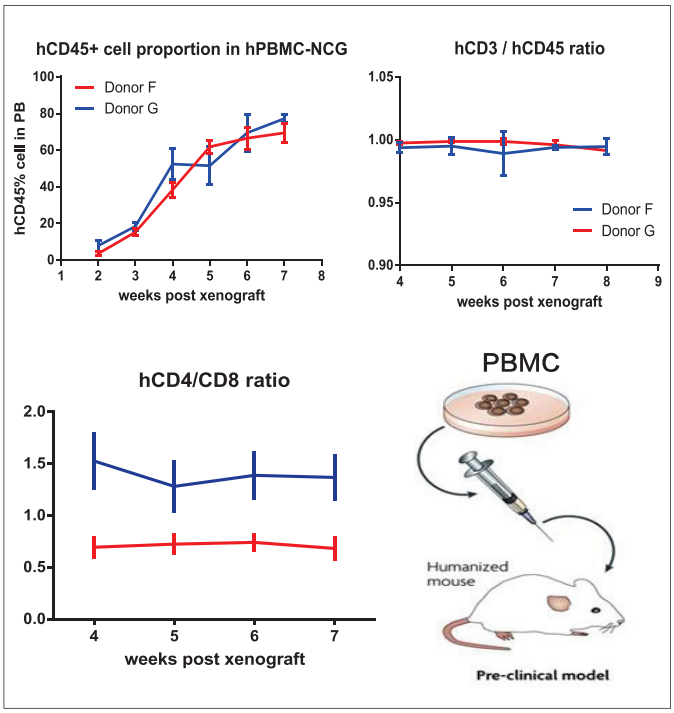
Hu-NCG

Hu-NCG, expressing a human immune system, is one of the most ideal models for testing cancer immunotherapies. Given the recent success of immune checkpoint inhibitors in the treatment of cancer, Hu-NCG efficacy is the future trend for intense research and preclinical therapeutic development.

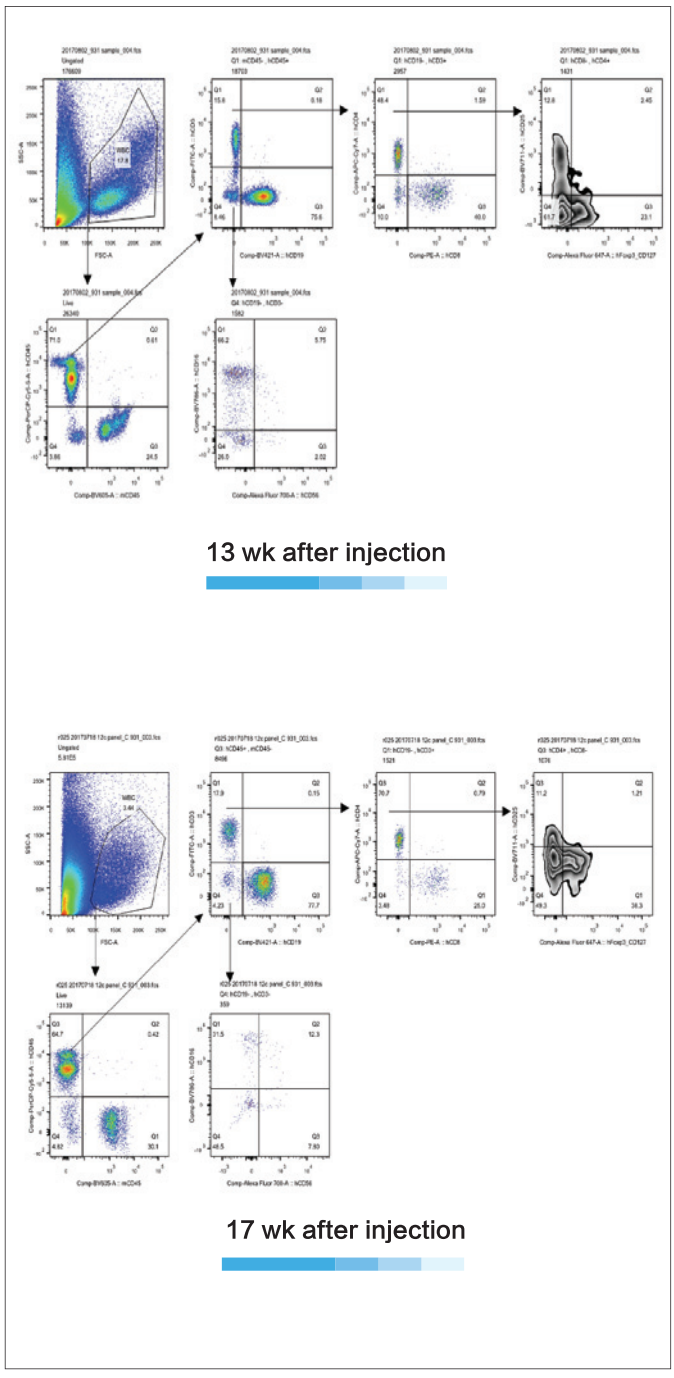
▶ Humanized HSC-NCG mice constructed CD34$^+$-hu-NCG



▶ Humanized PBMC-NCG mice construction



▶ Human cell Subpopulation in peripheral Blood



Model mouse supply

Mouse Model	Strain	Characteristic
Humanized mouse Model	B6JNju-Human OX40^{em1Cin}/Nju	Screening OX40 agonists
	BALB/c-hPDCD1^{em1Cin}/Nju	Screening tumor therapy drug
	B6/JNju-hPDCD1^{em1Cin}/Nju	Screening tumor therapy drug
	BALB/c-Tg(CD3E BAC)/Nju	Screening bispecific antibody
	NCG-HLA-A2.1/Nju	Hematopoietic stem cell transplantation
Immunodeficiency Model	NOD-Prkdc^{em26}Il2rg^{em26}/Nju	Severe immunodeficiency mouse
	Hairless NCG	Easy to observe tumor growth
	Balb/cJNju-Foxn1^{nu}/Nju	Nude mouse (lack of T cell)
	B6.129S7-Rag1^{tm1}/Nju	Lack of T cell and B cell
	NOD-Prkdc^{em26Cd}/Nju	Lack of T, B cell and lymphocyte
Diabetes Model	BKS-Lepr^{em2Cd}/Nju	After 8W, blood glucose continues to rise
	B6/JNju-Lep^{em1Cd}/Nju	Obesity research
	C57BL/6JNju DIO	Diet induced obesity
Atherosclerosis Model	B6/JNju-Apoe^{em1Cd}/Nju	At 3 months, fat grain can be found in mouse's neighboring arteries, the body got damaged and the injury increasing with the growth of age and reduction of lipid
	B6/JNju-Ldlr^{em1Cd}/Nju	Serum cholesterol level of homozygous mouse can attain about 200-400 mg/dl, when the mouse is fed by high-fat diet (>2000 mg/dl)
Tumor Model	B6/JNju-Apc^{MinC}/Nju	When fed by high-fat diet, heterozygous mouse occurs intestinal tumor in 100%

STAR mouse

BALB/c-hPDCD1^{em1Cin}/Nju

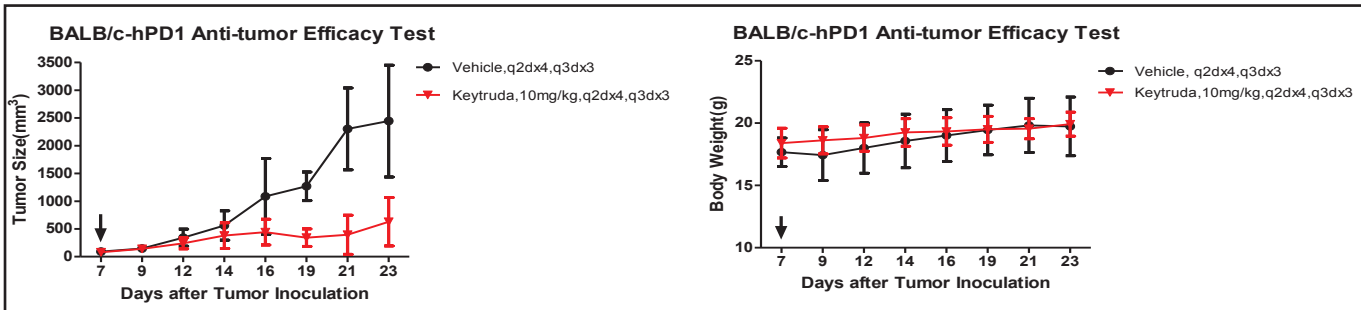
■ Expression of PD1 in humanized mouse physiologically ,comparable to WT.

■ Many tumor cell lines are derived from BALB/c, which means that BALB/c-hPD1 is suitable for relatively more types of tumor efficacy testing.

