Model Animal Research Center of Nanjing University
MOE key laboratory of model animal for disease study
National resource center for mutant mice

ANNUAL REPORT

2021

Director's Words

The pandemic has changed our society in many ways and urged us to rethink of various aspects of our life and work. However, one thing does not, and also will not, change at MARC is our passion for excellence in science. MARC has been devoted to biomedical research using animal models to make discoveries for a healthy life. Over the last decade, we've become stronger in developing genetically-modified animals for modeling human diseases. Through introducing state-of-art genome editing technologies, we not only accelerate generation of genetically-modified animals, but also start to develop more precise and complex models for studying human diseases. As a member of the International Mouse Phenotyping Consortium (IMPC), we've also established a state-of-art platform for mouse phenomics, which help us to get better understanding of gene functions in a post-genomic era. To improve our abilities for in-depth functional studies, we've expanded other facilities at MARC, including the imaging core, metabolomics core, and flow cytometry core. With these state-of-art core facilities, MARC scientists have tackled some longstanding scientific questions, and made several important discoveries this year. For example, in a study published in the Nature, Dr. Yun Shi's group together with teams led by Drs. Yan Zhao and Kai Zhang from Institute of Biophysics of Chinese Academy of Sciences deciphered the structure of GluK2-NETO2 complex and elucidated the molecular mechanism how NETO2 regulates Kainate receptors. Looking forward to the year ahead, I believe that more discoveries will be made in our ever-expanding research fields, from genetics and developmental biology to cancer biology, metabolic biology and neurobiology. We will continue to fulfil our mission at MARC pursuing first-class science for improvement of human health with the help of animal models.

My second term as the director of MARC starts from 2021. The privileges working with a great team of devoted professors, talented students and brilliant supporting personnel give me great confidence of a bright future for MARC. The success of MARC is also owed to great supports from friends of ours over years. I wish every MARCer and all friends a happier 2022!

Shuai Chen
Director

CONTENTS

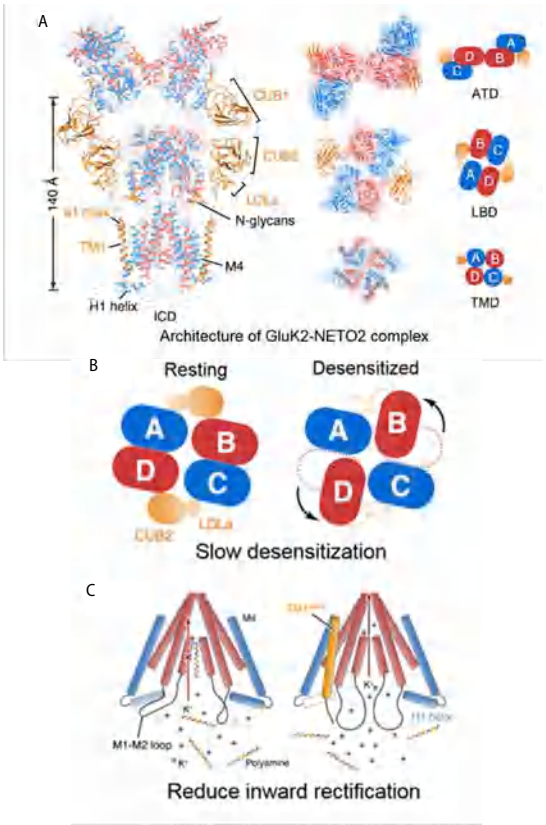
Research Highlight in 2021

Group Yun Shi

Kainate receptor modulation by NETO2

Lingli He, Jiahui Sun, Yiwei Gao, Bin Li, Yuhang Wang, Yanli Dong, Weidong An, Hang Li, Bei Yang, Yuhan Ge, Xuejun Cai Zhang*, Yun Stone Shi* & Yan Zhao*

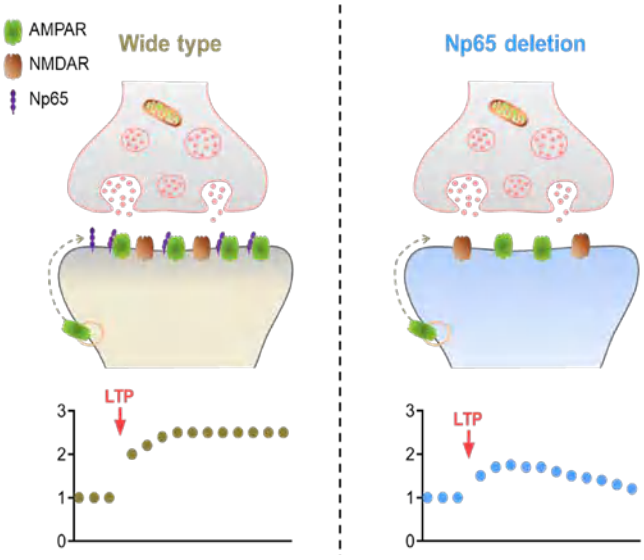
Glutamate-gated kainate receptors are ubiquitous in the central nervous system of vertebrates, mediate synaptic transmission at the postsynapse and modulate transmitter release at the presynapse. In the brain, the trafficking, gating kinetics and pharmacology of kainate receptors are tightly regulated by NETO proteins. Here we report cryo-electron microscopy structures of homotetrameric GluK2 in complex with NETO2 at inhibited and desensitized states, illustrating variable stoichiometry of GluK2-NETO2 complexes, with one or two NETO2 subunits associating with GluK2. We find that NETO2 accesses only two broad faces of kainate receptors, intermolecularly crosslinking the lower lobe of ATDA/C, the upper lobe of LBDB/D and the lower lobe of LBDA/C, illustrating how NETO2 regulates receptor-gating kinetics. The transmembrane helix of NETO2 is positioned proximal to the selectivity filter and competes with the amphiphilic H1 helix after M4 for interaction with an intracellular cap domain formed by the M1-M2 linkers of the receptor, revealing how rectification is regulated by NETO2.



The amino-terminal domain of GluA1 mediates LTP maintenance via interaction with neuroplastin-65

Chao-Hua Jianga, Mengping Wei, Chen Zhang* & Yun Stone Shi*

Long-term potentiation (LTP) has long been considered as an important cellular mechanism for learning and memory. LTP expression involves NMDA receptor-dependent synaptic insertion of AMPA receptors (AMPARs). However, how AMPARs are recruited and anchored at the postsynaptic membrane during LTP remains largely unknown. In this study, using CRISPR/Cas9 to delete the endogenous AMPARs and replace them with the mutant forms in single neurons, we have found that the amino-terminal domain (ATD) of GluA1 is required for LTP maintenance. Moreover, we show that GluA1 ATD directly interacts with the cell adhesion molecule neuroplastin-65 (Np65). Neurons lacking Np65 exhibit severely impaired LTP maintenance, and Np65 deletion prevents GluA1 from rescuing LTP in AMPARs-deleted neurons. Thus, our study reveals an essential role for GluA1/Np65 binding in anchoring AMPARs at the postsynaptic membrane during LTP.

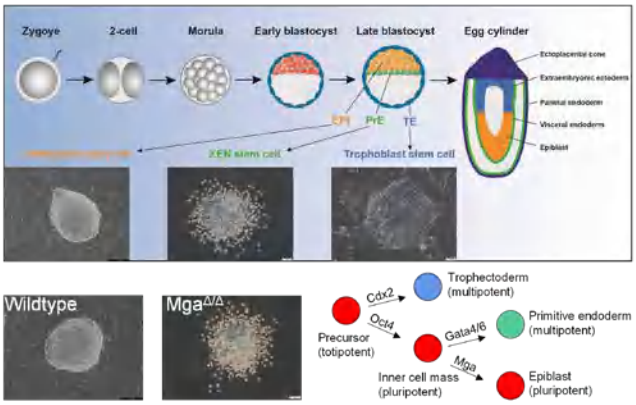


Group Jinzhong Qin

Mga safeguards embryonic stem cells from acquiring extraembryonic endoderm fates

Jinzhong Qin*, Congcong Wang†, Yaru Zhu†, Ting Su†, Lixia Dong, Yikai Huang, Kunying Hao

Polycomb group (PcG) proteins form multi-protein complexes that impact stem cell identity and fate decisions by still largely unexplored mechanisms. Here, by performing a CRISPR-based loss-of-function screen in embryonic stem cells (ESCs), we identify PcG gene Mga involved in the repression of endodermal transcription factor Gata6. We report that deletion of Mga results in peri-implantation embryonic lethality in mice. We further demonstrate that Mga null ESCs exhibit impaired self-renewal and spontaneous differentiation to primitive endoderm (PE). Our data support a model in which Mga might serve as a scaffold for PRC1.6 assembly and guide this multimeric complex to specific genomic targets including genes that encoding endodermal factors, Gata4, Gata6 and Sox17. Our findings uncover an unexpected function of Mga in ESCs, where it functions as a gatekeeper to prevent ESCs from entering into the PE lineage by directly repressing expression of a set of endoderm differentiation master genes.

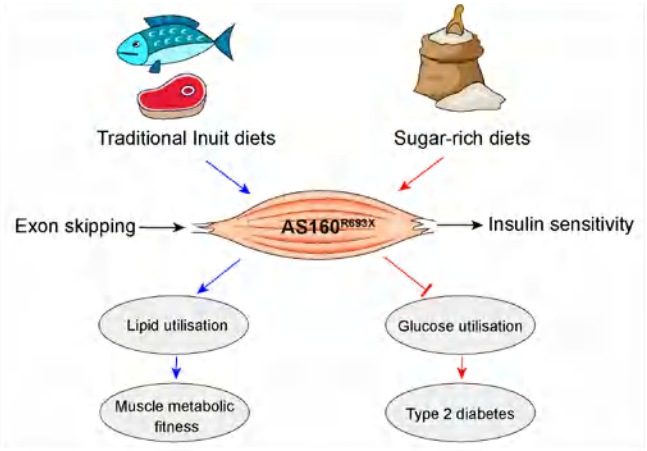


Group Shuai Chen and Hongyu Wang

Tissue-specific splicing and dietary interaction of a mutant *As160* allele determine muscle metabolic fitness in rodents

Xinyu Yang, Qiaoli Chen, Qian Ouyang, Ping Rong, Weikuan Feng, Chao Quan, Min Li, Qing Jiang, Hui Liang, Tong-Jin Zhao, Hong Yu Wang and Shuai Chen

Ethnic groups are physiologically and genetically adapted to their diets. Such genetic/dietary interactions may play an important role in the metabolic regulation as well as the pathogenesis of metabolic diseases. A common AS160^{R684X} variant has recently been identified in Greenlandic and North American Inuit with an allele frequency of 17-27%, which confers muscle insulin resistance and type 2 diabetes. Whether this mutation evolutionarily confers adaptation in Inuit and how it causes metabolic disorders upon dietary changes are unknown due to limitations in human studies. To delineate pathogenic mechanism of this AS160 mutation, we develop a genetically-modified rat model bearing an orthologous AS160^{R693X} mutation, which mimics human patients, exhibiting postprandial hyperglycemia and hyperinsulinemia. Importantly, a sugar-rich diet aggravates metabolic abnormalities in AS160^{R693X} rats. The AS160^{R693X} mutation diminishes a dominant long-variant AS160 without affecting a minor short-variant AS160 in skeletal muscle, which suppresses muscle glucose utilisation but induces fatty acid oxidation. This fuel switch suggests a possible adaptation in Inuit who traditionally had lipid-rich hypoglycemic diets. Finally, up-regulation of the short-variant AS160 through exon-switching restores glucose utilisation in rat myocytes and a mouse model. Our findings have implications for development of precision treatments for patients bearing the AS160^{R684X} mutation.



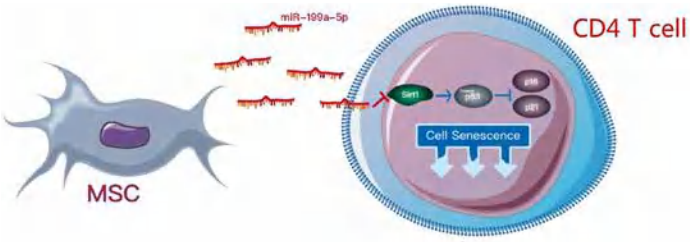
Working model of the genetic/dietary interaction in regulation of metabolic fitness and diseases.

Group Yan Li

Mesenchymal stem cell therapy ameliorates lupus through increasing CD4+ T cell senescence

Tao Cheng, Shuai Ding, Shanshan Liu, Yan Li*, Lingyun Sun*

Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) transplantation has been proved to be an effective therapeutic approach to treat systemic lupus erythematosus (SLE) by immunomodulation. Our study questions are focused on its inhibition effects of T-cell proliferation by inducing cell unresponsiveness and promoting premature senescence. The cellular senescence was monitored based on the activation of p21, p16, p53 and its key upstream regulator NAD-dependent protein deacetylase sirtuin-1 (Sirt1). Accordingly, we showed that hUC-MSCs transplantation ameliorated lupus symptoms and increased senescence of splenic CD4+ T cells through Sirt1/p53 signaling in MRL/lpr mice. Considering MSCs frequently communicate with surrounding immune cells by releasing miRNAs, we predicted and then identified miR-199a-5p from hUC-MSCs targets MRL/lpr splenic CD4+ T cells Sirt1 downregulation. Moreover, systemic delivery of miR-199a-5p in MRL/lpr mice has been proved to increase splenic CD4+T-cell senescence, mimicking the therapeutic effects of transplanted hUC-MSCs. In conclusion, we have identified miR-199a-5p as one of the mechanisms employed by hUC-MSCs to alleviate lupus disease associated pathologies in MRL/lpr mice, which is attributable for promoting splenic CD4+T cell senescence through Sirt1/p53 pathway. These findings not only extend our knowledge of the immunoregulatory function of hUC-MSCs in autoimmune disease, but also offer miR-199a-5p as a potential biomarker or therapeutic target to further explore in the future.

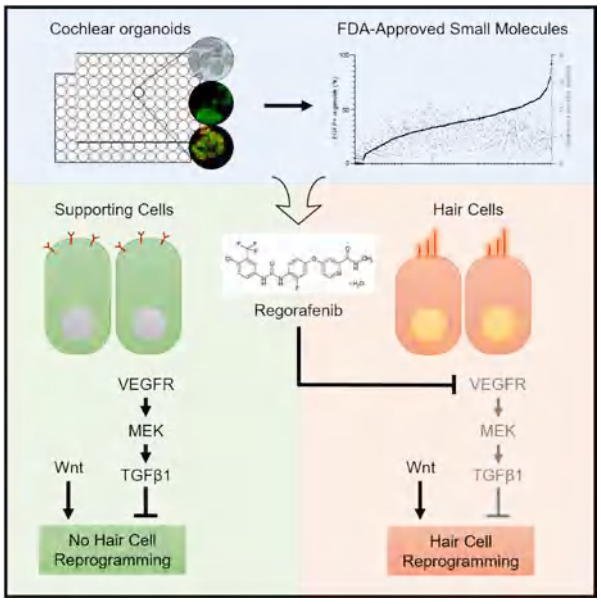


Group Guoqiang Wan

High-throughput screening on cochlear organoids identifies VEGFR-MEK-TGFB1 signaling promoting hair cell reprogramming

Qing Liu Linqing Zhang Min-Sheng Zhu and Guoqiang Wan

Hair cell degeneration is a major cause of sensorineural hearing loss. Hair cells in mammalian cochlea do not spontaneously regenerate, posing a great challenge for restoration of hearing. Here, we establish a robust, high-throughput cochlear organoid platform that facilitates 3D expansion of cochlear progenitor cells and differentiation of hair cells in a temporally regulated manner. High-throughput screening of the FDA-approved drug library identified regorafenib, a VEGFR inhibitor, as a potent small molecule for hair cell differentiation. Regorafenib also promotes reprogramming and maturation of hair cells in both normal and neomycin-damaged cochlear explants. Mechanistically, inhibition of VEGFR suppresses TGFB1 expression via the MEK pathway and TGFB1 downregulation directly mediates the effect of regorafenib on hair cell reprogramming. Our study not only demonstrates the power of a cochlear organoid platform in highthroughput analyses of hair cell physiology but also highlights VEGFR-MEK-TGFB1 signaling crosstalk as a potential target for hair cell regeneration and hearing restoration.



Student of the Year

Liwei Xiao

Liwei Xiao received his Bachelor's degree of Biological Technology in 2014 from School of Biological and Food Engineering, Hefei University of Technology. He joined Dr. Zhenji Gan's lab at the year of 2015 to study mitochondrial remodeling and metabolic diseases.

For the past few years, his work focused on the function and mechanistic actions of FNIP1 in skeletal muscle mitochondrial metabolism. Using both gain-of-function and loss-of-function strategies in mice and muscle cells, he and his colleagues discovered an essential function of adaptor protein FNIP1 in the coordinated regulation of the mitochondrial and structural programs controlling muscle fitness. These findings provide a new mechanism by which mitochondrial function and muscle fiber type are concordantly regulated and have important implications for possible therapeutic manipulation of muscle mitochondrial function muscle fiber type for maintaining muscle fitness in a variety of chronic disease states.