■ Syngeneic tumor model：CT26、4T1、Madison109 etc.

■ Ideal model for evaluation of immune checkpoint PD1 inhibitor, combinatorial test with other molecules.

■ Tumor efficacy experiment



△The BALB/c-hpd1 mice are homozygote inoculated with CT26 cell line and the PD1 targeted antibody (Keytruda) is effective in inhibiting tumor growth

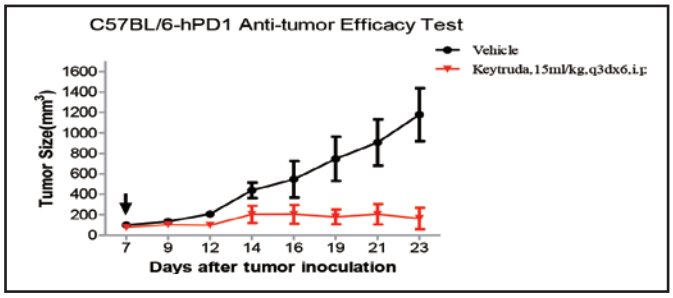
C57BL/6Nju-hPDCD1^{em1Cin}/Nju

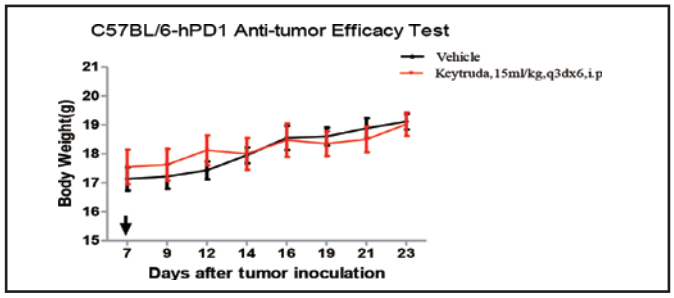
■ Expression of PD1 in humanized mouse physiologically ,comparable to WT

■ Syngeneic tumor model：Mc38、B16-F10、Pan02 etc.

■ Ideal model for evaluation of immune checkpoint PD1 inhibitor, combinatorial test with other molecules.

■ PD1 Targeting Antibody anti-tumor Validation





SD-Mdr1a^{em1Cd}Mdr1b^{em6Cd}/Nju

■ Carries a disruption of multi-drug resistance genes Abcb1a and Abcb1b encoding p-glycoprotein 3 and p-glycoprotein 1 respectively.

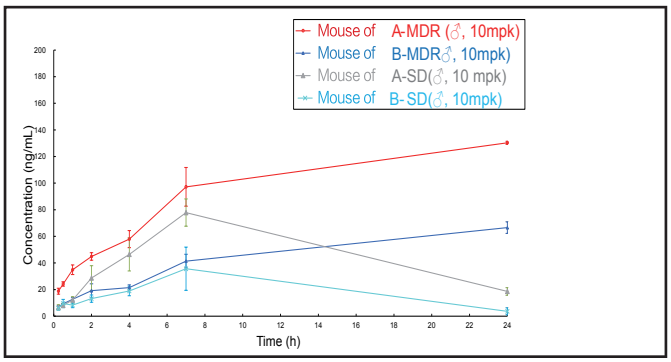
■ P-glycoprotein plays a critical role in efflux for both brain and liver.

■ Homozygous null Mdr1a rats display increased exposure to CNS drugs in the brain, as well as increased bioavailability in the plasma for P-gp-specific substrates.

■ Useful in a wide range of central nervous system research including neurotoxicology, drug transport, oral bioavailability and multi-drug resistance studies.

■ Susceptible to developing a severe, spontaneous intestinal inflammation phenotypically similiar to human inflammatory bowel disease (IBD).

SD-Mdr1a^{em7Cd}/Nju



B6/JNju-OX40^{em2Cd}/Nju

Expressing bioactive hOX40 protein physiologically.

Syngeneic tumor cell line: MC38, B6F10, etc.

Ideal model for antitumor efficacy evaluation of hOX40 agonists and inhibitor.

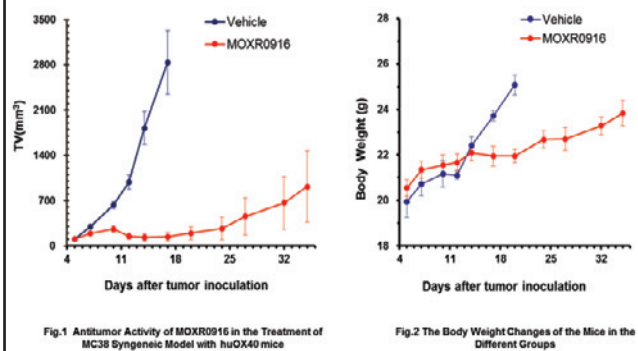


Fig.1 Antitumor Activity of MOXR0916 in the Treatment of MC38 Syngeneic Model with huOX40 mice

Fig.2 The Body Weight Changes of the Mice in the Different Groups

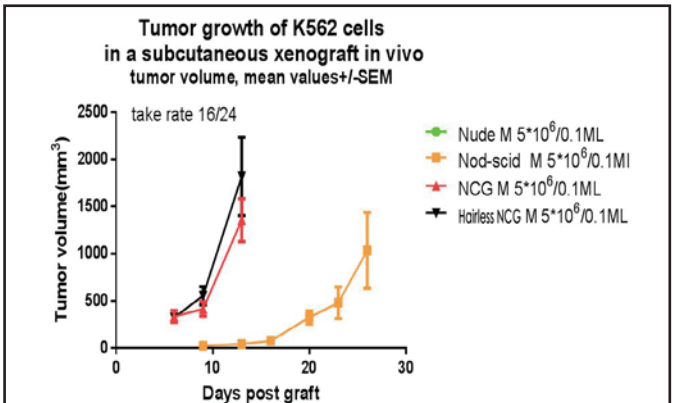
▲MOXR0916 can recognize hOX40 targets and effectively inhibit tumor growth

Hairless NCG

Hairless，ideal for tumor biology and xenograft research.

Severely immuno-deficient (T、B and NK cells deficiency) comparable to NCG.

Foxn1 intact, permits thymus reconstruction and is advantageous for HSCs reconstitution.



▲K562 can form tumor on hairless NCG and have the same successful rate on NCG

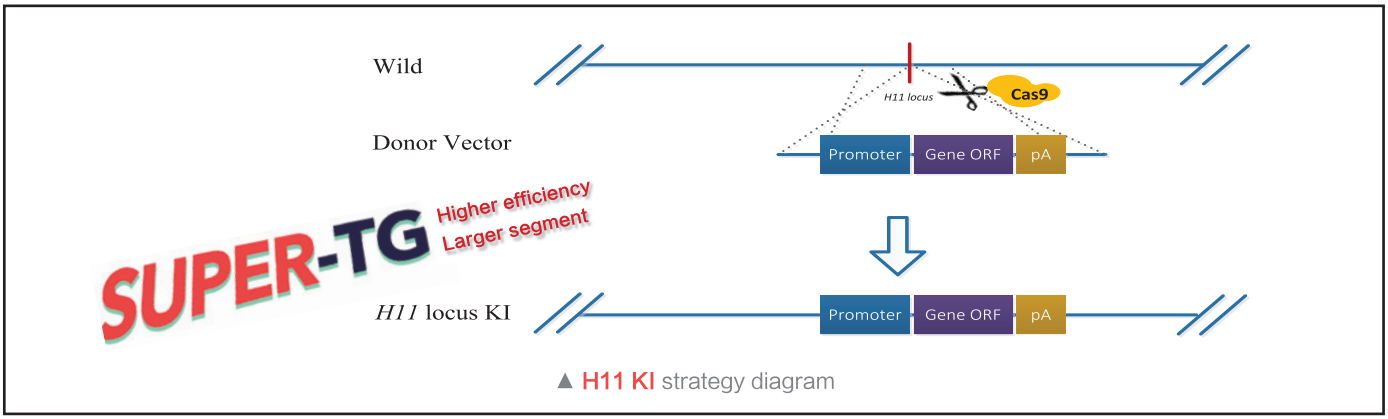
Transgenic/Knockout Mice Services

In 2017, more than 450 projects of TG/KO/CKO/KI are completed in NBRI.

■ CRISPR/Cas9 technology

Advantages:

High-quality	Cas9 KO success rate is 100%, Cas9 cKO / KI success rate is 95%.
Zero off-target	NBRI's leading-edge technology reduces the off-target effect, the project off-target rate until now is 0%.
Multi-modification	Target gene multiple sites or multiple genes knocked out simultaneously, successfully operate 23Mbps long targeting fragment.
Multi-Background	C57BL / 6J, C57BL / 6N, NOD / LTJ, BABL / C, etc.