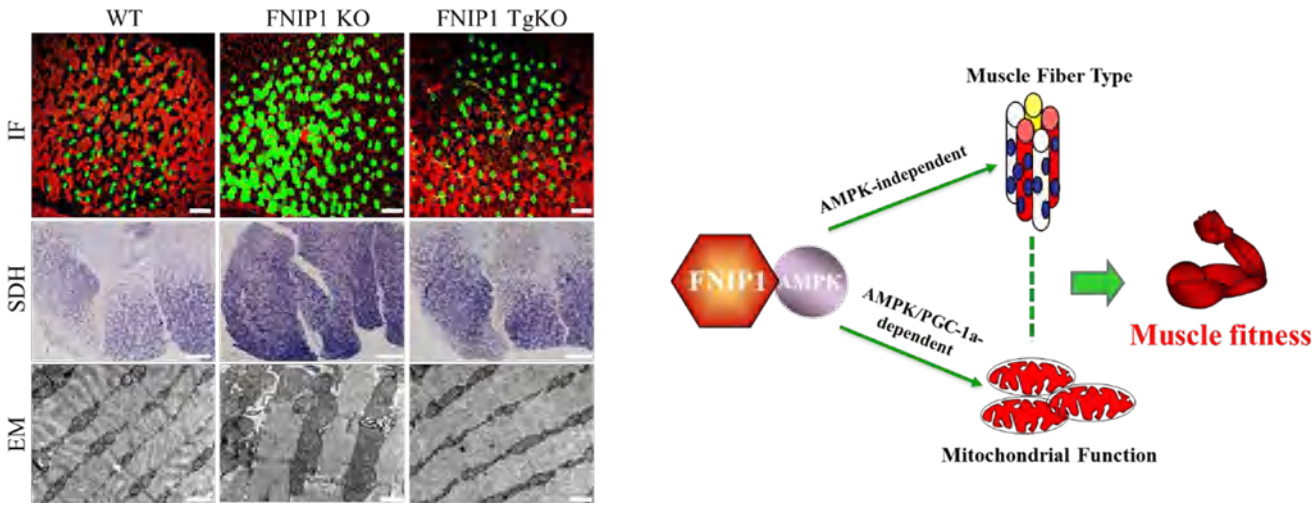
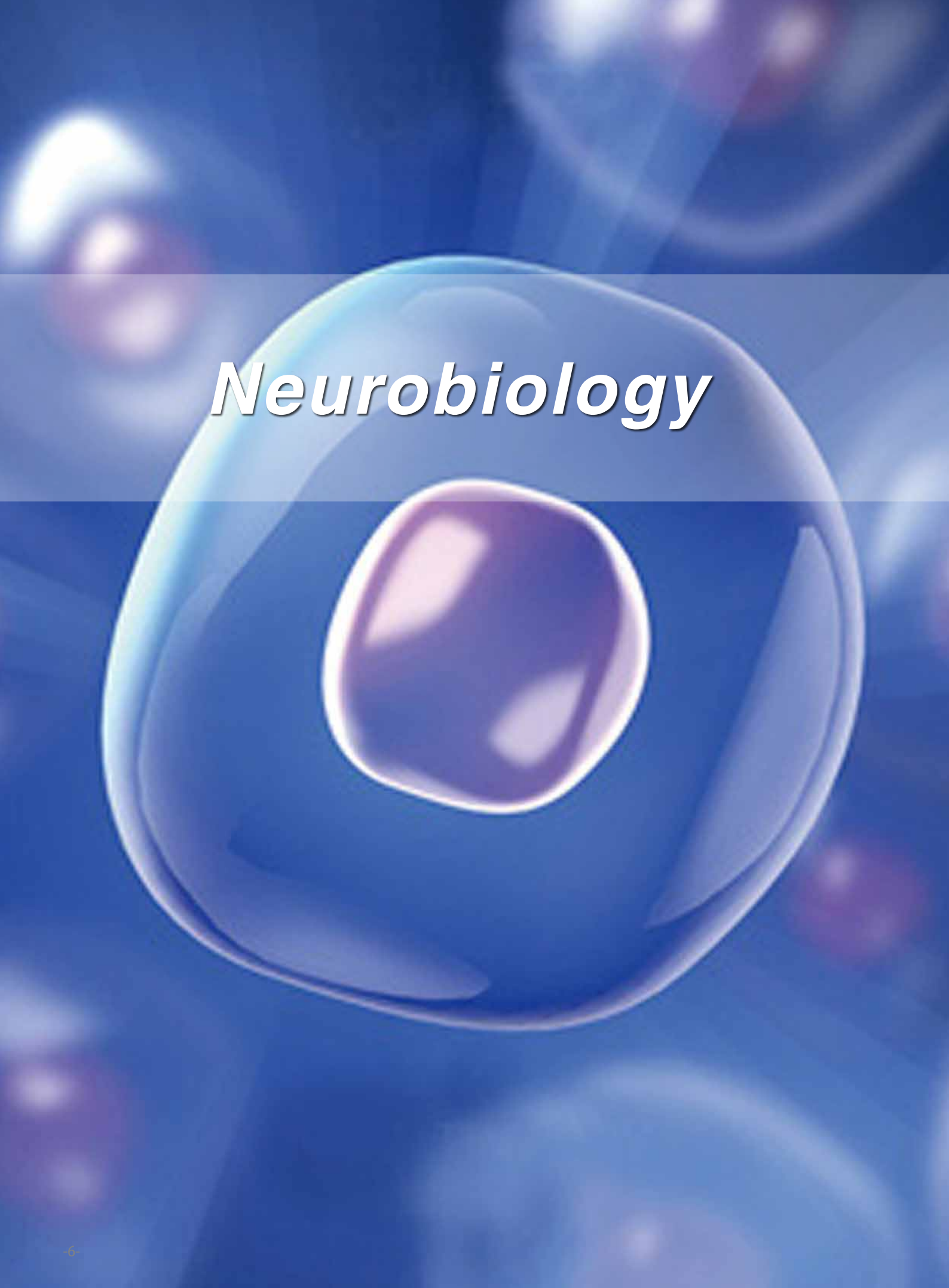


Figure1. FNIP1 as a dominant factor that coordinates mitochondrial and muscle fiber type programs that govern muscle fitness.

Selected publications

1. Xiao L, Liu J, Sun Z, Yin Y, Mao Y, Xu D, Liu L, Xu Z, Guo Q, Ding C, Sun W, Yang L, Zhou Z, Zhou D, Fu T, Zhou W, Zhu Y, Chen XW, Li JZ, Chen S, Xie X, Gan Z. AMPK-dependent and -independent coordination of mitochondrial function and muscle fiber type by FNIP1. PLoS Genet. 2021 Mar 29;17(3):e1009488.
2. Liu J, Liang X, Zhou D, Lai L, Xiao L, Liu L, Fu T, Kong Y, Zhou Q, Vega RB, Zhu MS, Kelly DP, Gao X, Gan Z. Coupling of mitochondrial function and skeletal muscle fiber type by a miR-499/Fnip1/AMPK circuit. EMBO Mol Med. 2016 Oct 4;8(10):1212-1228.


Neurobiology

Yun Shi , Ph.D

Yun Shi received Ph.D degree in physiology at Georgia State University under the mentoring of Dr. Chun Jiang at Atlanta, USA in 2007. His Ph.D work focus on the function and regulation of vascular KATP channels. He then had postdoctoral training with Dr. Roger Nicoll in UCSF where he worked on synaptic plasticity. 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 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.

Current research interests in our lab include: 1. The fundament of synaptic plasticity such as LTP and LTD. 2. Diseases associated with glutamate signal pathway. 3. Physiological functions of the mechanosensitive cation channel Tmem63 family.

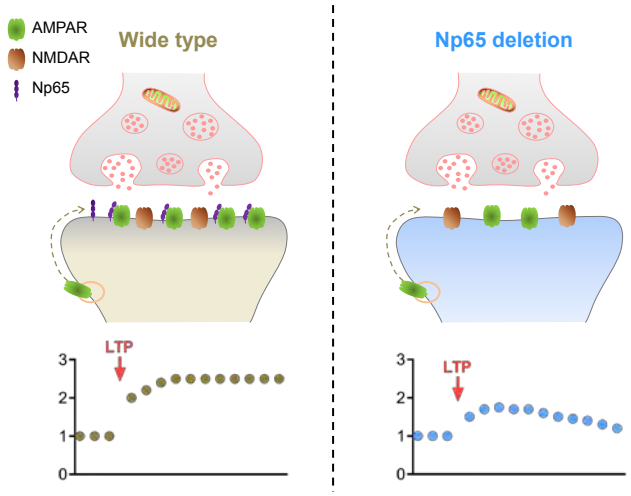


Figure 1. The critical role of Np65 in LTP maintenance (PNAS 2021).

Left, AMPARs enter synapse after LTP induction. Np65 bind the extracellular domain of AMPARs, which ankles AMPARs in synapse and maintain LTP. Right, deletion of Np65 impairs LTP maintenance.

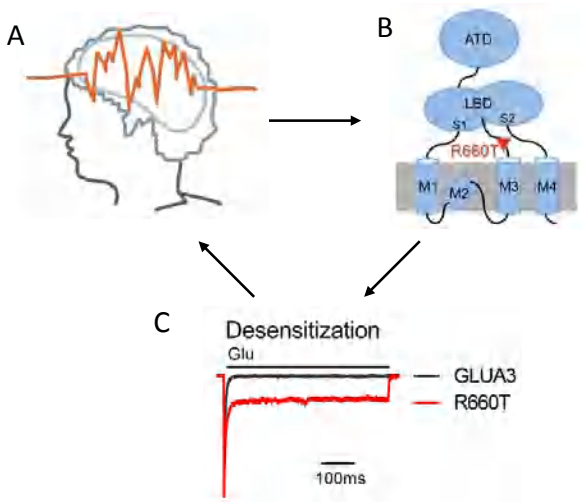


Figure 2. A mutant in AMPAR subunit GluA3 causes epilepsy (Plos Genetics 2021).

A. A female patient having severe epilepsy.

B. She carried a rare variant R660T in GluA3.

C.GluA3_R660T has elevated activity, which explained her epileptic syndrome.

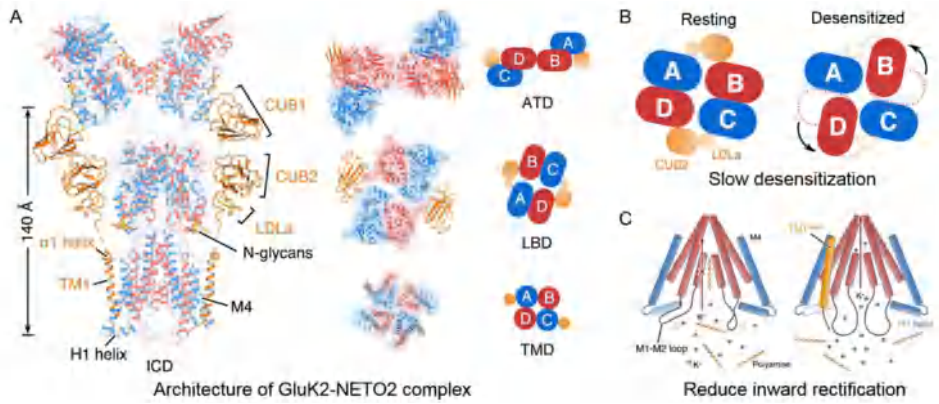


Figure 3. GluK2-NETO2 CryoEM Structure (Nature 2021).

A. The architecture of GluK2-NETO2 complex.

B. The structural mechanism for NETO2 regulation of GluK2 desensitization.

C. The mechanism for NETO2 regulation of GluK2 inward rectification.

Selected publications

1. He L, Sun J, Gao Y, Li B, Wang Y, Dong Y, An W, Li H, Yang B, Ge Y, Zhang XC*, Shi YS*, Zhao Y*. (2021) Kainate receptor modulation by Neto2. Nature. 599(7884):325-329.
2. Sun JH, Chen J, Ayala Valenzuela FE, Brown C, Masser-Frye D, Jones M, Romero LP, Rinaldi B, Li WL, Li QQ, Wu D, Gerard B, Thorpe E*, Bayat A*, Shi YS*. (2021) X-linked neonatal-onset epileptic encephalopathy associated with a gain-of-function variant p.R660T in GRIA3. PLoS Genet. 17(6):e1009608.
3. Peng SX, Wang YY, Zhang M, Zang YY, Wu D, Pei J, Li Y, Dai J, Guo X, Luo X, Zhang N, Yang JJ, Zhang C, Gao X, Liu N*, Shi YS*. (2021) SNP rs10420324 in the AMPA receptor auxiliary subunit TARP γ-8 regulates the susceptibility to antisocial personality disorder. Sci Rep. 11(1):11997.
4. Jiang CH, Wei M, Zhang C*, Shi YS*. (2021) The amino-terminal domain of GluA1 mediates LTP maintenance via interaction with neuroplastin-65. PNAS. 118(9): e2019194118.
5. Wu D, Zang YY, Shi YY, Ye C, Cai WM, Tang XH, Zhao L, Liu Y, Gan Z, Chen GQ, Xu Y, Yang JJ*, Shi YS*.(2020). Distant Coupling between RNA Editing and Alternative Splicing of the Osmosensitive Cation Channel Tmem63b. J Biol Chem. 295(52):18199-18212.
6. Du H, Ye C, Wu D, Zang YY, Zhang L, Chen C, He XY, Yang JJ, Hu P, Xu Z, Wan G*, Shi SY*. (2020) The cation channel TMEM63B is an osmosensor required for hearing. Cell Reports. 31(5):107596.
7. Li YJ, Duan GF, Sun JH, Wu D, Ye C, Zang YY, Chen GQ, Shi YY, Wang J, Zhang W*, Shi YS*.(2019) Neto proteins regulate gating of the kainate-type glutamate receptor GluK2 through two binding sites. J Biol Chem. 294(47):17889-17902.
8. Duan GF, Xu S, Ye Y, Tao W, Nicoll RA, Shi YS*, and Sheng N*.(2018) Signal peptide represses GluK1 surface and synaptic trafficking through binding to amino-terminal domain. Nature Communications. 9(1):4879.
9. Niu Y, Dai Z, Liu W, Zhang C, Yang Y, Guo Z, Li X, Xu C, Huang X, Wang Y, Shi YS*, Liu JJ*. (2017) Ablation of SNX6 leads to defects in synaptic function of CA1 pyramidal neurons and spatial memory. eLife. 6. pii: e20991.
10. 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. PNAS.113(38):E5645-54.

Group members

Former graduate students

Yanjun Li, Zhejiang University;
Jiang Chen, Drum Tower Hospital;
Guifang Duan, Peking University;
Han Du, Getein Biotech;
Dan Wu, Drum Tower Hospital;
Chang Ye, Nanjing University;
Jiahui Sun;
Shixiao Peng.

Graduate students

Chaohua Jiang
Yueying Wang
Xiaoyu Teng
Qingqing Li
Guolin Yang
Shiyu Zhang
Wenmin Cai
Tianzi Zhang
Yuhan Ge
Yangyang Chen
Jingjing Tu

Visiting students

Xiaohui Tang
Xiaoyu Yin
Yinchun Lin

Postdoc

Chang Ye

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 diseases

1. Molecular mechanisms by which Akt regulates oligodendrocyte differentiation.

As an important protein kinase, Akt has been implicated in diseases with white matter (WM) abnormalities. To study whether and how Akt may regulate OL development, we generated oligodendrocyte (OL) lineage cells-specific Akt1/Akt2/Akt3 triple conditional knockout (Akt cTKO) mice (Fig.1). We show that deletion of Akt three isoforms causes down-regulation of Sox10 and decreased levels of phosphorylated FoxO1 (pFoxO1) in the brain (Fig.2). In vitro analysis reveals that expression of FoxO1 with mutations on phosphorylation sites for Akt significantly represses the Sox10 promoter activity (Fig.2). Together, we have identified a novel phosphorylation-dependent mechanism for Sox10 expression and OL differentiation.

2. Essential role of Pen-2 in governing the differentiation of oligodendrocyte precursor cells to astrocytes.

Whereas the role of γ-secretase in neurogenesis has been intensively studied, little is known about its role in astrogliogenesis. Recent evidence has demonstrated that astrocytes can be generated from OL precursor cells (OPCs). We generated OL lineage cells specific presenilin enhancer 2 (Pen-2) cKO mice (Fig.3). We show that conditional inactivation of Pen-2 in OL lineage causes enhanced generation of GFAP-expressing astrocytes (Fig.3). Mechanistic analysis reveals that deletion of Pen-2 inhibits the Notch signaling to up-regulate signal transducer and activator of transcription 3 (Stat3) (Fig.4). These findings suggest that Pen-2 may control the differentiation of OPCs to astrocytes through the Stat3 signaling.

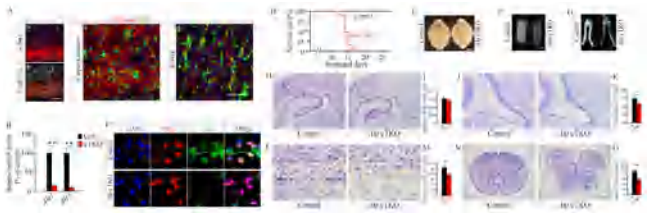


Figure 1. Deficient white matter development in Akt cTKO mice. (A-C) Characterization of Akt cTKO mice. (D) Survival rate. (E-G) Morphology of the brain, the spinal cord (SC) and the optic nerve (ON) in Akt cTKO mice. (H-O) Nissl analyses for the cortex (H), the fimbria (J), the corpus callosum (CC) (L) and the spinal cord (SC) (N).