▲ H11 KI strategy diagram

■ Traditional ES technology

Advantages:

Self-developed Del-itself technique	save 3 months modeling time
Develop BAV targeting technology	300kb segment knoked-in achievement
Rich experience in model preparation	The first CKO model of China was successfully produced in 2005.Until now, more than 700 cases of ES target model have been successfully completed.
Common use background	129S6/SvEvTac, C57BL/6N

■ Transgenic technology

Advantages:

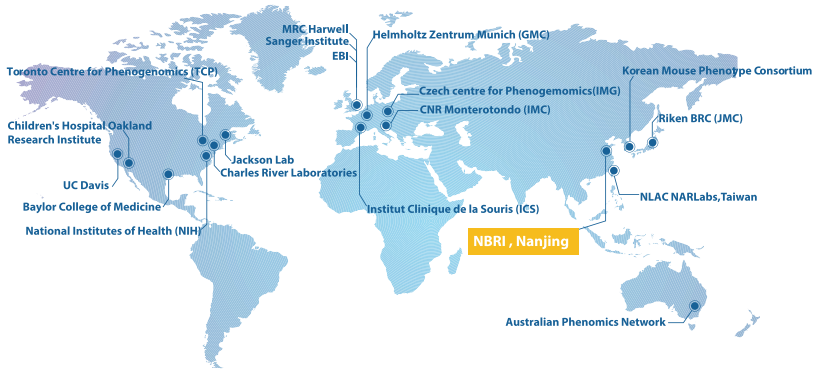
High success rate, 100%;

BAC transgenic technology can achieve the transgenic model of over 300kb;

Multi-background selection: C57BL/6J, NOD/LTJ, FVB, BABL/CJ, C57BL/ 6N, etc.

Phenotyping Service

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



AMPC Asian Mouse Phenotyping Consortium

IMPC INTERNATIONAL MOUSE PHENOTYPING CONSORTIUM

Mouse phenotype screening service

Metabolism
Metabolic cages
Glucose tolerance test

Angiocarpy
Echocardiographic
Electrocardiogram (ecg)

Bone
Bone mineral density
X-ray

Blood and immunity
Both FACS, ELISA
Routine blood

Neurobehavioral
PPI, SHIRPA
Water maze, opening

Growth
Morphological detection
Embryos detection

Perception
Ophthalmorphic observation
Hearing reaction

Tumor platform

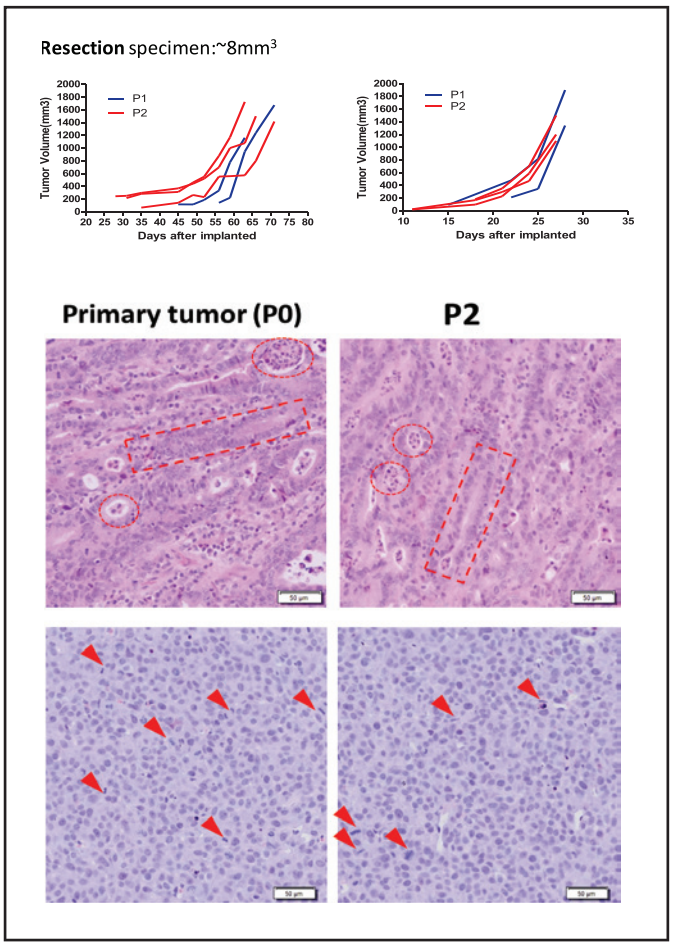
NBRI has successfully established a variety of PDX models including gastrointestinal cancer, liver cancer, pancreatic cancer, lung cancer, myeloma, sarcoma and hematological cancer, these models have been used in the study of disease mechanism, efficacy test and clinical treatment control. The humanized mouse models have been developed to test the efficacy of immunomodulatory drugs such as PD-1 / PD-L1 antibodies, CTLA4 antibodies and the efficacy of cell therapies such as CAR-T. NBRI has also built HIV virus infection model for related research based on it.

PDX modeling and drug screening evaluation

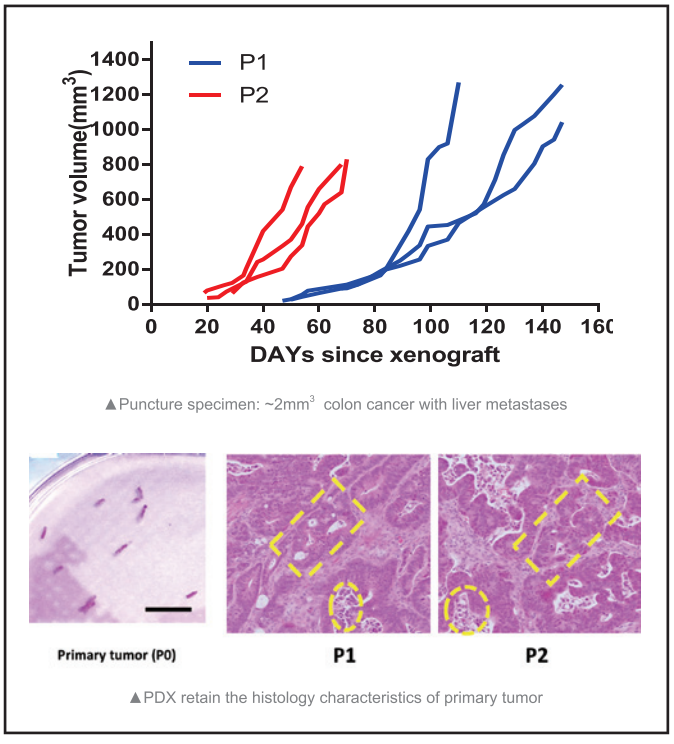
PBMC, HSC and other humanized mice producing

Tumor immunity checkpoint humanized drug screening

1、PDX modeling (surgical sample)



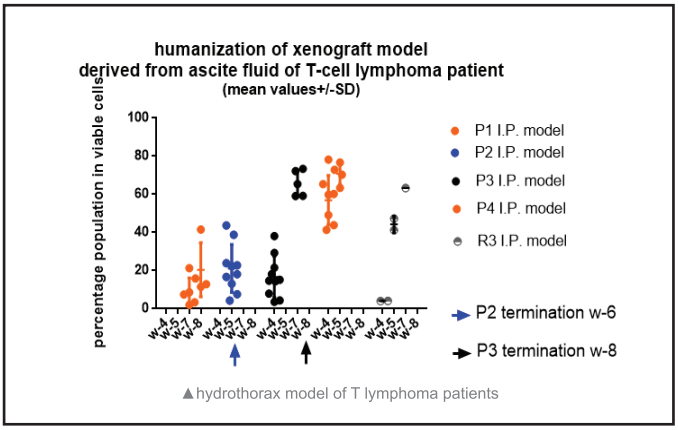
2、PDX modeling (biopsy samples)