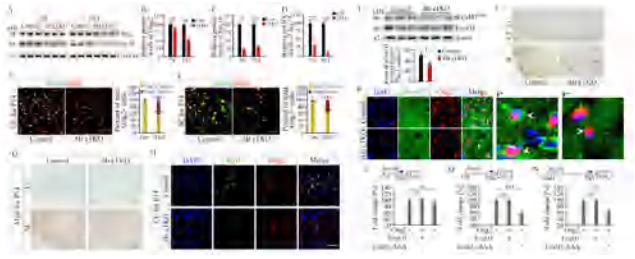


Figure 2. Down-regulation of Sox10 in Akt cTKO mice. (A-D) Decreased expression of Sox10 in Akt cTKO mice at P4 and P14. (E-F) Number of Sox10+/Olig2+ cells in the CC and the SC in Akt cTKO mice. (G,H) IHC analysis of Myrf+ cells in the CC and the SC in Akt cTKO mice at P14. (I-K) Western and IHC analyses on pFoxO1 in Akt cTKO mice. (L-M) Analysis of the promoter activity of Sox10 using cultured N2a cells.

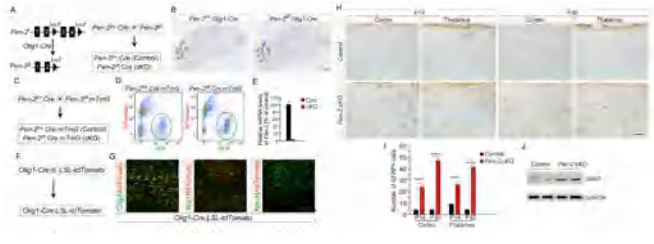


Figure 3. Enhanced generation of astrocytes in OL lineage cellS specific Pen-2 cKO mice. (A-D) Characterization of OL lineage cells specific Pen-2 cKO mice. (F,G) Co-staining of Olig2/tdTomato in Olig1-Cre;LSL-tdTomato mice. (H) IHC on GFAP in Pen-2 cKO mice. (I) Number of GFAP+ cells in Pen-2 cKO mice at P14 and P30. (J) Western analysis on GFAP in Pen-2 cKO mice.

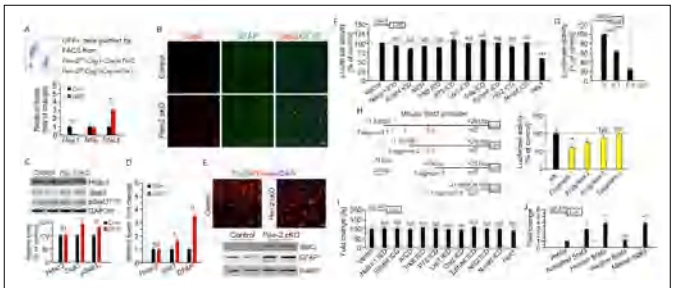


Figure 4. Increased expression of Stat3 in Pen-2 cKO mice. (A) Q-PCR analyses on Hes1, Nfia and Stat3. (B) IHC of Stat3/GFAP in Pen-2 cKO mice. (C) Western analysis on Hdac3, Stat3 and pStat3. (D) Q-PCR analyses on Hdac3, Stat3 and GFAP. (E) Western analyses on Stat3 and GFAP using cultured Pen-2 cKO OPCs. (F-H) Analysis of the promoter activity of Stat3 using cultured N2a cells. (I,J) Analysis of the promoter activity of GFAP.

Recent publications (*, Corresponding author)

1. Wang H, Liu M, Ye Z, Zhou C, Bi H, Wang L, Zhang C, Fu H, Shen Y, Yang J, Hu Y*, Chen G*. Akt regulates Sox10 expression to control oligodendrocyte differentiation via phosphorylating FoxO1. Journal of Neuroscience 2021 (41): 8163-8180.
2. Hou J, Bi H, Ye Z, Huang W, Zou G, Zou X, Shi Y, Shen Y, Ma Q, Kirchhoff F, Hu Y*, Chen G*. Pen-2 negatively regulates the differentiation of oligodendrocyte precursor cells into astrocytes in the central nervous system. Journal of Neuroscience 2021 (41):4976-4990.
3. Wang H, Liu M, Zou G, Wang L, Duan W, He X, Ji M, Zou X, Hu Y*, Yang J*, Chen G*. Deletion of PDK1 in oligodendrocyte lineage cells causes white matter abnormality and myelination defect in the central nervous system. Neurobiology of Disease 2021 (148): 105212.
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Group members

Group leader

Guiquan Chen

Graduate students

Huiru Bi
Yingqian Xia
Xiaolian Ye
Mengjia Liu
Yizhi Zhang
Xudong Cai
Liyang Yao

Former members of the lab

Shanshan Cheng
Long Wang
Congyu Xu
Chen Zhang
Jinxing Hou
Chaoli Huang
Tingting Liu
He Wang

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

Exposure to benzo(a)pyrene (BaP, a common environmental pollutant) is associated with cognitive impairments and some Alzheimer's disease (AD)-like pathological changes. However, it is largely unknown whether BaP exposure participates in the disease progression of AD. We investigated effects of BaP exposure on AD progression and its underlying mechanisms. BaP or vehicle was administered to 4-month-old APP/PS1 transgenic mice and wildtype (WT) mice for two months. AD-like pathological and biochemical alterations and learning and memory ability were examined one month after 2-month BaP exposure.

BaP exposure induced progressive decline in spatial learning/memory and exploratory behaviors in APP/PS1 mice and WT mice, and APP/PS1 mice showed severer behavioral deficits than WT mice. BaP exposure promoted neuronal loss, Aβ burden and Aβ plaque formation in APP/PS1 mice, but not in WT mice. Gene expression profiling showed most robust alteration in genes and pathways related to inflammation and immune-regulatory process, Aβ secretion and degradation, and synaptic formation in WT and APP/PS1 mice after BaP exposure. Consistently, the cortex and the hippocampus of WT and APP/PS1 mice displayed activation of microglia and astroglia and upregulation of inducible nitric oxide synthase (iNOS), glial fibrillary acidic protein (GFAP) and NADPH oxidase (three widely-used neuroinflammatory markers) after BaP exposure. Furthermore, BaP exposure aggravated neurodegeneration induced by aged Aβ peptide (a mixture of monomers, oligomers and fibrils) in primary neuron-glia cultures through enhancing NADPH oxidase-derived oxidative stress. collectively, our study showed that chronic exposure to environmental pollutant BaP induced, accelerated and exacerbated the progression of AD, in which elevated neuroinflammation and NADPH oxidase-derived oxidative insults were key pathogenic events.

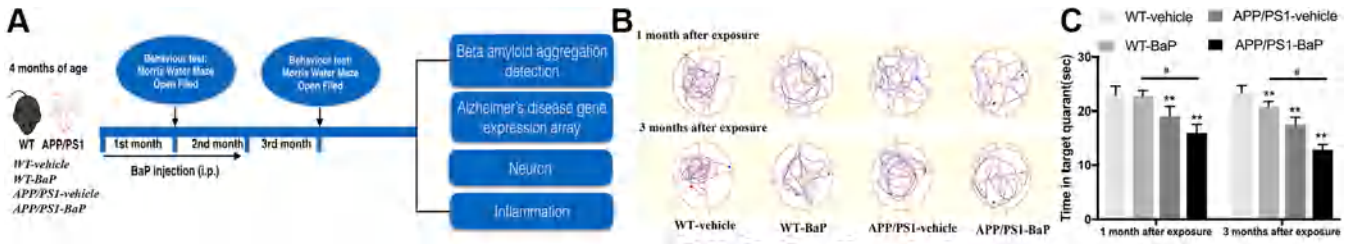

Figure 1. BaP exposure accelerated progressive spatial and working memory decline in the Morris water maze test.

A, Scheme of experimental design for in vivo studies. B, Representative path maps of each group in the probe trail. C, Time spent in the target quadrant in the probe trail. E, Entries to the platform quadrant in the probe trail. N=10/group. Data are mean ± SEM, *P < 0.05 and **P < 0.01 compared with WT-Vehicle control; #P < 0.05 compared with WT-BaP group.

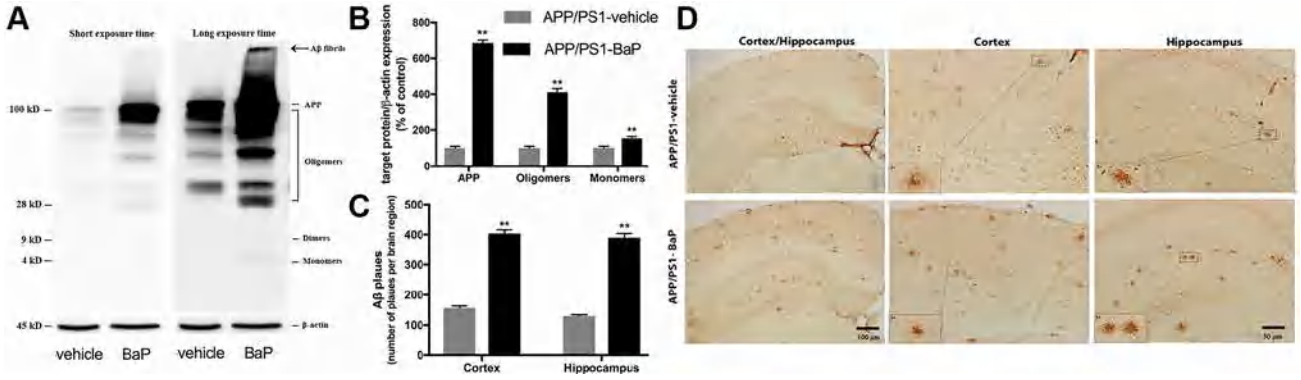

Figure 2. BaP exposure exacerbated Aβ burden and plaque formation in APP/PS1 mice.

A, Levels of RIPA soluble Aβ species and APP were detected by Western blot using 6E10 antibody for human Aβ/APP. B. The ratio of densitometry values of Aβ monomer (measured in the image with long exposure time), oligomers and APP (measured in the image with short exposure time) was normalized to β-actin. C, Total number of Aβ plaques was counted. D, Representative images of immunohistochemical staining of human Aβ using 6E10 antibody in the cortex and the hippocampus of APP/PS1 mice. Data are expressed as a percentage of APP/PS1-Vehicle control and are mean ± SEM of 3-4 mice in each treatment group. Significance was determined by t-test. **P < 0.01 compared with APP/PS1-Vehicle. Arrow: Aβ fibrils stuck in the loading well of the SDS-PAGE gel and then transferred to the PVDF membrane.

Selected publications(* Corresponding author)

1. Liu D, Zhao Y, Qi Y, Gao Y, Tu DZ, Wang Y, Gao H-M*, Zhou H* (2020) Benzo(a)pyrene exposure induced neuronal loss, plaque deposition, and cognitive decline in APP/PS1 mice. Journal of Neuroinflammation 17(1):258
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7. Zhou H, Liao J, Aloor J, Nie H, Wilson B, Fessler M, Gao H-M*, Hong J-S (2013) CD11b/CD18 is a novel receptor mediating extracellular dsRNA-induced immune responses. Journal of Immunology 190:115-125
8. 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)

Group members

Principal investigator

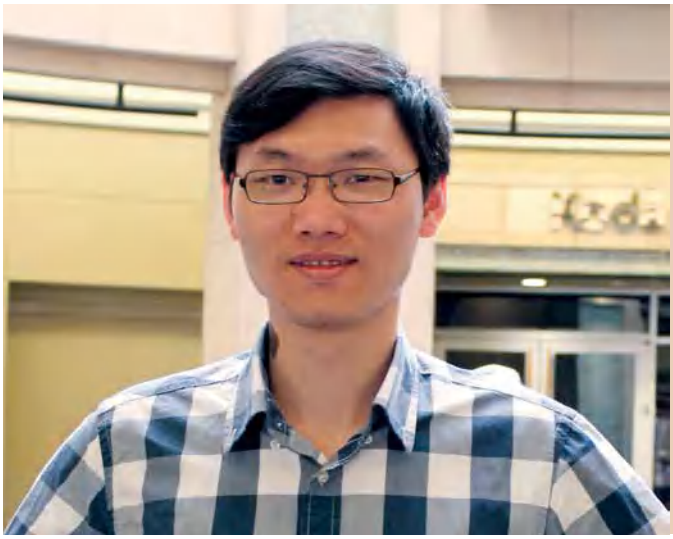
Huiming Gao

Graduate students

Ru Yang
Hui Li
Wei Huang
Xingqian Liu
Mengnan Yang
Yanlin Huang

Lab alumni

Yun Gao
Dezhen Tu
Tian Guan



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. Wan lab works on the genetics of hearing and deafness, development and regeneration of cochlear sensory cells and synapses, as well as applications of cochlear organoid models for hearing research.

Contact Information

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

Development and Regeneration of Auditory Sensory Cells and Synapses

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

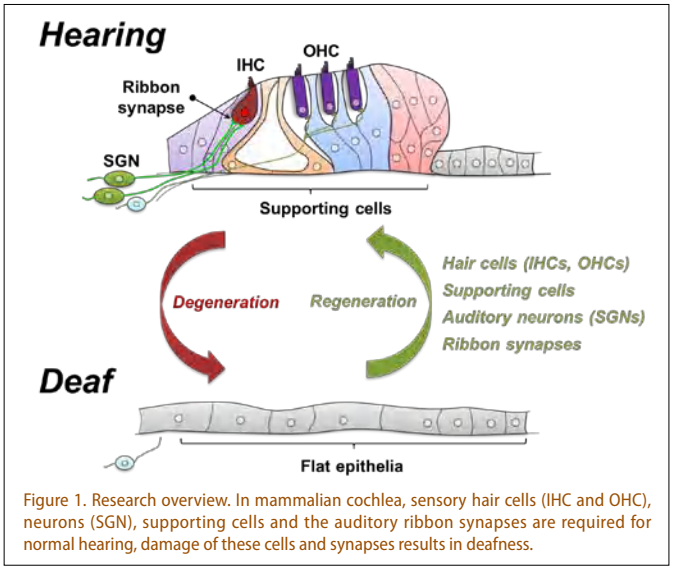


Figure 1. Research overview. In mammalian cochlea, sensory hair cells (IHC and OHC), neurons (SGN), supporting cells and the auditory ribbon synapses are required for normal hearing, damage of these cells and synapses results in deafness.

(1) Novel regulators of hair cell development and reprogramming using cochlear organoids

Loss of hair cells is the primary cause of sensorineural hearing loss. Unlike fish, birds and amphibians, mammalian hair cells do not regenerate, posing great challenge in restoration of auditory function in deaf humans. Due to the scarcity of cochlear sensory cells and lack of appropriate cell culture models, high throughput screening (HTS) for regulators of hair cells is severely limited. To circumvent this problem, we established a robust, high throughput cochlear organoid platform that facilitates 3D expansion of cochlear progenitor cells and differentiation of hair cells in a temporary-regulated manner. High throughput screening of the FDA-approved drug library identified Regorafenib, a VEGFR inhibitor, as a potent small molecule for hair cell differentiation. Regorafenib also promotes reprogramming and maturation of hair cells in both normal and neomycin-damaged cochlear explants. Mechanistically, inhibition of VEGFR suppresses TGFβ1 expression via MEK pathway and TGFβ1 downregulation directly mediates the effect of Regorafenib on hair cell reprogramming. Our study not only demonstrates the power of cochlear organoid platform in high throughput analyses of hair cell physiology, but also highlights VEGFR-MEK-TGFβ1 signaling crosstalk as a potential target for hair cell regeneration and hearing restoration (Figure 2).

(2) Progenitors and small molecule cocktails for cochlear sensory neuron reprogramming

In the mammalian cochlea, spiral ganglion neurons (SGNs) relay the acoustic information to the central auditory circuits. Degeneration of SGNs is a major cause of sensorineural hearing loss and severely affects the effectiveness of cochlear implant therapy. Cochlear glial cells are able to form spheres and differentiate into neurons in vitro. However, the identity of these progenitor cells is elusive, and it is unclear how to differentiate these cells towards functional SGNs. In this study, we found that Sox2+ subpopulation of cochlear glial cells preserves high potency of neuronal differentiation. Interestingly, Sox2 expression was downregulated during neuronal differentiation and Sox2 overexpression paradoxically inhibited neuronal differentiation. Our data suggest that Sox2+ glial cells are potent SGN progenitor cells, a phenotype independent of Sox2 expression. Furthermore, we identified a combination of small molecules that not only promoted neuronal differentiation of Sox2- glial cells, but also removed glial cell identity and promoted the maturation of the induced neurons (iNs) towards SGN fate. In summary, we identified Sox2+ glial subpopulation with high neuronal potency and small molecules inducing neuronal differentiation towards SGNs (Figure 3).

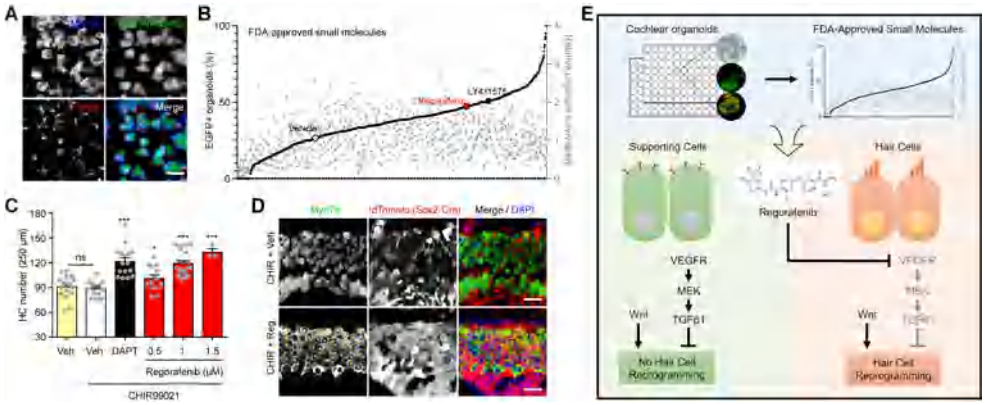


Figure 2. High throughput screening on cochlear organoids identifies VEGFR-MEK-TGFβ1 signaling promoting hair cell reprogramming. (A) Confocal images of EGFP+ hair cells in differentiated DIV26 Pou4f3(EGFP/+) reporter organoids (CHIR99021 + LY411575). Hair cells were co-labelled with Myo7a and stereocilia labelled with F-actin. Scale bar, 20 μm. (B) Screening of the FDA-approved small molecules identifies Regorafenib promoting hair cell differentiation in Pou4f3(EGFP/+) cochlear organoids. (C) Hair cell counts of cochlear explants treated with DAPT or Regorafenib at middle turn. (D) Regorafenib promoted hair cell reprogramming from Sox2+ supporting cells in cochlear explants lineage-traced by Sox2-CreER. Yellow asterisks indicate co-labelled cells. Scale bars, 20 μm. (E) A working model of VEGFR-MEK-TGFβ1 signaling axis in promoting hair cell reprogramming. (Published in Stem Cell Rep. 2021).

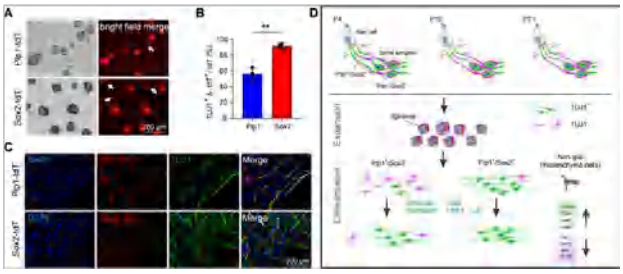


Figure 3. Cochlear Sox2+ glial cells as potent progenitors for spiral ganglion neuron reprogramming induced by small molecules. (A) Representative images of the Sox2-tdT and Plp1-tdT positive spheres culture 5 div. The white arrow represents the Plp1-tdT or Sox2-tdT negative spheres. Representative images (C) and ratios (B) of TUJ1+ neurons differentiated from Sox2-tdT or Plp1-tdT positive cells. (D) A working model of glial progenitors and small molecules for SGN reprogramming. (Published in Front Cell Dev Biol. 2021)

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

1. Liu, Q., Zhang, L., Zhu, M.S., and Wan, G. (2021). High-throughput screening on cochlear organoids identifies VEGFR-MEK-TGFB1 signaling promoting hair cell reprogramming. Stem Cell Reports, 16:2257-2273.
2. Chen, Z., Huang, Y., Yu, C., Liu, Q., Qiu, C., and Wan, G. (2021). Cochlear Sox2+ glial cells are potent progenitors for spiral ganglion neuron reprogramming induced by small molecules. Frontiers in Cell and Developmental Biology, 9:728352.
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5. Du, H.*, Ye, C.*, Wu, D.*, Zang, Y.Y., Zhang, L., Chen, C., He, X.Y., Yang, J.J., Hu, P., Xu, Z., Wan, G.# and Shi, Y.S.# (2020). The cation channel TMEM63B is an osmosensor required for hearing. Cell Reports, 31(5):107596.

Graduate students

Post-doc researcher

Guang-Jie Zhu

Graduate students

Zhen Chen
Sihao Gong
Yihan He
Yuhang Huang
Qing Liu
Cui Qiu
Chaorong Yu
Linqing Zhang

Rotation student

Guoli Zhong

Organogenesis

Jiong Chen Ph.D.

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

Contact Information

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

Understanding the driving forces underlying collective cell Migration

Cells do not always migrate individually; they often migrate collectively as a cluster, a sheet, or a strand under physiological, developmental and cancer metastatic conditions. Collective cell migration has recently received much attention from cell and developmental biologists, and it has emerged as an important field of study with many characteristics distinct from those of single cell migration. As a new field, collective migration still has many fundamental questions unresolved. For example, what intrinsic factors or signals pre-determine the migratory fate of a group of cells that will later collectively detach and migrate away from the host tissue (likened to a group of runners pre-selected from a larger group of candidate runners)(Figure 1)? How can the group of cells communicate with each other and collectively know the front vs. back, top vs. bottom and inside vs. outside during migration (Figures 2 and 3)? Finally, what powers the group to migrate collectively (Figure 3)?

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

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

Control of front-back polarity. It is known that the chemotactic migration of border cells is guided by the guidance receptor PVR, in response to extracellular signals secreted from oocyte. But, how guidance signaling sets up the front-back polarity of the entire border cell cluster is not well understood. We've made an interesting discovery that the guidance receptor PVR mediates the asymmetric distribution of exocyst and recycling endosome to set up the front-back polarity. (Wan et al., Development, 2013). Furthermore, we find that molecules crucial in apical-basal polarity, including aPKC and Crumbs complex, are required for the establishment of front-back polarity (Fig 3; Wang et al., Development, 2018). In addition, we find interesting coordination among the front-back, apical-basal and inside-outside polarities within the border cell cluster.

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

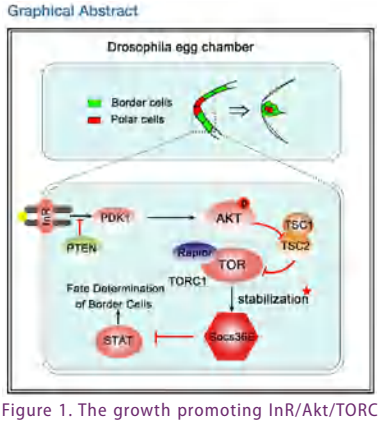


Figure 1. The growth promoting InR/Akt/TORC1 pathway attenuates migratory cell fate of border cells by down-regulation of JAK/STAT and destabilization of Socs36E. (from Kang et al., 2018)

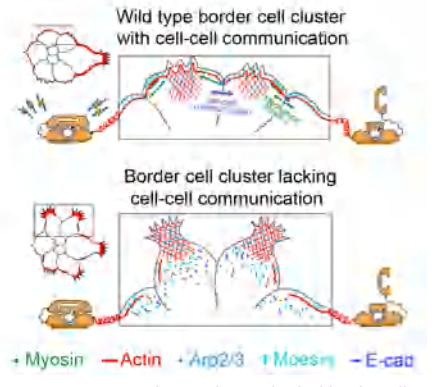


Figure 2. Diagram showing how individual border cells communicate with each other via supracellular actin-myosin network. Disruption of the supracellular network results in lack of cell-cell communication and defects in collective cell migration (from Wang H. et al., 2020)

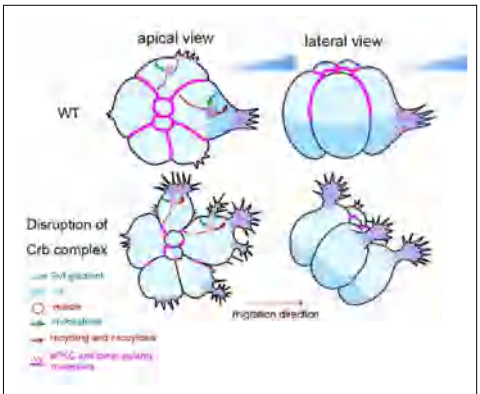
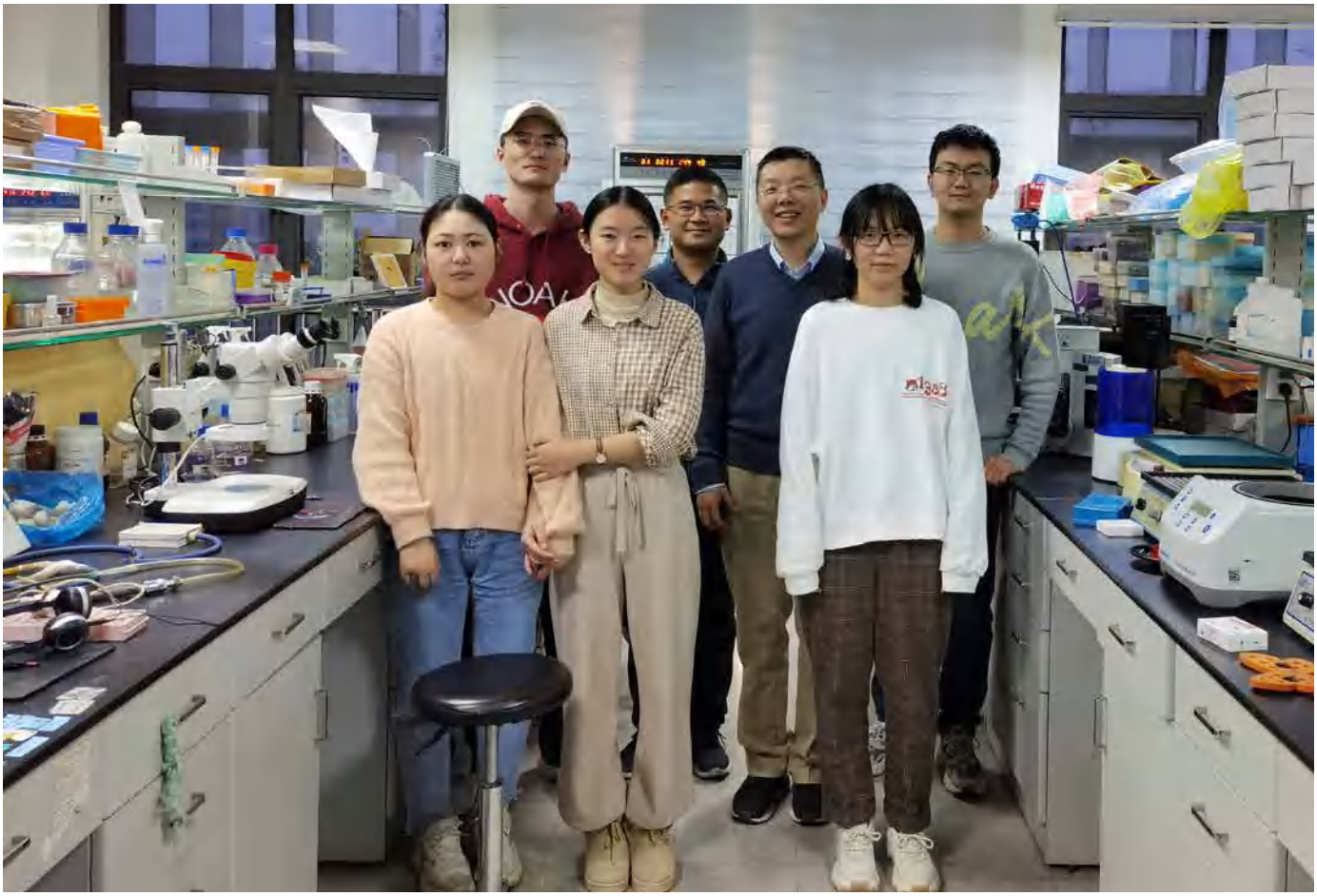


Figure 3. Diagram showing WT and Crb complex-deficient border cells in apical and lateral views. The model proposes how the two pools of aPKC and three distinct cell polarities are established by the Crb and Par complexes and endocytic recycling machinery. (from Wang et al., 2018)

Selected Publications

1. Wang, X., Wang, H. *, Liu, L., Li, S., Emery, G. and Chen, J. * Temporal coordination of collective migration and lumen formation by antagonism between two nuclear receptors. Iscience (2020)
2. Wang, H. *, Guo, X., Wang, X., Wang, X. and Chen, J. * Supracellular actomyosin mediates cell-cell communication and shapes collective migratory morphology. Iscience (2020)
3. Guo, X., Luo, J., Wang, H.*, and Chen, J*. SERCA regulates collective cell migration by maintaining cytoplasmic Ca2+ homeostasis, Journal of Genetics and Genomics (2019)
4. Luo, J.*, Zhou P., Guo, X., Wang, D., and Chen, J *. The polarity protein Dlg5 regulates collective cell migration during Drosophila oogenesis, accepted by PLoS One (2019)
5. Luo, J., Shen, P.* and Chen, J.*. A modular toolset of phiC31-based fluorescent protein tagging vectors for Drosophila. Fly (2019)
6. Wang, H., Qiu, Z., Xu, Z., Chen, S., Luo, J., Wang, X.*and Jiong Chen*. aPKC is a key polarity molecule coordinating the function of three distinct cell polarities during collective migration, Development (2018)
7. Kang, D., Wang, D., Xu, J., Quan, C., Guo1, X., Wang, H., Luo, J., Yang, Z., Chen, S.*, Chen, J.*. The InR/Akt/TORC1 growth-promoting signaling negatively regulates JAK/STAT activity and migratory cell fate during morphogenesis, Developmental Cell (2018)
8. Wu, J., Wang, H., Xuan, G., Chen, J.* Cofilin-mediated actin dynamics promotes actin bundle formation during Drosophila bristle development Molecular Biology of the Cell (2016)
9. Wan, P., Wang, D., Luo, J., Chu, D., Wang, H., Zhang, L., and Chen, J.* Guidance receptor promotes asymmetric distribution of Exocyst and recycling endosome during collective cell migration Development (2013).
10. Chu, D., Pan, H., Wan, P., Wu, J., Luo, J., Zhu, H. and Chen, J.* AIP1 Acts with Cofilin to Control Actin Dynamics during Epithelial Morphogenesis Development 139:3561-3571. (2012)
11. Zhang, L., Luo, J., Wan, P., Wu, J., Laski, F. and Chen, J.* Regulation of cofilin phosphorylation and asymmetry in collective cell migration during morphogenesis. Development 138, 455-64. (2011)



Group members

Associate Research Fellow:
WANG Heng, Ph.D

Graduate Students:
QU Chen
DONG Zhixiang
KAN Yating
PEI Ruoyi
WANG Xinyi

Former Graduate Students:

ZHANG Lijun (Ph.D)	KANG Di (Ph.D)	WU Mengqi (Ph.D)
CHU Dandan (Ph.D)	WANG Heng (Ph.D)	Xu Zehao (Ph.D)
WAN Ping (Ph.D)	GUO Xuan (Ph.D)	XU Jianbing (MS)
WU Jing (Ph.D)	WANG Dou (Ph.D)	QIU Zhiqian (MS)
LUO Jun (Ph.D)	WANG Xianping (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

Regulation of hedgehog signaling

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.

E3 ligase Herc4 regulates Hedgehog signaling through promoting Smoothened degradation

Hedgehog (Hh) signaling plays conserved roles in controlling embryonic development, its dysregulation causes many diseases including cancers. The G protein-coupled receptor Smoothened (Smo) is the key signal transducer of the Hh pathway, whose posttranslational regulation has been shown to be critical for its accumulation and activation. Ubiquitination has been reported to be an essential posttranslational regulation of Smo. Here, we identify a novel E3 ligase of Smo, Herc4, which binds to Smo and regulates Hh signaling by controlling Smo ubiquitination and degradation. Interestingly, our data suggest that Herc4-mediated Smo degradation is regulated by Hh in PKA-primed phosphorylation dependent and independent manners.

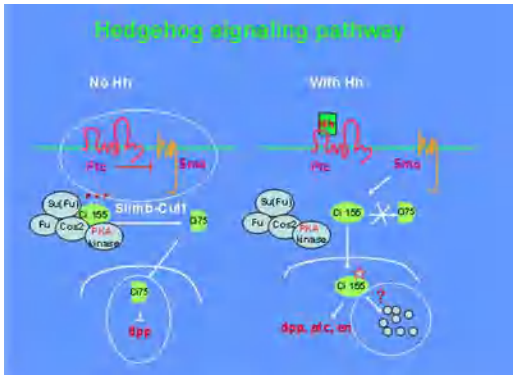


Fig1. Hedgehog signaling pathway in Drosophila.