▲Puncture specimen: ~2mm^3 colon cancer with liver metastases

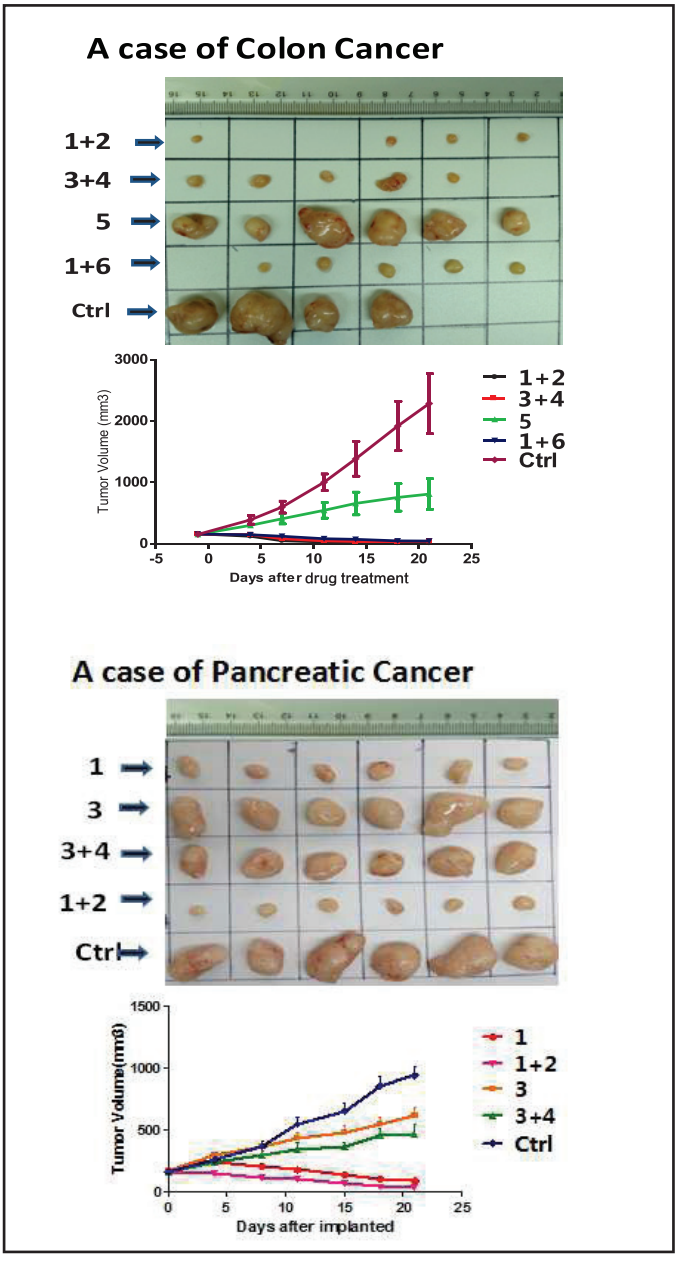
▲PDX retain the histology characteristics of primary tumor

3、PDX modeling (Pleural effusion sample)



▲hydrothorax model of T lymphoma patients

4、PDX pharmacodynamics experiment

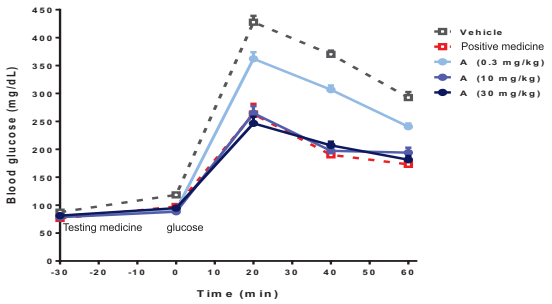


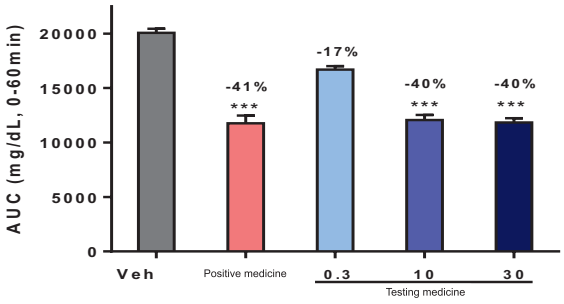
Metabolic platform

1. Acute glucose tolerance test

■ obesity ■ blood fat ■ complication
■ diabetes

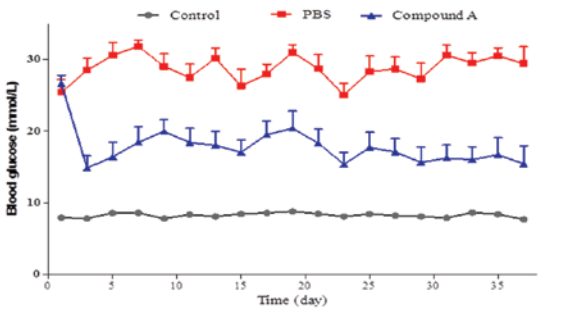
28 days subchronic efficacy test; Random blood glucose and fasting glucose test; Glucose tolerance test (GTT); Islet tolerance test (ITT);Blood biochemical and cytokines (TNF－a, il-6, c-peptide, Glucagon,Leptin, insulin, etc) test.



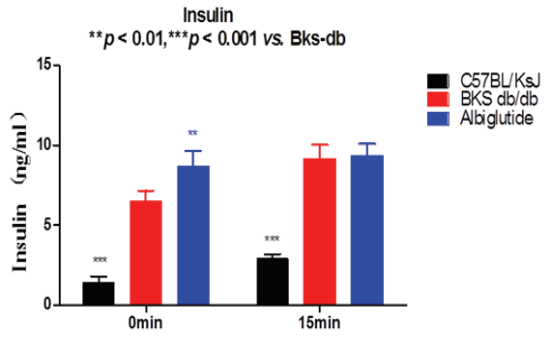


N=5，***p <0.001 vs vehicle，one way ANOVA followed by Dunnett's test

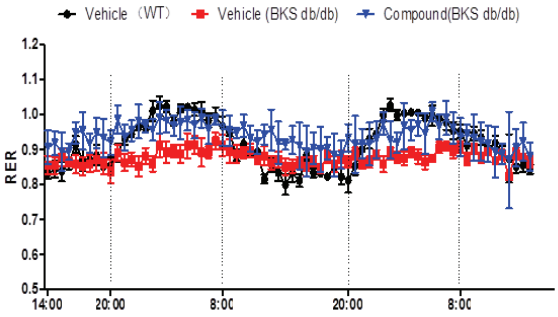
2. Type II diabetes model pesticide effect experiment



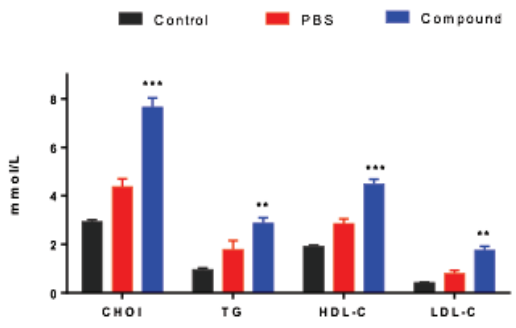
▲ hypoglycemic effect of BKS-db diabetes mice after drug intervention



▲ Albiglutide raising the levels of serum insulin in BKS-db mice



▲ The change of respiratory exchange rate explained the change of respiratory substrate of BKS db/db by test samples



▲ Result of blood biochemistry

Breeding and cryopreservation

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

In 2017, NRCMM has finished cryopreservations of 585 strains. At the same time, NBRI provides customers with services of genotyping and maintaining live colonies. In addition, NBRI provides custom breeding services, such as strain rederivation, fast expansions via IVF and strain rescue, in order to meet specific requirments.

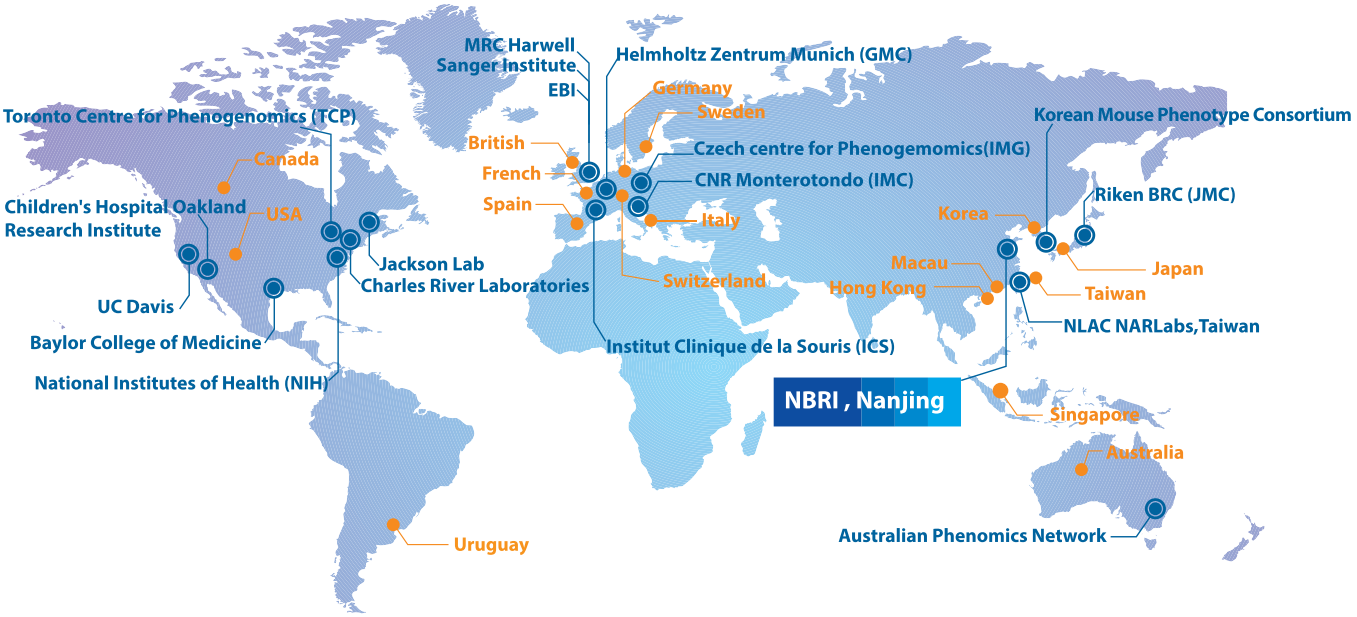
Animal health monitoring program and veterinary severice

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

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

Import and export service

In the recent 15 years, NBRI has imported more than 700 strains of live mouse and frozen material from 15 countries and exported more than 100 strains to 17 countries.