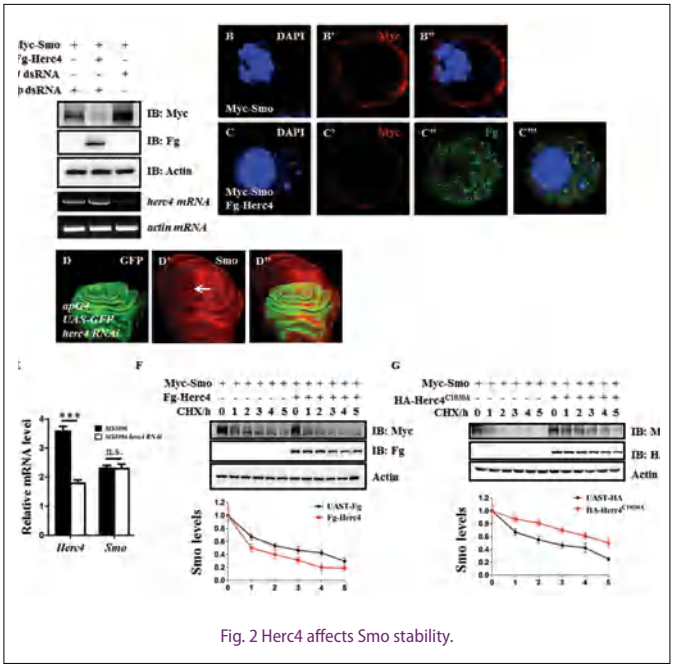


Fig. 2 Herc4 affects Smo stability.

(A) Overexpression of Fg-Herc4 downregulated Myc-Smo protein level. Knockdown of herc4 upregulated Myc-Smo protein. herc4-dsRNA could effectively knock down herc4 mRNA level in S2 cells (bottom two panels). (B-C‴) S2 cells transfected with indicated constructs were stained by Myc, Flag antibody and DAPI. Of note, Herc4 inhibited Smo cell membrane accumulation (compare Figures 2C-C‴ with Figures 2B-B″). The nuclei were showed by DAPI staining. (D-D″) Knockdown of herc4 with apG4 increased the anterior compartment Smo protein level of the wing disc. Arrows indicate the increase of Smo. (E) The relative mRNA level of smo in wing discs. (F-G) Western blots of lysates from S2 cells expressing indicated proteins and treated with CHX for the indicated time intervals. Quantification analyses were shown below. The results were presented as means±s.d. of values from three independent experiments. Of note, Herc4 could promote Smo degradation (F). Herc4^{C1030A} could hamper Smo degradation (G).

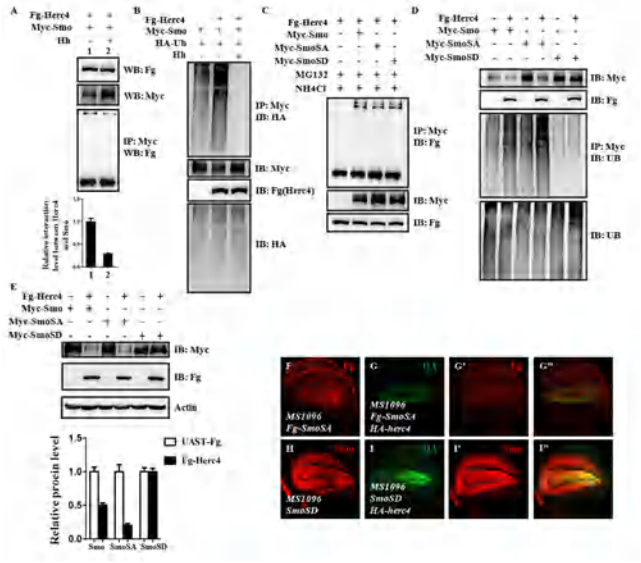


Fig. 3 Hh inhibits Herc4-mediated Smo ubiquitination.

(A) Hh treatment inhibited the interaction of Herc4 and Smo. (B) Hh decreased Smo ubiquitination mediated by Herc4. (C) S2 cells were transfected with indicated plasmids and treated with the proteasome/lysosome inhibitors MG132 and NH4Cl. Fg-Herc4 interacts equally with Myc-Smo, Myc-SmoSA and Myc-SmoSD. (D) From cell based ubiquitin assay, Herc4 upregulated the ubiquitination level of Smo and SmoSA, but did not affect the ubiquitination level of SmoSD. (E) Overexpression of Herc4 in S2 cells apparently decreased the protein level of Smo and SmoSA, but did not affect SmoSD protein level. (F-G″) Overexpression of Herc4 in wing discs with MS1096-gal4 decrease the expression level of Fg-SmoSA. (compare Figure 3G′ with 3F). (H-I‴) Overexpression of Herc4 in wing discs with MS1096-gal4 has no effect on the protein level of SmoSD. (compare Figure 3I′ with 3H).

Selected Publications

1. Zhang Q*.Jiang J*. (2021) Regulation of Hedgehog Signal Transduction by Ubiquitination and Deubiquitination. Int. J. Mol. Sci. 22(24), 13338;
2. Jiang W#, Yao X#, Shan Z, Li W, Gao Y, Zhang Q*. (2019) E3 ligase Herc4 regulates Hedgehog signalling through promoting Smoothened degradation. J Mol Cell Biol. 11(9):791-803.
3. Zhou Z#, Yao X#, Pang S, Chen P, Jiang W, Shan Z, Zhang Q*. (2018) The deubiquitinase UCHL5/UCH37 positively regulates Hedgehog signaling by deubiquitinating Smoothened. J Mol Cell Biol. 10(3):243-257.
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5. Zhou Z#, Yao X#, Li S#, Xiong Y, Dong X, Zhao Y, Jiang J*, Zhang Q*. (2015) Deubiquitination of Ci/Gli by Usp7/HAUSP Regulates Hedgehog Signaling. Dev Cell. 34(1):58-72.
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

Graduate Students

Zhaoliang Shan
Hui Zuo
Chunhua Jian
Shengnan Li
Xiuxiu Ti
Ying Wang
Yuxue Gao
Yuecen Sun

Former graduate students

Chen Liu (Ph.D)
Siya Su (Ph.D)
Zizhang Zhou (Ph.D)
Shu Pang (MS)
Ping Chen (Ph.D)
Weirong Jiang (MS)
Xia Yao (Ph.D)
Wenting Li (MS)

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 and identified a few novel genes that play essential roles in Xenopus embryonic development. He received the degree Dr. rer. nat. and graduated summa cum laude in 2002. 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. In October 2008, he was offered the professor at MARC and set up the laboratory for developmental biology and cancer biology. The results in his group suggest that the property of neural stemness is the key to understand tumorigenicity and differentiation potential. He proposes that "Tumorigenesis represents a process of loss of original cell identity and gain of properties of neural stemness" and "Neural stemness represents the ground or basal state of cell tumorigenicity and differentiation potential."

Contact Information

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

Neural stemness represents the ground state of cell tumorigenicity and differentiation potential

Our previous studies demonstrated that cancer cells share characteristics of neural stem/progenitor cells because 1) cancer cells exhibit neuronal differentiation potential and 2) cancer cells share regulatory networks with neural stem/progenitor cells. This led us to the proposal that tumorigenesis may represent a process of loss of original cell identity and gain of neural stemness. However, it remains to be elucidated whether the property of neural stemness is the source of cell tumorigenicity. In this year, in the first work we show that neural stem cells have the potential of tumorigenicity and pluripotent differentiation; when a somatic cell, the myoblast C2C12 cell, was dedifferentiated by knocking out the key muscle cell differentiation gene, Myod1, the cell gained the property of neural stemness, tumorigenicity and pluripotent differentiation potential (Figure 1); and the evolutionary advantage of neural state pre-determined the potential of tumorigenicity and differentiation potential of neural stemness. In the second work, we show that the machineries for the basic cell physiological functions, including cell cycle, ribosome, proteasome, epigenetic modification, etc., which are enriched in neural stem cells or embryonic neural cells during vertebrate embryogenesis and promote cancers, are concertedly regulated to maintain neural stemness in cancer cells and neural stemness (Figure 2). In the third work, by using the principle of developmental biology, we demonstrate that a general principle to suppress cell tumorigenicity should be the use of non-neural pro-differentiation factors to repress neural stemness in tumorigenic cells. The fourth work is a summary of our own results on neural stemness, tumorigenicity and differentiation potential, in combination with historical findings in developmental and cancer biology and evolution, in particular the "neural default model" of embryonic pluripotent cells in developmental biology. I propose that neural stemness represents the ground state of tumorigenicity and differentiation potential. Neural stemness is a cell property that unifies developmental biology and cancer biology (Figure 3). For detailed information, see our papers listed below.

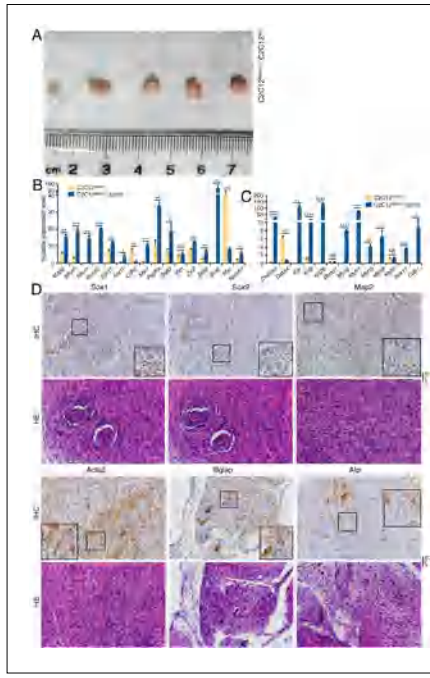

Figure 1. Myoblast C2C12 cells with knockout of Myod1 gene showed tumorigenicity (A) and differentiation potential (B-D).

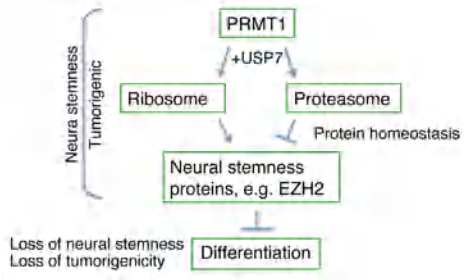

Figure 2. A model depicting the coordinated regulation of the ribosome and proteasome by PRMT1 in the maintenance of neural stemness in cancer cells and neural stem cells.

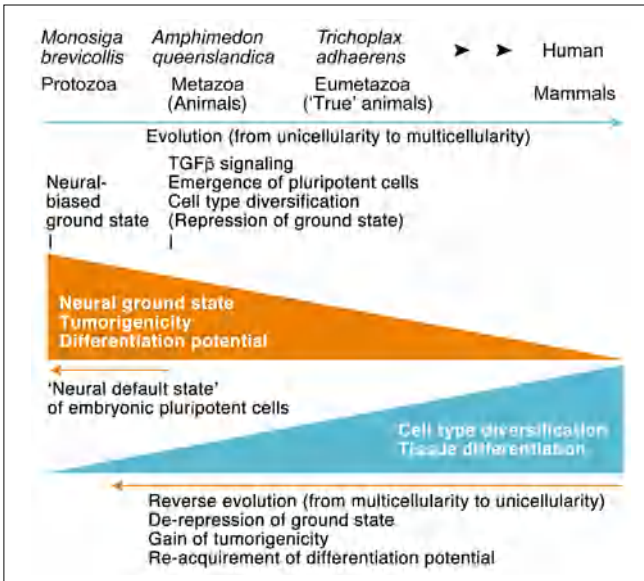

Figure 3. Neural-biased unicellular state as the ground state of tumorigenicity and differentiation potential

Selected publications (*Correspondence author)

1. Cao Y*. Neural is Fundamental: Neural Stemness as the Ground State of Cell Tumorigenicity and Differentiation Potential. Stem Cell Rev Rep. 2021 Oct 29. doi: 10.1007/s12015-021-10275-y. Epub ahead of print. PMID: 34714532.
2. Chen L, Zhang M, Fang L, Yang X, Cao N, Xu L, Shi L, Cao Y*. Coordinated regulation of the ribosome and proteasome by PRMT1 in the maintenance of neural stemness in cancer cells and neural stem cells. J Biol Chem. 2021 Oct 4;297(5):101275.
3. Yang X, Cao N, Chen L, Liu L, Zhang M, Cao Y*. Suppression of Cell Tumorigenicity by Non-neural Pro-differentiation Factors via Inhibition of Neural Property in Tumorigenic Cells. Front Cell Dev Biol. 2021 Sep 14;9:714383.
4. Xu L, Zhang M, Shi L, Yang X, Chen L, Cao N, Lei A, Cao Y*. Neural stemness contributes to cell tumorigenicity. Cell Biosci. 2021 Jan 19;11(1):21.
5. Lei A, Chen L, Zhang M, Yang X, Xu L, Cao N, Zhang Z, Cao Y*. EZH2 Regulates Protein Stability via Recruiting USP7 to Mediate Neuronal Gene Expression in Cancer Cells. Front Genet. 2019 May 3;10:422.
6. 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)
7. 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.
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9. 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.
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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
Lei Anhua, PhD
Xu Liyang, PhD
Shi Lihua, MSc
Chen Lu, PhD
Cao Ning, MSc
Yang Xiaoli, PhD

Graduate students:
Zhang Min
Liu Yang
Wang Qi (Rotation)
Wang Chen (Rotation)

Technicians:
Ma Haihua

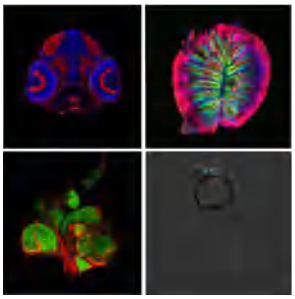
Xin Lou Ph.D.

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

Contact Information

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

Vertebrate Organogenesis and regeneration



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

Currently, our research focuses on the following aspects

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

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

2)IDENTIFICATION OF NOVEL REGULATORS OF ORGANOGENESIS.

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

Selected Publications

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

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

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

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

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

Group members

Lab Head	Graduate Students
Xin Lou	Yuxi Yang
	Xue Zhang
	Xiaoxue Pu
	YuanYuan Wei

Qingshun Zhao, Ph.D

Qingshun 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 gene that is responsible for RA synthesis in zebrafish early development (Liang et al, 2008). Like mammals, zebrafish possesses a third *cyp26* gene (*cyp26c1*) (Gu et al., 2005) in addition to *cyp26a1* and *cyp26b1*. The Cyp26c1 metabolizes RA but not retinol or retinal in a similar way to Cyp26a1, the major gene that is responsible for RA metabolism in zebrafish early development (Gu et al., 2006). Like *cyp26a1*, proper expression of *cyp26c1* at early developmental stage is essential for the development of anterior–posterior axis and left–right symmetry in zebrafish embryos (Gu et al., 2005; Gu et al., 2006). Analyzing the promoter of *cyp26a1*, we reveal that zebrafish *cyp26a1* possesses three conserved RAREs in response to RA signal and therefore maintains RA homeostasis in a feedback way (Hu et al., 2008; Li et al., 2012). Other than Cyp26s that can limit RA signaling, Ncor1 (nuclear receptor co-repressor) is essential for patterning the anterior–posterior axis of zebrafish hindbrain by actively repressing RA signaling (Xu et al., 2009). Consistent with these results, *znfl1* whose expressions are in response to RA signaling, mediate the roles of RA in patterning zebrafish posterior neuroectoderm by acting upstream of *pou5f3* and *sall4* (Dong et al., 2017). Additionally, Znfl1s regulate left-right asymmetry patterning through controlling the expression of *fgfr1a* (Li et al., 2019).

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* and upstream to *scl* in a dose dependent manner (Liang et al., 2012). Furthermore, zebrafish microRNA miR-210-5p inhibits primitive myelopoiesis by silencing *foxj1b* and *slc3a2a* mRNAs downstream of *gata4/5/6* transcription factor genes (Jia et al., 2019). Moreover, RA is also essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos (Li et al., 2016). Additionally, Ncor1 and Ncor2 play essential but distinct roles in zebrafish primitive myelopoiesis (Li et al., 2014). On the other hand, the differentiation of ventral mesoderm is affected by environmental factors, excessive sodium nitrite affects zebrafish valve leaflet formation by producing too much NO signaling (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 demonstrate that *foxc1a* is essential for somitogenesis by controlling Fgf and Notch signaling through restricting the expression of *aldh1a2* in zebrafish paraxial mesoderm directly (Li et al., 2015) and plays essential roles in zebrafish cardiogenesis by directly activating the expression of *nkx2.5*, encoding a transcriptional regulator of cardiac progenitor cells (Yue et al., 2018), and directly inhibiting the expression of *aldh1a2* in *foxc1a*-expressing cells (Gu et al., Unpublished data). In human cells, we demonstrate that FOXC1 does regulate human NKX2-5 expression in a dose-dependent manner via direct binding to its proximal promoter. A comparison of FOXC1 mutant function in the rat cardiac cell line H9c2 and zebrafish embryos suggested that the zebrafish embryos might serve as a more representative model system than the H9c2 cells. Three of the Axenfeld-Rieger syndrome FOXC1 mutations tested increased whereas a fourth repressed the expression of NKX2-5 implying that mutant FOXC1s might play etiological roles in CHD by abnormally regulating NKX2-5 in the patients. To sum up, zebrafish embryos can serve as a useful in vivo platform for rapidly evaluating disease-causing roles of mutated genes (*Zhang et al., 2020*).