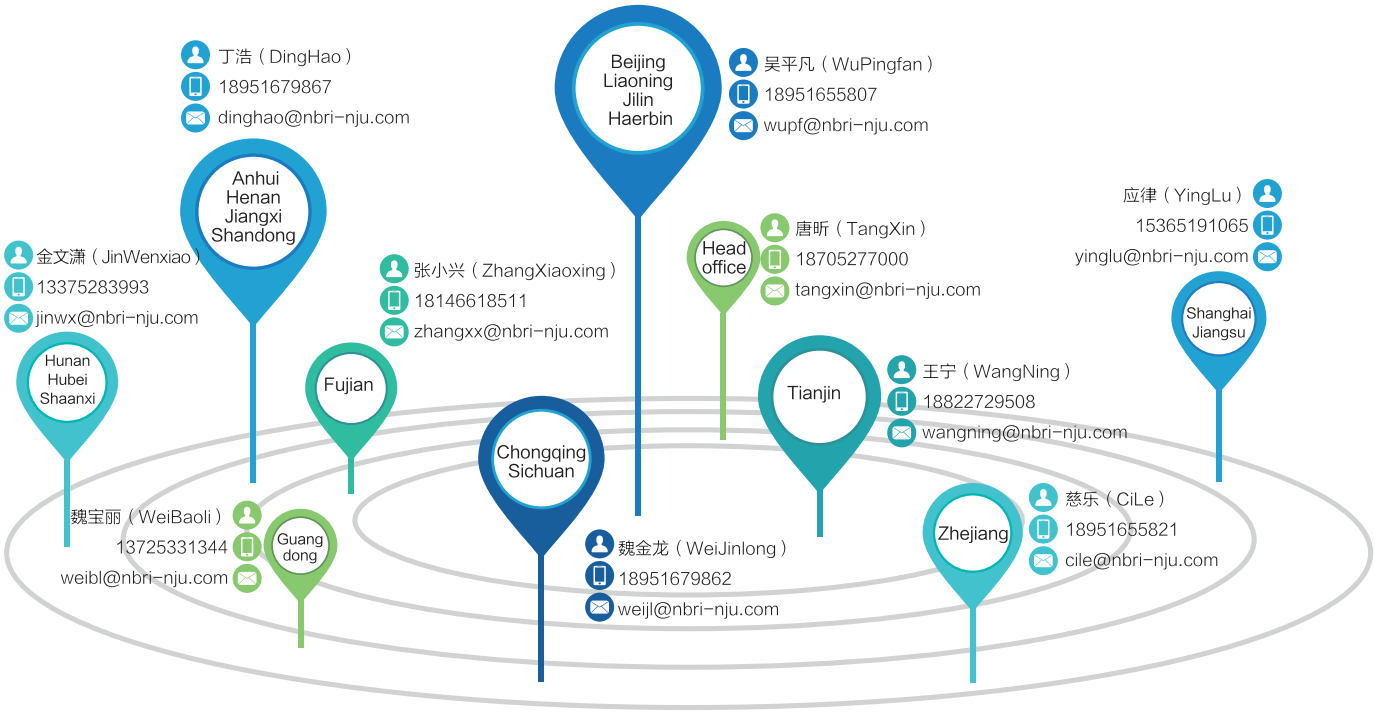
Covering areas and organizations of one stop import and export service of NBRI

Contact Us

南京大学—南京生物医药研究院
Nanjing Biomedical Research Institute of Nanjing University

Tel：+86 025-58641559　　Fax：+86 025-58641551　　Website：www.nbri-nju.com
Email：services@nbri-nju.com　　Add：12 Xue-Fu Road, Nanjing 210061, P.R.China

Sales department tel



Mouse platform Tel

+86 025-58641520　+86 025-58641559　+86 025-58641580

Publications in 2017

1.	Zhou Z, Yao X, Pang S, Chen P, Jiang W, Shan Z, Zhang Q (2017) The deubiquitinase UCHL5/UCH37 positively regulates Hedgehog signaling by deubiquitinating Smoothened. J Mol Cell Biol [Epub ahead of print]
2.	Yang L, Xue Y, Liu J, Zhuang J, Shen L, Shen B, Yan J, Guo H (2017) Long noncoding RNA ASAP1-IT1 promotes cancer stemness and predicts a poor prognosis in patients with bladder cancer. Neoplasma [Epub ahead of print]
3.	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 (2017) A regulatory circuit HP1gamma/miR-451a/c-Myc promotes prostate cancer progression. Oncogene [Epub ahead of print]
4.	Zhang CX, Zhang Q, Xie YY, He XY, Xiang C, Hou XS, Zhou Y, Chen L, Zhang GX, Liu G (2017) Mouse Double Minute 2 Actively Suppresses p53 Activity in Oocytes during Mouse Folliculogenesis. American Journal of Pathology 187: 339-351
5.	Feng XJ, Yu W, Li XD, Zhou FF, Zhang WL, Shen Q, Li JX, Zhang C, Shen PP (2017) Apigenin, a modulator of PPAR gamma, attenuates HFD-induced NAFLD by regulating hepatocyte lipid metabolism and oxidative stress via Nrf2 activation. Biochemical Pharmacology 136: 136-149
6.	Li QQ, Dai BY, Yao Y, Song K, Chen DY, Jiang Q (2017) Chronic Kidney Dysfunction Can Increase the Risk of Deep Vein Thrombosis after Total Hip and Knee Arthroplasty. BioMed research international: 8260487
7.	Kong F, Wang M, Huang X, Yue Q, Wei X, Dou X, Peng X, Jia Y, Zheng K, Wu T, Yan J, Li J (2017) Differential regulation of spermatogenic process by Lkb1 isoforms in mouse testis. Cell Death & Disease 8: e3121
8.	Wang H, Zhang BF, Zhang TT, Wang L, Zou XY, Xu Y, Chen L, Chen GQ (2017) Impaired Spatial Learning is Associated with Disrupted Integrity of the White Matter in Akt3 Knockout Mice. Cns Neuroscience & Therapeutics 23: 99-102
9.	Liu TT, Ye XL, Zhang JP, Yu TT, Cheng SS, Zou XC, Xu Y, Chen GQ, Yin ZY (2017) Increased adult neurogenesis associated with reactive astrocytosis occurs prior to neuron loss in a mouse model of neurodegenerative disease. Cns Neuroscience & Therapeutics 23: 885-893
10.	Xiao Q, Zhang GX, Wang HJ, Chen L, Lu SS, Pan DJ, Liu G, Yang ZZ (2017) A p53-based genetic tracing system to follow postnatal cardiomyocyte expansion in heart regeneration. Development 144: 580-589
11.	Hou NN, Yang YX, Scott IC, Lou X (2017) The Sec domain protein Scfd1 facilitates trafficking of ECM components during chondrogenesis. Developmental Biology 421: 8-15
12.	Chen QL, Rong P, Xu DJ, Zhu SS, Chen L, Xie BX, Du Q, Quan C, Sheng Y, Zhao TJ, Li P, Wang HY, Chen S (2017) Rab8a Deficiency in Skeletal Muscle Causes Hyperlipidemia and Hepatosteatosis by Impairing Muscle Lipid Uptake and Storage. Diabetes 66: 2387-2399
13.	Chen QL, Xie BX, Zhu SS, Rong P, Sheng Y, Ducommun S, Chen L, Quan C, Li M, Sakamoto K, MacKintosh C, Chen SA, Wang HY (2017) A Tbc1d1 (Ser231Ala)-knockin mutation partially impairs AICAR- but not exercise-induced muscle glucose uptake in mice. Diabetologia 60: 336-345
14.	Niu Y, Dai ZH, Liu WX, Zhang C, Yang YR, Guo ZZ, Li XY, Xu CC, Huang XH, Wang YC, Shi YS, Liu JJ (2017) Ablation of SNX6 leads to defects in synaptic function of CA1 pyramidal neurons and spatial memory. Elife 6: e20991
15.	Song AY, Jiang SJ, Wang QH, Zou JH, Lin ZY, Gao X (2017) JMJD3 Is Crucial for the Female AVPV RIP-Cre Neuron-Controlled Kisspeptin-Estrogen Feedback Loop and Reproductive Function. Endocrinology 158: 1798-1811
16.	Niu ZY, Tang JJ, Zhang WL, Chen YJ, Huang YH, Chen B, Li JH, Shen PP (2017) Caspase-1 promotes monocyte-macrophage differentiation by repressing PPAR gamma. Febs Journal 284: 568-585
17.	Chen L, Chen QL, Rong P, Wang HY, Chen S (2017) The energy sensing LKB1-AMPK alpha 1 pathway regulates IGF1 secretion and consequent activation of the IGF1R-PKB pathway in primary hepatocytes. Febs Journal 284: 2096-2109
18.	Xu CY, Yu LJ, Hou JX, Jackson RJ, Wang H, Huang CL, Liu TT, Wang QH, Zou XC, Morris RG, Spires-Jones TL, Yang ZZ, Yin ZY, Xu Y, Chen GQ (2017) Conditional Deletion of PDK1 in the Forebrain Causes Neuron Loss and Increased Apoptosis during Cortical Development. Frontiers in Cellular Neuroscience 11: 330
19.	Gong J, Wang X, Zhu CW, Dong XH, Zhang QX, Wang XN, Duan XC, Qian FP, Shi YW, Gao Y, Zhao QS, Chai RJ, Liu D (2017) Insm1a Regulates Motor Neuron Development in Zebrafish. Frontiers in Molecular Neuroscience 10: 274
20.	Dong XH, Li JY, He LQQ, Gu C, Jia WS, Yue YY, Li J, Zhang QX, Chu LL, Zhao QS (2017) Zebrafish Znfl1 proteins control the expression of hoxb1b gene in the posterior neuroectoderm by acting upstream of pou5f3 and sall4 genes. Journal of Biological Chemistry 292: 13045-13055
21.	Zhang Z, Lei AH, Xu LY, Chen L, Chen YL, Zhang XN, Gao Y, Yang XL, Zhang M, Cao Y (2017) Similarity in gene-regulatory networks suggests that cancer cells share characteristics of embryonic neural cells. Journal of Biological Chemistry 292: 12842-12859
22.	Zhao WK, Tong H, Huang YK, Yan Y, Teng HJ, Xia Y, Jiang Q, Qin JZ (2017) Essential Role for Polycomb Group Protein Pcgf6 in Embryonic Stem Cell Maintenance and a Noncanonical Polycomb Repressive Complex 1 (PRC1) Integrity. Journal of Biological Chemistry 292: 2773-2784
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24.	Xing LJ, An Y, Shi GS, Yan J, Xie PC, Qu ZP, Zhang ZH, Liu ZW, Pan DJ, Xu Y (2017) Correlated evolution between CK1 delta Protein and the Serine-rich Motif Contributes to Regulating the Mammalian Circadian Clock. Journal of Biological Chemistry 292: 161-171

25.	Xu L, Liu XY, Sheng N, Oo KS, Liang JX, Chionh YH, Xu J, Ye FZ, Gao YG, Dedon PC, Fu XY (2017) Three distinct 3-methylcytidine (m(3)C) methyltransferases modify tRNA and mRNA in mice and humans. Journal of Biological Chemistry 292: 14695-14703
26.	Xue CB, Ren HC, Zhu H, Gu XK, Guo Q, Zhou Y, Huang J, Wang SR, Zha GB, Gu JH, Yang YM, Gu Y, Gu XS (2017) Bone marrow mesenchymal stem cell-derived acellular matrix-coated chitosan/silk scaffolds for neural tissue regeneration. Journal of Materials Chemistry B 5: 1246-1257
27.	Shi TS, Lu K, Shen SY, Tang QL, Zhang KJ, Zhu XB, Shi Y, Liu XL, Teng HJ, Li CJ, Xue B, Jiang Q (2017) Fenofibrate decreases the bone quality by down regulating Runx2 in high-fat-diet induced Type 2 diabetes mellitus mouse model. Lipids in Health and Disease 16: 201
28.	Wan GQ, Corfas G (2017) Transient auditory nerve demyelination as a new mechanism for hidden hearing loss. Nature Communications 8: 14487
29.	Niu ZY, Shi Q, Zhang WL, Shu YX, Yang NF, Chen B, Wang QS, Zhao XY, Chen JJ, Cheng N, Feng XJ, Hua ZC, Ji JG, Shen PP (2017) Caspase-1 cleaves PPAR gamma for potentiating the pro-tumor action of TAMs. Nature Communications 8: 766
30.	Shen L, Zhang F, Huang RM, Yan J, Shen B (2017) Honokiol inhibits bladder cancer cell invasion through repressing SRC-3 expression and epithelial-mesenchymal transition. Oncology Letters 14: 4294-4300
31.	Yuan XW, Sun XT, Shi XL, Wang H, Wu GY, Jiang CP, Yu DC, Zhang WW, Xue B, Ding YT (2017) USP39 promotes colorectal cancer growth and metastasis through the Wnt/beta-catenin pathway. Oncology Reports 37: 2398-2404
32.	Sun TZ, Li XD, Shen PP (2017) Modeling amplified p53 responses under DNA-PK inhibition in DNA damage response. Oncotarget 8: 17105-17114
33.	Wang QH, Jiang SJ, Song AY, Hou SY, Wu QF, Qi LJ, Gao X (2017) HOXD-AS1 functions as an oncogenic ceRNA to promote NSCLC cell progression by sequestering miR-147a. Oncotargets and Therapy 10: 4753-4763
34.	Zhu KA, Liu MH, Fu Z, Zhou Z, Kong Y, Liang HW, Lin ZG, Luo J, Zheng HQ, Wan P, Zhang JF, Zen K, Chen J, Hu FL, Zhang CY, Ren J, Chen X (2017) Plant microRNAs in larval food regulate honeybee caste development. Plos Genetics 13: e1006946
35.	Jiang C, Diao F, Sang YJ, Xu N, Zhu RL, Wang XX, Chen Z, Tao WW, Yao B, Sun HX, Huang XX, Xue B, Li CJ (2017) 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 13: e1006535
36.	Zhang GX, Xie YY, Zhou Y, Xiang C, Chen L, Zhang CX, Hou XS, Chen J, Zong H, Liu G (2017) p53 pathway is involved in cell competition during mouse embryogenesis. Proceedings of the National Academy of Sciences of the United States of America 114: 498-503
37.	Yan Y, Zhao WK, Huang YK, Tong H, Xia Y, Jiang Q, Qin JZ (2017) Loss of Polycomb Group Protein Pcgf1 Severely Compromises Proper Differentiation of Embryonic Stem Cells. Scientific Reports 7: 46276
38.	Li L, Yu F, Shi JP, Shen S, Teng HJ, Yang JQ, Wang XS, Jiang Q (2017) In situ repair of bone and cartilage defects using 3D scanning and 3D printing. Scientific Reports 7: 9416
39.	Zhou S, Lu WL, Chen L, Ge QT, Chen DY, Xu ZH, Shi DQ, Dai J, Li JX, Ju HX, Cao Y, Qin JZ, Chen S, Teng HJ, Jiang Q (2017) AMPK deficiency in chondrocytes accelerated the progression of instability-induced and ageing-associated osteoarthritis in adult mice. Scientific Reports 7: 43245
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41.	Chen J, Du YN, He XY, Huang XX, Shi YS (2017) A Convenient Cas9-based Conditional Knockout Strategy for Simultaneously Targeting Multiple Genes in Mouse. Scientific Reports 7: 517
42.	Han YY, Huang WW, Liu JK, Liu DD, Cui YY, Huang RM, Jun Y, Lei M (2017) Triptolide Inhibits the AR Signaling Pathway to Suppress the Proliferation of Enzalutamide Resistant Prostate Cancer Cells. Theranostics 7: 1914-1927
43.	Zhuang JL, Shen L, Yang L, Huang XJ, Lu Q, Cui YY, Zheng X, Zhao XZ, Zhang DZ, Huang RM, Guo HQ, Yan J (2017) TGF beta 1 Promotes Gemcitabine Resistance through Regulating the LncRNA-LET/NF90/miR-145 Signaling Axis in Bladder Cancer. Theranostics 7: 3053-3067
44.	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