Engineered endonuclease including ZFN, TALEN and CRISPR/Cas9 are powerful tools to create genome edited animals without species limitation. Employing 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), and the *mstna* null yellow catfish exhibit double muscle phenotype with muscle hyperplasia (Zhang et al., 2019). By co-microinjecting *yfp-nanos3* mRNA with genome editing tools to make founders and then screen them with the help of tentatively fluorescent-labeled PGCs, we invent a new method that significantly increases the ease and speed of generating heritable knockin animals with CRISPR/Cas9 (Dong et al., 2014). Using this method, we develop "two-step strategy" to generate an *aldh1a2* floxed zebrafish line (*aldh1a2*$^{flox/flox}$) by first inserting mloxP sites into its 3rd intron and then into its 4th intron. With the systemic expression of Cre in the eggs of *aldh1a2*$^{flox/flox}$ zebrafish, we obtained an aldh1a2 conventional knockout zebrafish line (*aldh1a2*$^{+/-}$) (Gu et al., Unpublished data). Interestingly, the embryos whose primordial germ cells are eliminated at early development grow up as all-male-like sterile zebrafish (Zhou et al., 2018). Collaborating with the groups of Professors Zhou and Zhu, we developed an alternative novel tool for DNA editing (SGN: structure-guided nuclease) without target sequence limitation (Xu et al., 2016). Unfortunately, our further efforts do not support that the system works in human colorectal carcinoma cell line (HCT116), nor in producing any germline transmission zebrafish mutants (Zhang et al., Unpublished data)

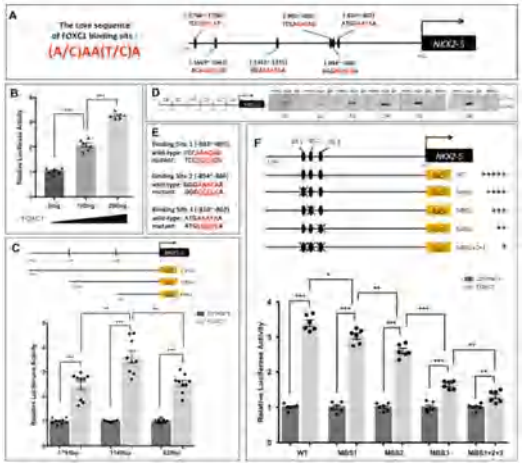


Figure 1 .FOXC1 directly regulates the expression of NKX2-5 by binding to its proximal promoter in H9c2 cells.

A, Schematic showing putative FOXC1 transcription factor binding sites in 1791 bp 5′-flanking sequence upstream of NKX2-5 translation start site (ATG). B, Results of Dual-Luciferase Reporter Assay showing the responses of NKX2-5 promoter to different doses of FOXC1. C, Schematic (top) showing the firefly luciferase reporter expression constructs comprising the different lengths of upstream regulatory sequence of NKX2-5, namely 1791 bp, 1149 bp or 630 bp, and the coding sequences of NKX2-5 or firefly luciferase, and the results (below) of Dual-Luciferase Reporter Assay on the three expression constructs. D, Schematic (left) showing the dissection of the 1149 bp regulatory sequences of NKX2-5 into S1-S6 regions and the results of ChIP-PCR assay (right) indicating that S5contains FOXC1-binding sites. E, The wild-type sequences and location of FOXC1-binding sites (BS) in S5 of NKX2-5 regulatory sequence (top), and the mutant FOXC1 binding sites (MBS) with changed core sequence. F, Schematic (top) showing the reporter expression constructs carrying wild-type BS or MBS of FOXC1, and the results (below) of Dual-Luciferase Reporter Assay on the five expression constructs. X-axis (B, C, F): The amount of overexpressed FOXC1 (B), the reporter expression constructs with different lengths of regulatory sequences (C), or the reporter expression constructs carrying wild-type BS or MBS of FOXC1 (F). Light grey columns (C, F): transfected with wild-type FOXC1;Dark grey columns (C, F): transfected with the same amount of functional null mutated FOXC1(p.Q70Hfs*8) as control. Y-axis (B, C, F): Relative activity of firefly luciferase reporter.

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

1) Qinxin Zhang, Dong Liang, Yunyun Yue, Luqingqing He, Nan Li, Dongya Jiang, Ping Hu, Qingshun Zhao*. 2020. Axenfeld-Rieger syndrome-associated mutants of the transcription factor FOXC1 abnormally regulate NKX2-5 in model zebrafish embryos. *The Journal of Biological Chemistry*, 295(33):11902-11913.

2) Wenshuang Jia, Dong Liang; Nan Li, Meijing Liu, Zhangji Dong, Jingyun Li, Xiaohua Dong, Yunyun Yue, Ping Hu, Jihua Yao, Qingshun Zhao*. 2019. Zebrafish microRNA miR-210-5p inhibits primitive myelopoiesis by silencing *foxj1b* and *slc3a2a* mRNAs downstream of *gata4/5/6* transcription factor genes. *The Journal of Biological Chemistry*, 294(8):2732-2743.

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

4) 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.

5) 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.

6) 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)

7) 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.

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Group members

Graduate Students

Luqingqing He

Nan Li

Zhaojunjie Zhang

Dongya Jiang

Yunfeng Zhang

Shuang Wang

Yuxuan Wei

Old Lab Members

Fang Xu, PhD (2007)
Ping Hu, PhD (2009)
Jie Bao, PhD (2009)
Dong Liang, PhD (2011)
Kui Li, PhD (2011)
Jingyun Li, PhD (2013)
Junbo Li, PhD (2013)
Zhangji Dong, PhD (2014)
Wenshuang Jia, PhD (2015)
Xiaohua Dong, PhD (2015)
Shasha Cao, PhD (2016)
Yunyun Yue, PhD (2017)
Meijing Liu, PhD (2018)
Chun Gu, PhD (2019)
Qinxin Zhang (2019)
Zhiying Zou, MS (2005)
Lu Sun, MS (2005)
Wei Song, MS (2006)
Xiaolin Wang, MS (2006)
Mei Zhang, MS (2008)

Metabolism and Immunity

Xiang Gao, Ph.D.

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

Contact Information

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

Physiological regulation and metabolic homeostasis

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

1. Uncovering that magnesium enhances survival of sepsis by blocking pyroptosis (Figure 1)

Hypomagnesemia is a significant risk factor for critically ill patients to develop sepsis, a life-threatening disease with a mortality rate over 25%. Our clinic data analysis showed that hypomagnesemia is associated with a decreased monocyte count in septic patients. At the cellular level, we found that Mg2+ inhibits pyroptosis. Specifically, Mg2+ limits the oligomerization and membrane localization of gasdermin D N-terminal (GSDMD-NT) upon the activation of either the canonical or non-canonical pyroptotic pathway. Mechanistically, we demonstrated that Ca2+ influx is a prerequisite for the function of GSDMD-NT. Mg2+ blocks Ca2+ influx by inhibiting the ATP-gated Ca2+ channel P2X7, thereby impeding the function of GSDMD-NT and inhibiting lipopolysaccharide (LPS)-induced non-canonical pyroptosis. Furthermore, Mg2+ administration protects mice from LPS-induced lethal septic shock. Together, our data reveal the underlying mechanism of how Mg2+ inhibits pyroptosis and suggest potential clinic applications of magnesium supplementation for sepsis prevention and treatment. (Wang et al, Cell Death Differ)

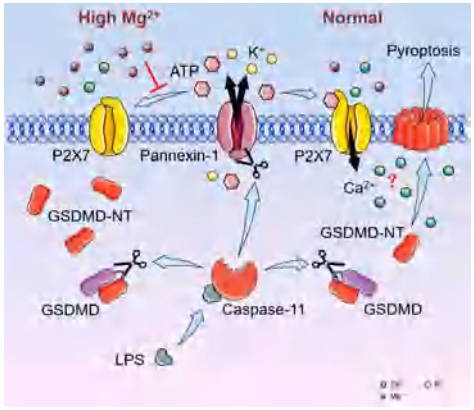

Figure 1. Magnesium enhances survival of sepsis by blocking pyroptosis

2. A SNP of bacterial blc disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption (Figure 2)

Alteration of commensal bacterial composition is associated with many inflammatory diseases. However, few studies pinpointed the specific bacterial genes that may suppress host immune responses against microbes. By screening 3,983 E. coli mutants, we discovered that 9 bacterial genes, when deleted, activate innate immunity in the host Caenorhabditis elegans. The gene encoding bacterial lipocalin (blc), among these 9 genes, shown a distinctive SNP in many clinic pathogenic bacteria. We found bacteria with this SNP, which converts the Blc G84 to Blc E84, are highly enriched in the fecal of inflammatory bowel disease (IBD) patients. Exposure to the BlcE84-encoding bacteria resulted in epithelial barrier disruption and immune activation both in worm and mouse. Detailed analysis indicated the infection of the BlcE84-encoding bacteria causes a significant decrease in lysophosphatidylethanolamine (LPE) levels in the intestine, and subsequently the disruption of gut epithelial integrity in mice. Consistently, the levels of LPE in IBD patients are significantly lower compared to that of health people. Finally, supplement of LPE, which activating the LPA1/PLCβ/PKC signaling, can reverse all the defects induced by the BlcE84-encoding bacteria. Our results identified a novel bacterial gene in E. coli that regulates gut integrity and immunity. (Zou et al, Ebiomedicine).

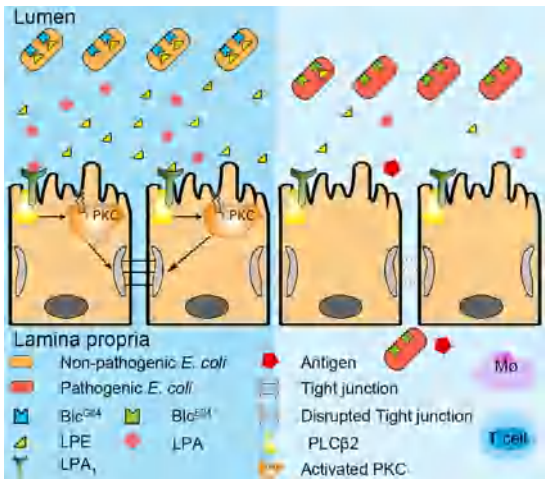

Figure 2. A SNP of bacterial blc disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption

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1. Gao, L., X. Dong, W. Gong, W. Huang, J. Xue, Q. Zhu, N. Ma, W. Chen, X. Fu, X. Gao, Z. Lin, Y. Ding, J. Shi, Z. Tong, T. Liu, R. Mukherjee, R. Sutton, G. Lu, and W. Li*, Acinar cell NLRP3 inflammasome and GSDMD activation mediates pyroptosis and systemic inflammation in acute pancreatitis. Br J Pharmacol, 2021.
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7. Wang, Y., P. Shi, Q. Chen, Z. Huang, D. Zou, J. Zhang, X. Gao*, and Z. Lin*, Mitochondrial ROS promote macrophage pyroptosis by inducing GSDMD oxidation. J Mol Cell Biol, 2019.
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Group members

Group Leader

Xiang Gao

Graduate Students

Xu Li
Jingwen Pei
Qiyao Liu
Qianyue Chen
Minli Sun
Lulu Kang
Yushu Wang
Manyun Li
Lu Li

Technicians

Jiafeng Zou
Jiaxiang Zou

Shuai Chen, Ph.D.

Dr. Shuai Chen received his Ph.D. degree 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 Metabolic Biology in 2012. He is the recipient for Distinguished Young Scholars from the National Natural Science Foundation (2020) and New Century Excellent Talents from the Ministry of Education (2013). He currently serves as Director of MARC and Director of Nanjing Biomedical Research Institute of Nanjing University (NBRI).

Contact Information

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

Metabolic Physiology and Diseases

Metabolic diseases including type 2 diabetes mellitus (T2DM), obesity and non-alcoholic fatty liver disease (NAFLD) have become prevalent world-wide in the last few decades, which urges a better understanding of their pathogenesis as well as new therapeutic strategies to combat these diseases. Insulin resistance is a common cause for the pathogenesis of these metabolic diseases, whose underlying mechanism is still not clear. Insulin actions exhibit a tissue/pathway-dependent manner. Therefore, the goal of my laboratory is to understand the molecular basis of tissue/pathway-specific insulin actions, the pathogenic mechanisms of metabolic diseases, and discoveries of leading compounds to combat these diseases. We are currently running three research programmes in the laboratory: (1) protein modifications in mediating insulin actions, (2) tissue/pathway-specific insulin actions and diabetic complications, (3) discoveries of therapeutic targets and agents for metabolic diseases.

The recent progresses of my lab is as follows:

1. Tissue-specific splicing and dietary interaction of a mutant As160 allele determine muscle metabolic fitness

The genetic/dietary interaction plays an important role in the metabolic regulation as well as the pathogenesis of metabolic diseases. A common AS160^{R684X} variant has recently been identified in Greenlandic and North American Inuit with an allele frequency of 17-27%, which confers muscle insulin resistance and type 2 diabetes. This mutation exists in a long splicing isoform of AS160 but not in a short splicing isoform. To delineate pathogenic mechanism of this AS160 mutation, we generated an AS160^{R691X} mouse model as well as an AS160^{R693X} rat model, in which the arginine residue homologous to human Arg^{684} site was mutated to a stop codon. Using these models, we show that the AS160^{R684X} mutation might be evolutionarily adaptive to lipid-rich hypoglycemic diets but cause diabetes on sugar-rich diets through selective inhibition of the long AS160 isoform, and up-regulation of the short form of AS160 through exon-switching may have potentials to restore muscle insulin sensitivity. (Yang XY., Chen QL., …, Wang H.Y.*, Chen S.* 2021 Diabetes).

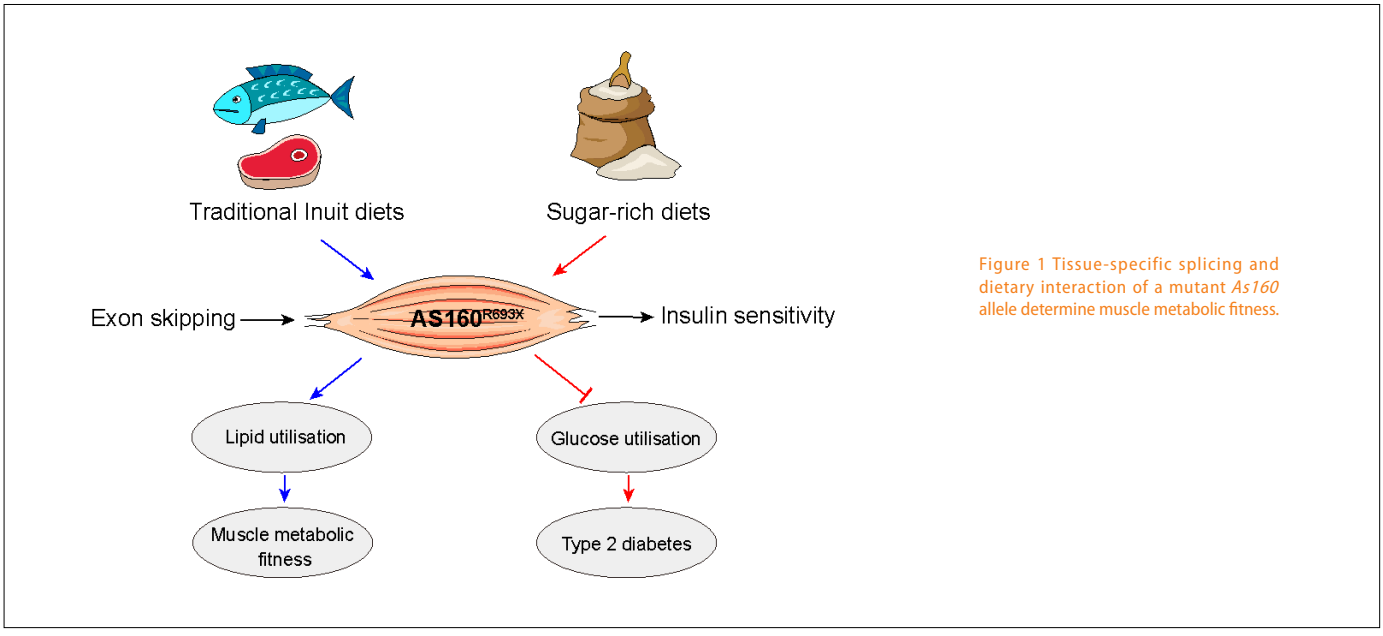

Figure 1 Tissue-specific splicing and dietary interaction of a mutant *As160* allele determine muscle metabolic fitness.

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2. Quan C, Du Q, Li M, Wang RZ, Su S, Zhu SS, Chen QL, Sheng Y, Chen L, Wang H, Campbell DG, MacKintosh C, Yang ZZ, Ouyang KF, Wang HY* and Chen S* (2020) A PKB-SPEG signaling nexus links insulin resistance with diabetic cardiomyopathy by regulating calcium homeostasis. Nat Commun 11(1): 2186 doi: 10.1038/s41467-020-16116-9 (* corresponding author)
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4. Quan C, Li M, Du Q, Chen QL, Wang H, Campbell D, Fang L, Xue B, MacKintosh C, Gao X, Ouyang KF, Wang HY and Chen S* (2019) SPEG controls calcium re-uptake into the sarcoplasmic reticulum through regulating SERCA2a by its second kinase-domain. Circ Res 124(5): 712-726 (* corresponding author)
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11. Chen Q.L., Quan C., Xie B.X., Chen L., Zhou S.L., Toth R., Campbell D.G., Lu S.S., Shirakawa R., Horiuchi H., Li C.J., Yang Z.Z., MacKintosh C., Wang H.Y.* and Chen S.*. (2014) GARNL1, a major RalGAP a subunit in skeletal muscle, regulates insulin-stimulated RalA activation and GLUT4 trafficking via interaction with 14-3-3 proteins. Cell Signal 26(8): 1636-1648 (* corresponding author)
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Group members

PI
Shuai Chen

Postdoc
Dr. Qiaoli Chen
Dr. Chao Quan
Dr. Ping Rong

Graduate Students
Qian Du
Sangsang Zhu
Wen Wei
Minjun Liu
Xinyu Yang
Qi Wang
Weikuan Feng
Kun Zhou
Ruizhen Wang
Ye Cao
Longfei Ding

Technicians
Dunyu Tian
Chuanxiu Liu

Former Lab Members
Dr. Bingxian Xie
Dr. Liang Chen
Dr. Min Li
Dr. Yang Sheng

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

Basic Biology of Aging

Aging is a process of function decline accompanied with increased mortality rate over time. 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. In order to characterize how IIS and TOR pathway interact with each other to modulate aging, we constructed a double mutant in DAF-2 (IGF-1 receptor) and TOR target RSKS-1 (ribosomal S6 kinase). Surprisingly, this daf-2 rsks-1 mutant shows a nearly 5-fold, synergistic lifespan extension (Figure 1A). Using transcriptome profiling, we demonstrated that the underlying mechanisms involve positive feedback regulation of the DAF-16/FOXO transcription factor via the key energy homeostasis regulator AMPK (Figure 1B, C). We then performed polysomal profiling coupled with mRNA-Seq to identify genes that are translationally regulated in the daf-2 rsks-1 mutant and characterize their roles in aging (Figure 1D). Eventually, we identified a translationally regulated non-autonomous mitochondrial stress response mechanism in the modulation of lifespan by insulin-like signaling and S6K (Figure 1E).

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) Cell non-autonomous regulation of mitochondrial stress response.

2) Lipid metabolism in dietary restriction-induced lifespan extension.

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

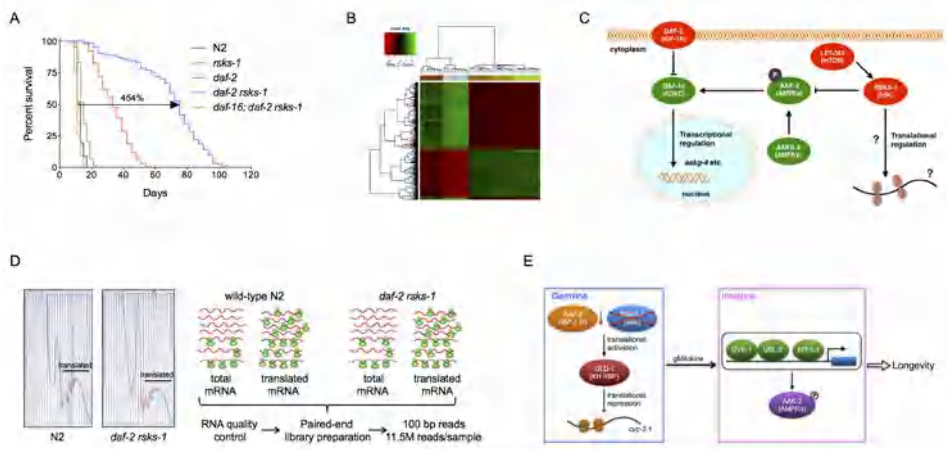

Figure 1. Functional genomics analysis of the super long-lived daf-2 rsks-1 mutant.
(A) Double mutations in DAF-2 (IGF-1 receptor) and RSKS-1 (ribosomal S6 kinase) leads to nearly 5-fold synergistic lifespan extension, which requires the DAF-16 (FOXO) transcription factor. (B) Transcriptome profiling 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 mRNA-Seq were performed to identify genes that are regulated at the translational level in the daf-2 rsks-1 mutant. (E) A model depicting the translational repression of CYC-2.1 (cytochrome c) by the RNA-binding protein GLD-1 in the germline non-autonomously activates mitochondrial unfolded protein response (UPRmt) and AMPK in the intestine via germline-produced mitokine (gMitokine) signaling, which leads to significant lifespan extension in the daf-2 rsks-1 mutant.

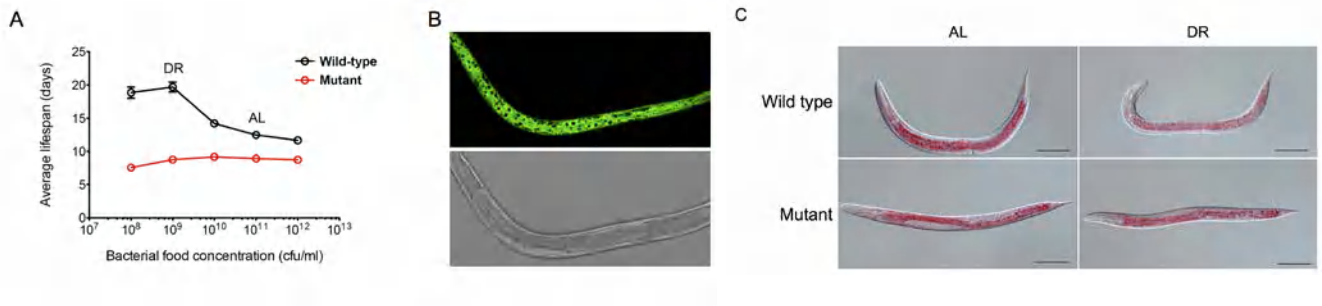

Figure 2. Characterization of lipid metabolism in dietary restriction-induced lifespan extension.

(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):

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5. Lei Hou#, Dan Wang#, Di Chen#, Yi Liu, Yue Zhang, Hao Cheng, Chi Xu, Na Sun, Joseph McDermott, William B. Mair, Jing-Dong J. Han, A Systems Approach to Reverse Engineer Lifespan Extension by Dietary Restriction. Cell Metabolism, 2016, 23(3): 529-540. (#, co-first authors)
6. Jianfeng Lan, Xuan Zhang, Di Chen*, Molecular Mechanisms of Dietary Restriction in Aging - Insights from Caenorhabditis elegans Research, Science China Life Sciences, 2015, 58(4): 352-358.
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Group members

Principle Investigator

Di Chen

Graduate Students

Zi Wang
Xiao Zang
Hanxin Zhang
Yiyan Zhang

Former Graduate Student

Jianfeng Lan (Ph.D.)
Xuan Zhang (MS)
Di Wu (Ph.D.)
Lina Zou (Ph.D.)

Technical Assistant

Zixing Wu

Chao-Jun Li, Ph.D

Chao-Jun Li received his Ph. D in Physiology from Nanjing University in 1994. He did his postdoctoral training at the Hong Kong University of Science and Technology from 1996-1998 and the Medical School of Yale University from 1999-2000. He worked as an extinguishing professor in Nanjing Normal University from 1994-2008 before he joined the Faulty of Model Animal Research Center (MARC), Nanjing University in 2008. He is now a professor of Cell Biology and a principal investigator in MARC and the Medical School of Nanjing University. He was elected as the vice-president of Chinese Society for Cell Biology since 2014.

Contact Information

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

Metabolic reprogramming and protein prenylation balance

Protein prenylation is a critical process for the membrane association of plenty of signaling proteins, including small G proteins. Geranylgeranyl diphosphate synthase (Ggps1) 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, called farnesylation and geranylgeranylation respectively. The prenylated proteins then are able to associate with membrane to initiate their function. We first identified Ggps1 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 its membrane association (Am J Path, 2011a, 2011b; J Biol Chem 2011; EMBO J, 2011). 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 (J Biol Chem 2020). We have found that Ggps1 regulated protein prenylation balance is involved in spermatogenesis and infertility (J Exp Med, 2013; Sci Rep, 2016; PLoS Genetics, 2017; CDDis 2019); hypertrophy and heart failure (J Path, 2015; Cardiovasc Res. 2018); lipid-induced muscle insulin resistance (J Biol Chem, 2015; FASEB J); intracellular vesicle formation (Nat Comms, 2020; J Path, 2016); pulmonary development (Am J Path, 2016) and NAFLD/HCC progression (J Path, 2018). Right now, we are exploring protein prenylation balance and the metabolic reprogramming like glucose/lipid shift during pathological and physiological processes.

1. GGPP allosterically activates FBP1 homotetramer to link cholesterol synthesis with glucose metabolism (Lei Fang; Chao-Jun Li)

In previous research, GGPP and its precursor FPP are mainly noticed as substrates of protein prenylation. Nevertheless, metabolite-protein interactions (MPIs) not just include covalent co- or post-translational modification but also exist non-covalent effects like co-factors, ligand-receptor, substrate-enzyme, and client-carrier relationships. And many of which represent key nodes in biochemical networks that regulate physiological processes and diseases. Herein, we have generated an extensive interaction network between GGPP and proteins in primary hepatocytes using biotin-streptavidin system (BAS)-based affinity purification combined with label-free quantification mass spectrometry. Further bioinformatics analysis revealed novel interactions between GGPP and several key enzymes in glucose and lipid metabolism. Driven by further validation experiments, we demonstrated that hepatic GGPP directly binds to a gluconeogenesis rate-limiting enzyme FBP1. GGPP binding extremely enhanced its gluconeogenic activity. Further X-ray crystalline experiments were performed to describe the structure of binding between GGPP and FBP1 and explore the mechanism how GGPP coupled cholesterol synthesis with glucose metabolism, which is critical to coordinate the metabolic programs to fit the demand of cells under stress.

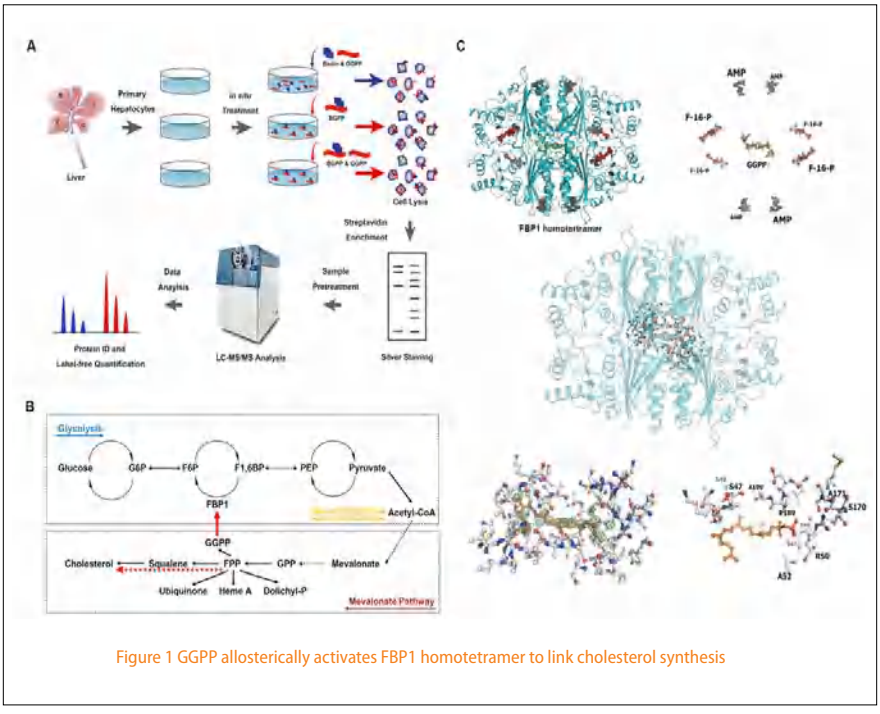

Figure 1 GGPP allosterically activates FBP1 homotetramer to link cholesterol synthesis

2. IKK-mediated regulation of the COP9 Signalosome via phosphorylation of CSN5 (Lei Fang)

The COP9 signalosome (CSN) is an evolutionarily conserved multi-subunit protein complex, which controls protein degradation through deneddylation and inactivation of cullin-RING ubiquitin E3 ligases (CRLs). Recently, the CSN complex has been linked to the NF-κB signaling pathway due to its association with the IKK complex. However, how the CSN complex is regulated in this signaling pathway remains unclear. Here, we have carried out biochemical experiments and confirmed the interaction between the CSN and IKK complexes. In addition, we have determined that overexpression of IKKα or IKKβ leads to enhanced phosphorylation of CSN5, the catalytic subunit for CSN deneddylase activity. Mutational analyses have revealed that phosphorylation at serine 201 and threonine 205 of CSN5 impairs CSN-mediated deneddylation activity in vitro. Interestingly, TNF-α treatment not only enhances the interaction between CSN and IKK but also induces an IKK-dependent phosphorylation of CSN5 at serine 201, linking CSN to TNF-α signaling through IKK. Moreover, TNF-α treatment affects the CSN interaction network globally, especially the associations of CSN with the proteasome complex, eukaryotic translation initiation factor complex, and CRL components. Collectively, our results provide new insights into IKK-mediated regulation of CSN associated with the NF-κB signaling pathway.

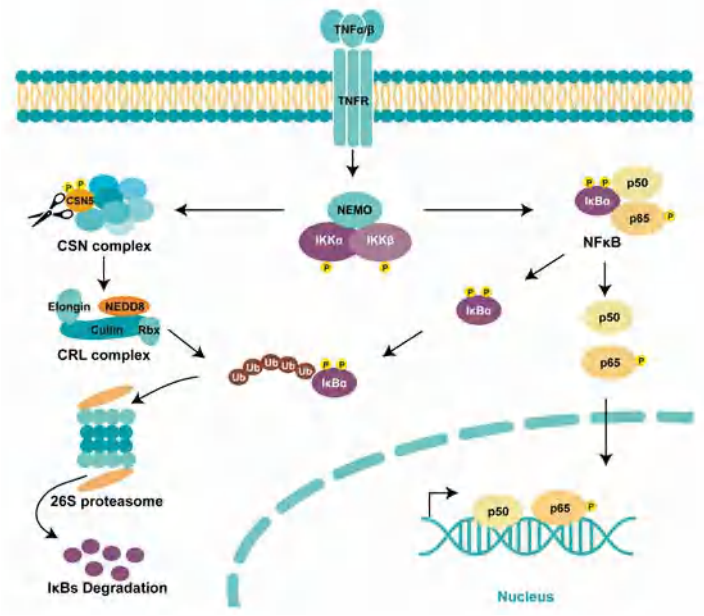

Figure 2 Working model for reciprocal regulation between the CSN complex and the NF-κB signaling pathway.

3. Ketone body regulates mitochondrial maturation and cell cycle arrest in neonatal heart stimulated by colostrum fatty acids (Chao-Jun Li)

Mitochondrial oxidative phosphorylation dominates the generation of ATP supplying for myocardial contraction and pump function in adult heart. While glycolysis is preferred in fetal heart, fetal cardiomyocytes' mitochondria undergo perinatal replacement by mature mitochondria. Meanwhile, heart regeneration capacity decreases dramatically after birth and vanishes in 1 week. Here we showed that the expression of HMGCS2, the rate-limiting enzyme in ketogenesis, and its downstream ketone body appeared transiently in postnatal heart. Fatty acid in maternal milk induced HMGCS2 expression in neonatal cardiomyocytes. The functional study showed that ketone body was essential for mitochondrial improvement induced by fatty acid in neonatal cardiomyocytes. Moreover, ketone body deficiency decreased mitochondrial respiration in neonatal heart. TEM analysis revealed that mitochondrial ultrastructure was disrupted and mitochondria were swelled in ketone body-deficient heart. Cardiac function was also impaired in postnatal heart. Simultaneously, cardiomyocyte proliferation was increased in ketone body-deficient heart. Further studies showed that histone acetylation was significantly reduced in ketone body-deficient mouse hearts. Transcriptomics and subsequent GSEA analysis showed that the expression of a large number of genes in the hearts of in ketone body-deficient mice was decreased, while the genes with increased expression were relatively few. The genes with decreased expression were mainly involved in mitochondrial oxidative phosphorylation and electron transport chain. Genes related to the cell cycle were mainly negative regulators of cell cycle. Besides, histone deacetylase (HDAC) inhibitor could also promote cardiomyocyte mitochondrial respiration and heart function but block cell proliferation. Our findings establish that the ketone body which appears transiently after birth regulates the expression of mitochondrial oxidative phosphorylation and cell cycle arrest-related genes by maintaining histone acetylation, and further promotes the perfection of cardiac mitochondrial function and cardiomyocyte cell cycle arrest in postnatal mouse.