Seminar in 2017

	Date	Speaker	Title	Unit
1	20170119	Nengyin Sheng	Secrets of the Excitatory Synapse: Lessons from Glutamate Receptors Trafficking	University of California, San Francisco
2	20170330	Guangshou Ou	Mechanisms Underlying Neuroblast Development in C. elegans	Tsinghua University
3	20170406	Li Yu	From autophagosome to migrasome	Tsinghua University
4	20170410	Guoping Shi	Role of IgE in cardiovascular diseases	Harvard Medical School
5	20170427	Chen Zhang	Frontiers in learning and memory	Peking University
6	20170427	Yihong Wan	Novel Regulators of Osteoporosis and Cancer Bone Metastasis	UT Southwestern Medical Center
7	20170508	Shuhai Lin	Mass spectrometry-based metabolomics and tumor metabolism	Shanghai Jiao Tong University
8	20170508	Antonio Vidal-Puig	Adipose tissue expandability and Metabolic Syndrome	MRC Metabolic Disease Unit
9	20170519	Zhixin Wang	Auto-activation of p21-activated protein kinase (PAK)	Tsinghua University
10	20170519	Jiawei Wu	Molecular recognition between kinases and phosphatases in MAPK signaling	Tsinghua University
11	20170525	Min Han	Gut-initiated food evaluation systems that sense specific nutrients to regulate animals' reproductive development, food uptake, and foraging behaviors	University of Colorado
12	20170525	Yohei Niikura	Centromere, kinetochore, and cell division in cancer	Greehey Children's Cancer Research Institute UT Health Science Center San Antonio
13	20170531	Dong Wang	The glance of RNA world - Where, Who, What?	Harbin Medical University
14	20170608	Ping Wang	Protein Ubiquitination and Cell Signaling	TongJi University
15	20170629	Jun Huang	DNA repair and human diseases	Zhejiang University
16	20170921	James T. Stull	Wimps, Heart Failure and Aortic Aneurysms	University of Texas Southwestern Medical Center
17	20171023	William Wisden	How do we sleep	Imperial College London, U.K.
18	20171023	Xiao Yu	Neural circuits underlying sleep-wake regulation	Imperial College London, U.K.
19	20171027	Gabriel Corfas	A new mechanism of receptor tyrosine signaling and its role in brain development	University of Michigan
20	20171030	Jian Zuo	Cochlear hair cell regeneration and protection	St. Jude Children's Research Hospital
21	20171101	Bin Liang	Lipid Metabolism under Stress	Kunming Institute of Zoology,CAS
22	20171103	Kai Ge	Enhancer chromatin modification in differentiation, development and cancer	National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK),
23	20171103	Xiaofeng Qin	Profiling antigenic and immunological status of tumor micro enviroment for guiding cancer immunotherapy	Suzhou Institute of Systems Medicine
24	20171103	Yi Yang	Single cell metabolism imaging and the optical manipulation techniques	East China University of Science and Technology

	Date	Speaker	Title	Unit
25	20171113	Jeffrey Robbins	Proteotoxicity and Heart Disease	Co-Director of the Heart Institute
26	20171115	Chengran Xu	To understand the regulation of cell differentiation during development from the perspective of Omics	Peking University
27	20171124	James (Jim) Woodgett	Use of mouse models to probe the multiple roles of cellular signaling via glycogen synthase kinase-3	University of Toronto
28	2017-12-1	Hongyun Tang	Regulatory role of fatty acids in reproductive development and maintenance of muscle integrity	HHMI and MCDB of CU Boulder
29	2017-12-7	Xiaoyang Dou	Gene molecular regulatory network	Institute of Computing Technology,Chinese Academy of Sciences

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 2017, the following lecture series and courses were given by MARC staff at Nanjing University (unless indicated otherwise).

Information genomics:

Minsheng Zhu

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 PIs in MARC

Frontier of Cell Biology

Jun Huang (Zhejiang University)

Ping Wang (Tongji University)

Min Han (Howard Hughes Medical Institute and University of Colorado at Boulder)

Chen Zhang (Beijing University)

Li Yu (Tsinghua University)

Guangshuo Ou (Tsinghua University)

Mechanism of Development

Jiong Chen

Ying Cao

Zhongzhou Yang

Qingshun Zhao

Medical Genetics (Shanghai Jiaotong University)

Xiang Gao

MARC seminar in Genetics

All PIs in MARC

MARC seminar in Developmental Biology

All PIs in MARC

PhD Theses

MARC students successfully defended the following PhD thsese in 2017

PhD Theses:

Group Qingshun Zhao

Yunyun Yue

The role of FOXC1 in vertebrate cardiogenesis

Group Jianghuai Liu

Yuanyuan Tong

Type I IFN, TAM, CD115, differentiation, cancer

Group Jun Yan

Xiaojing Huang

Heterochromatin protein 1γ overexpression in bladder cancer cells limits response to chemotherapy

Group Xiaosong Gu

Hui Zhu

Molecular mechanisms of Schwann cells promoting peripheral nerve regeneration via tunneling nanotubes and insulin-like growth factor-1

Chengbin Xue

Repair of Peripheral Nerve Defects Using Tissue Engineered Nerve and Molecular Mechanisms underlying Functional Reconstruction

Group Guiquan Chen

Congyu Xu

Forebrain conditional inactivation of PDK1 leads to learning deficit and microcephaly

Group Xingxu Huang

Bian Hu

CRISPR/Cas9 mediated gene editing in primary human T cells and T cells with chimeric antigen receptor targeting CD133

Group Zhenji Gan

Jing Liu

The coordinate control mechanism of skeletal muscle energy metabolism and structural programs

Group Shuai Chen

Bingxian Xie

The Inactivation of RabGAP Function of AS160 Promotes Lysosomal Degradation of GLUT4 and Causes Postprandial Hyperglycemia and Hyperinsulinemia

Liang Chen

Regulating the secretion of IGF1 mediates the progress of metabolic associated diseases

Qiaoli Chen

Rab8a deficiency in skeletal muscle causes hyperlipidemia and hepatosteatosis via impairment of muscle lipid uptake and storage

Group Ying Cao

Zan Zhang

Solid cancer cells can differentiate into neuron-like cells and share a regulatory network with embryonic neural cells

Group Jiong Chen

Zehao Xu

Role of energy metabolism in border cell collective migration

Heng Wang

Apical Complex Molecules Play Essential and Novel Roles in Collective Cell Migration

Group Gen Liu

Yinyin Xie

MitoTimer transgenic mice reveal tightly coupled and metabolically regulated mitochondrial quality control in skeletal muscles

Group Yun Shi

Yanjun Li

Preferential Assembly of Heteromeric GluA1/A2 Receptor is Determined by GluA1 Signal Peptide

Group Minsheng Zhu

Tao Tao

Trio Regulates Neurite Outgrowth through a Coupling Mechanism with a Membrane Trafficking Signaling Regulation

Jie Sun

CPI-17 Regulated Calcium Sensitization of Vascular Smooth Muscles Is Required for Obesity Related Hypertension

Group Chaojun Li

Zhong Chen

Functional study of protein geranylgeranylation in the mouse heart development during mid-gestation

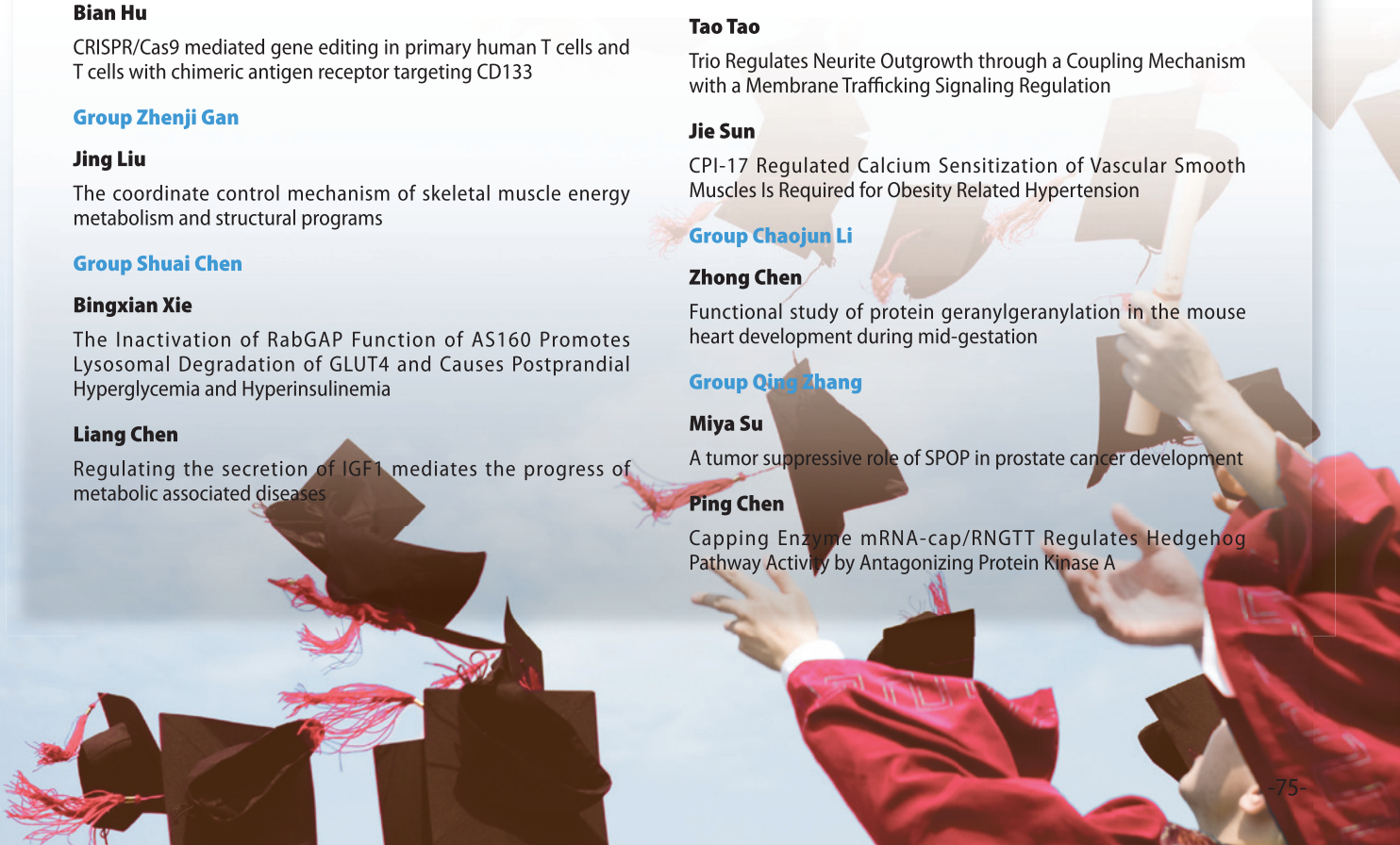
Group Qing Zhang

Miya Su

A tumor suppressive role of SPOP in prostate cancer development

Ping Chen

Capping Enzyme mRNA-cap/RNGTT Regulates Hedgehog Pathway Activity by Antagonizing Protein Kinase A



The 8th China Conference of Development Biology 2017

The 8th China Conference of Development Biology, organized by Model Animal Research Center of Nanjing University, was held on 21-23 March, 2017, in Nanjing, China. This meeting themed with organogenesis and regeneration, sponsored by Chinese Society for Cell Biology (CSCB) and Society for Cell Differentiation and Development of CSCB, was aimed to provide the chance for ideas exchange and cooperation within investigators working in development biology. Dr Minsheng Zhu, Dr Zhongzhou Yang and Dr Xiang Gao were in charge of the conference, and around 60 invited scientists and experts were attended the meeting.

The open talk was given by Dr Xiang Gao, the dean of Nanjing Biomedical Research Institute of Nanjing University. Outstanding leading scientists and well known experts from worldwide shared their knowledge and talked about various aspects related to topic of Stem Cell Development, Reproduction and Early Embryogenesis, Gene Editing, Development and Disease, Organogenesis and Tissue Regeneration. Participants spoke highly of speeches in the conference which represent the current state in recent scientific advance and high level in the field of development biology study.

Laboratory Open Day 2017

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 "follow science, chase dream" organized by MARC, NBRI, State Key Laboratory of Pharmaceutical Biotechnology and Jiangsu Society for Cell and Developmental Biology was held on 14th May, 2017. More than 120 persons including primary pupil and their parents, middle school students and other biological enthusiasts attended the activities to get close to adorable animals and try to know laboratory work, exploring scientific mysteries.

Prof. Zhongzhou Yang, the president of Society for Cell Differentiation and Development of CSCB, gave an introduction about the history and development of MARC. He chatted with participants and then took interview from Nanjing News. Prof. Shuai Chen, the Secretary General of Jiangsu Society for Cell and Developmental Biology, appeared in person and gave an interview to Science and Technology News.

Laboratory Open Day aimed to get public understand the lab work and get interesting in research, and inspire their motivation in science. The open day includes four sessions: animals model exhibition, scientific booklets collection, video play and poster display for MARC research work. Plenty of fancy lab experiments were available for people to be involved in. After known the sacrifices of animals for scientific work, all the attendants showed their respect to those lovely creatures. This activity was reported by medias.

The 3rd National Symposium on Muscle 2017

The 3rd National Symposium on Muscle organized by MARC was held on 28th June, 2017. Scientists from famous research institutes, Purdue University, Huazhong Agricultural University, Guangdong institute of Microbiology, Jilin University, Nanjing Normal University, The Chinese University of Hong Kong, Chinese Academy of Agricultural Sciences, South China University of Technology, Chinese Academy of Medical Sciences, Nanjing University, Zhejiang University, China Agricultural University, Chinese Academy of Sciences, Fudan University, Xiamen University and Shaanxi Normal University, attended this conference.

The meeting was chaired by Prof. Zhenji Gan and Prof. Xiang Gao. Prof. Gao, the founder of MARC and Dean of NBRI, gave an opening speech.

Speakers, Shihuan Kuang, Heng Wang, Liwei Xie, Ping Huang, Huaqun Chen, Huating Wang, Yubo Zhang, Xiaozhong Shi, Shuai Chen, chengyong Shen, Yong Zhang, Zhonglin Tang, Zhenji Gan, Zhuoxian Meng, Qingyong Meng, Yang Yu and Ping Hu were gathering together to share and discuss the new development and achievements on the frontier muscle research.

This conference provided the chance for scientists in muscle research field to exchange academic ideas, and share their work with audiences and network with colleagues, peers and like-minded individuals working in the field of muscle.

2017 The Academic Annual Meeting of Jiangsu Society for Cell and Development Biology

Nanjing, 28 June, 2017-Over 180 participants from Nanjing Agricultural University, Nanjing University of Science and Technology, China Pharmaceutical University, Nanjing University, Nanjing Medical University, Suzhou University, Southeast University, Jiangnan University, Nanjing Normal University and Nantong University, took part in the academic annual meeting of Jiangsu Society for Cell and Developmental Biology.

The meeting was chaired by Prof. Minsheng Zhu, the director of MARC and president of Jiangsu Society for Cell and Developmental Biology.

The guest speakers, Prof. Yuanchao Wang and Prof. Jianfa Zhang made interesting and inspiring speeches followed by talks giving by scientists from cell and development field in Jiangsu Province.

The audiences spoke highly of the brilliant talks delivered by the conference, and felt like be inspired by a wide range of the speakers.

2017 Annual Conference of MARC

The 2017 MARC Annual Conference was held in the third floor of MARC canteen from November 8th to9th. This conference was organized by Dr. Di Chen and Dr. Yun Shi. More than 200 scientists and students from Nanjing University and Nantong University attended the conference.

Dr. Minsheng Zhu, director of MARC, made welcome remarks at the opening ceremony, in the following sessions, PIs presented latest research progresses and scientific ideas from their laboratories, followed by lively discussions between the speakers and the audiences. About 74 posters were presented by senior students to exhibit their research results. In the Teacher-Student interaction session, interested issues and topics were discussed.

At last, Pei Wang from Dr. Minsheng Zhu's laboratory, was awarded the 2017 Student of MARC for his excellent research. Xijun Liang from Dr. Zhenji Gan's laboratory , Qiaoli Chen from Shuai Chen's laboratory, Wukui Zhao from Jinzhong Qin's laboratory and Zan Zhang from Ying Cao's laboratory were nominated. In addition, eleven students received 2017 Outstanding Poster Prize.

2017 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 8th Summer Camp from July 10th to 14th this summer. 53 excellent undergraduates were selected from a pool of 299 applicants from 81 universities nationwide.

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

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

We respectively held academic salons, dedicates PIs with summer camp students communicate with each other at three nights .Moreover, 2 of our outstanding graduate students, Qiaoli Chen and Mingyang Jiang, 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.

2017 Students Union

In MARC, we have rich and wonderful activities between the professors and students. The about three 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 2017, the annual table tennis and badminton game came at the appointed time in May and Dec this year, respectively, and being attractive as usually. Sports will not only spice up our life, but also more importantly inspire passion and enthusiasm for physical exercises, which is more than necessary for scientific students like us. 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.

Also, our lectures with invited speakers were quite abundant this year. Plenty of renowned scientists, have given lectures here. And more speakers form non-science background, such as experts of literature, arts and social science were invited as well.

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