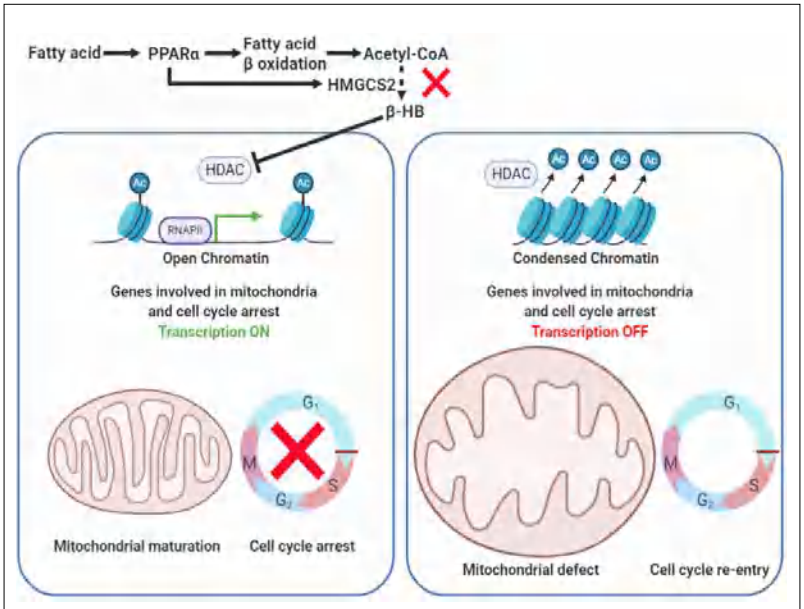

Figure 3: Working model for ketone body regulating mitochondrial function and cell cycle in postnatal heart

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

1. Danyang Chong#, Zhong Chen#, Shan Guan, Tongyu Zhang, Na Xu, Yue Zhao*, Chaojun Li*. Geranylgeranyl pyrophosphate regulates endothelial cell proliferation and anti- apoptosis during vascular development in the mouse embryo through mediating protein geranylgeranylation. J Genet Genomics. 48(2021):300-311.
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6. Liu J#, Jiang S#, Zhao Y#, et al., Xue B*, Li C*. Geranylgeranyl diphosphate synthase (Ggps1) regulates non-alcoholic fatty liver disease (NAFLD)-fibrosis progression by determining hepatic glucose/fatty acid preference under high-fat diet conditions. J Pathol. 2018 Nov;246(3):277-288. (Commentary by Fullerton MD. Does prenylation predict progression in NAFLD? J Pathol. 2018; Oct 30)
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Group members

Group Leader
Chaojun Li

Associate professor

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

Research Associate

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

Graduate Students:

Jing-Zi Zhang (Ph.D.)
Dan-Yang Chong (Ph.D.)
Yong-Juan Sang (Ph.D.)
Kang Li (Ph.D.)
Tong-Yu Zhang (Ph.D.)
Meng-Fei Zhao (Ph.D.)
Dan-Dan Bu (Ph.D.)
Yang-Qing Li (Ph.D.)
Hai-Quan Wang (Ph.D.)
Bing-Hao Wang (M.Phil.)
Xin-Ying Wang (M.Phil)
Chao-Fan Yang (M.Phil.)
Yan Guo (M.Phil.)
Xin-Yu Li (M.Phil.)
Xiao-Chen Wang (M.Phil.)

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.

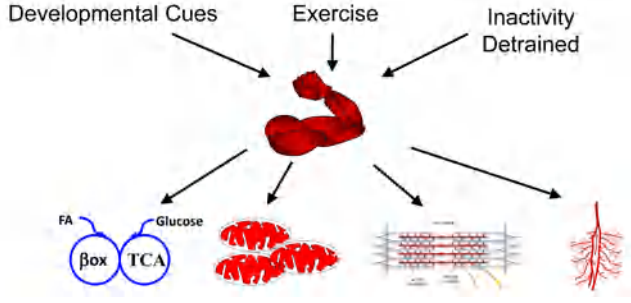

Fig 1. The muscle fitness is determined by developmental as well as physiological inputs. These inputs coordinately control the programs including fuel burning, mitochondrial ATP production, contraction, and angiogenesis.

Histone methyltransferase MLL4 controls myofiber identity and running endurance

Skeletal muscle fitness is an important determinant of health and disease and muscle depends critically on the precise orchestration of contractile and metabolic gene expression programs to direct fiber type specification and to ensure muscle performance. However, exactly how such fiber type-specific patterns of gene expression are established and maintained remains unclear. Myofibers are generally classified as slow-twitch (type I) and fast-twitch (type II) with different contractile and energy metabolism functions. Type I myofibers are rich in mitochondria, relying largely on mitochondrial oxidative metabolism and resistant to fatigue, whereas type II myofibers generally contain fewer mitochondria, have lower oxidative capacity and are fatigue sensitive, and can be sub-classified as either type IIa, IIx, or IIb in rodents based on the type of myosin heavy chain (MHC) isoform expressed. Recently, we investigated the role of histone methyltransferase MLL4, an enhancer regulator enriched in slow myofibers, in controlling muscle fiber identity as well as muscle performance. We show that MLL4 is required for establishing and maintaining slow type I fiber program to ensure running endurance. MLL4 directly binds to enhancers and functions as a coactivator of MEF2 to drive the slow-oxidative type I fiber gene program. In addition, the MLL4 regulatory circuit is associated with muscle fiber type remodeling in humans. These results uncover a pivotal role for MLL4 in specifying structural and metabolic identities of myofibers that govern muscle performance (Fig. 2).

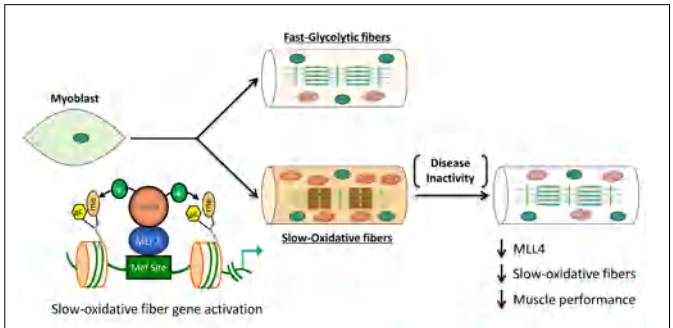

Fig 2. The schematic depicts a proposed model for the MLL4 regulatory mechanism that specifies muscle fiber identity and muscle performance.

Injury-induced IRE1α activation is required for skeletal muscle regeneration to preserve muscle mass after injury.

Skeletal muscle can undergo a regenerative process from injury or disease to preserve muscle mass and function, which is critically influenced by cellular stress responses. Inositol-requiring enzyme 1 (IRE1) is an ancient endoplasmic reticulum (ER) stress sensor and mediates a key branch of the unfolded protein response (UPR). Here, we show that IRE1α serves as a myogenic regulator in skeletal muscle regeneration in response to injury and muscular dystrophy. Utilizing cardiotoxin (CTX)-induced acute muscle injury mouse model as well as primary myoblasts in culture, we provided strong data that demonstrate injury-induced IRE1α activation is required for skeletal muscle regeneration to preserve muscle mass after injury. We also uncovered novel molecular mechanisms that IRE1α employs its RNase-dependent RIDD action to downregulate the expression of the mRNA encoding Myostatin, a critical negative regulator of muscle regeneration and growth. Further we showed that this IRE1α/Myostatin regulatory circuit is implicated in the disease progression of muscular dystrophy. Our findings reveal the physiological importance of IRE1α's RIDD activity in the control of muscle regeneration, and its dysregulation may represent an unrecognized mechanism linking ER stress to muscle degenerative diseases (Fig. 3).

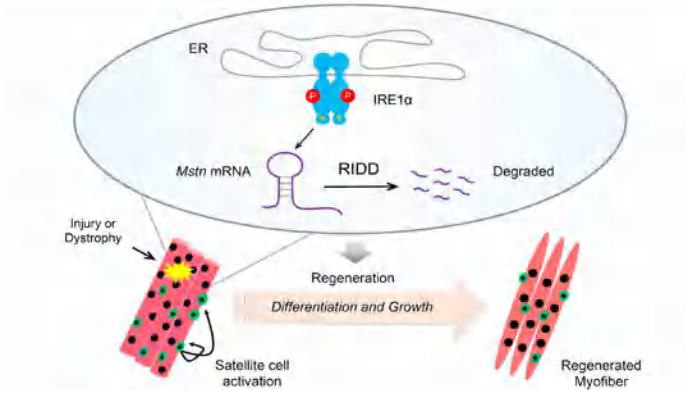

Fig 3. IRE1α regulates skeletal muscle regeneration through RIDD suppression of Myostatin. Upon injury-induced or dystrophy-associated stress, IRE1α RNase is activated in muscle cells to decay the mRNA encoding Myostatin, thereby promoting myoblast differentiation and myotube growth during the skeletal muscle regeneration response.

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Group members

Group Leader

Zhenji Gan

Research Associates

Tingting Fu
Dengqiu Xu

Graduate students

Zhisheng Xu
Yujing Yin
Zongchao Sun
Chenyun Ding
Qiqi Guo
Wanping Sun
Yan Mao
Likun Yang
Zheng Zhou
Yuhuan Jia
Xinyi Chen

Former Graduate Students

Jing Liu (Ph.D.)
Xijun Liang (Ph.D.)
Tingting Fu (Ph.D.)
Lin Liu (Ph.D.)
Liwei Xiao (Ph.D.)
Qian Zhou (MS)

Technical Assistant

Danxia Zhou

Hong-Yu Wang, Ph.D.

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

Contact Information

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

Mechanisms of metabolic disorder

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.

Group members

Group Leader

Hong-Yu Wang

Graduate students

Qian OuYang
Shu Su
Derong Liang
Jiamou Chen

Selected publications:

1. Yang XY, Chen QL, Ouyang Q, Rong P, Feng WK, Quan C, Li M, Jiang Q, Liang H, Zhao T-J, Wang H-Y and Chen S. Tissue-Specific Splicing and Dietary Interaction of a Mutant As160 Allele Determine Muscle Metabolic Fitness in Rodents. 2021 Diabetes Aug; 70(8): 1826-1842.
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7. Chen L, Chen Q, Rong P, Wang HY* and Chen S*. (2017) The energy sensing LKB1-AMPKα1 pathway regulates IGF1 secretion and consequent activation of the IGF1R-PKB pathway in primary hepatocytes. FEBS J. 2017 Jul; 284(13):2096-2109 (*corresponding author)
8. Chen Q, Xie B, Zhu S, Rong P, Sheng Y, Ducommun S, Chen L, Quan C, Li M, Sakamoto K, MacKintosh C, Chen S* and Wang HY* (2016) A TBC1D1Ser231Ala knockin mutation partially impairs 5-aminoimidazole-4-carboxamide-1-ß-D-Ribofurano-side- but not exercise-induced muscle glucose uptake in mice. Diabetologia 60(2): 336-345 (*corresponding author)
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10. Chen L, Chen Q, Xie B, Quan C, Sheng Y, Zhu S, Rong P, Zhou S, Sakamoto K, MacKintosh C, Wang HY*, Chen S*. (2016) Disruption of the AMPK-TBC1D1 nexus increases lipogenic gene expression and causes obesity in mice via promoting IGF1 secretion. Proc Natl Acad Sci U S A. Jun 28; 113(26): 7219-24. (*corresponding author)
11. Wang HY*, Quan C, Hu B, Xie, BX, Du Y, Chen L, Yang W, Yang L, Chen Q, Shen B, Hu B, Zheng ZH, Zhu HB, Huang XX, Xu GW, Chen S. (2016) A lipidomics study reveals hepatic lipid signatures associating with deficiency of the LDL receptor in a rat model. Biol Open. Jul 15; 5(7): 979-86. (☆ corresponding author)
12. Li M, Quan C, Toth R, Campbell DG, MacKintosh C*, Wang HY*, Chen S*. (2015) Fasting and systemic insulin signaling regulate phosphorylation of brain proteins that modulate cell morphology and link to neurological disorders. J Biol C 2015 Dec 11; 290 (50): 30030-41. (*corresponding author)
13. Quan, C, Xie BX, Wang HY*, Chen S*. (2015) PKB-Mediated Thr649 Phosphorylation of AS160/TBC1D4 Regulates the R-Wave Amplitude in the Heart. PLoS One. Apr 29;10(4):e0124491(*corresponding author)

Yan LI Ph.D.

Dr. Yan Li acquired his Ph.D in 2012 from Singapore-MIT alliance program under the supervision of MIT Professor Jianzhu CHEN. From 2012 to 2016, he completed post-doctoral training with Prof. James DI SANTO in Institut Pasteur, Paris, and was promoted as an assistant researcher in 2016. In 2018, he was hired as a professor in Nanjing University and a principal investigator at the State Key Laboratory of Pharmaceutical Biotechnology. In 2019, he was qualified as a doctoral supervisor, a principal investigator at the Innovation Institute of Nanjing University, awarded as Jiangsu Province " Innovative and Entrepreneurial Talent " and "Distinguished Professor", and received the grant support from the National Key R&D Program Youth Program (formerly the Youth 973 Program); in 2020, he was awarded the leading talent of Jiangsu Innovative and Entrepreneurial Team Program; in 2021, he was awarded National Science Fund for Excellent Young Scholars. His main research results have been published in prestigious journals such as Cell, Nature Methods, Nature Communications, etc., and have been invited for presentation at international conferences 9 times, and filed one international patent. Dr. Yan Li is currently a member of the Immune Cell Branch of the Chinese Society of Cell Biology, a member of the Chinese Anti-Cancer Association, a board member of the Jiangsu Society of Cell and Developmental Biology, an associate editor of the Frontiers in Immunology humanized mouse topic, and a member of the Scientific Committee of the International Workshops on Humanized Mice. In addition, he has reviewed manuscripts for journals such as PNAS, European Journal of Immunology, and Frontiers in Immunology/Mircrobiology.

Contact Information

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

Next-generation humanized mouse models for translational medicine

In order to accelerate the translation from basic biological discoveries into clinical treatments, our team has developed a series of mouse strains and experimental methods, which chimerized the human immune system and a variety of human tissues into mice, and solve scientific questions that are difficult to answer by traditional mouse models and clinical trials.

The current research fields of our team include (Figure 1) :

(1) Development of humanized mouse models for fully human antibody discovery and vaccine evaluation.

(2) HLA fully matched immune system + liver/lung doubly humanized mouse model.

(3) Immunotherapy for tumors and autoimmune diseases.

(4) Regulation of human immune development and function via gut microbiota derived metabolites.

At present, the laboratory is supported by the National Natural Science Foundation of China/Ministry of Science and Technology, Jiangsu Provincial Department of Science and Technology/Education and Nanjing University. We have undertaken some projects including the National Key Research Plan, Jiangsu Innovative and Entrepreneurial Team, and the Fundamental Research Funds for the Central Universities.

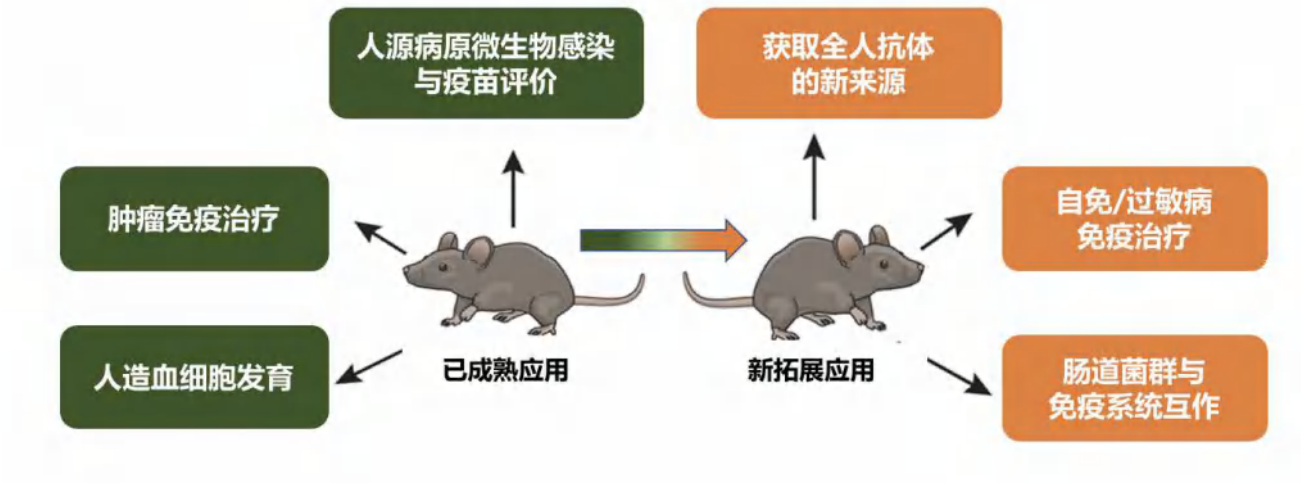

Figure 1 Current research fields in the laboratory

Selected Publications

1. Ana Cardoso, Ana Catarina Martins, Ana Raquel Maceiras, Wei Liu, Isabel Castro, Antonio G. Castro, Antonio Bandeira, James P. Di Santo, Ana Cumano, Yan Li, Paulo Vieira, and Margarida Saraiva (2021). Interleukin-10 induces interferon-g-dependent emergency myelopoiesis. Cell Reports. 37, 109887.
2. Yan Yan, Wei Zhao, Wei Liu, Yan Li, Xu Wang, Jingna Xun, Chantsalmaa Davgadorj (2021). CCL19 enhances CD8+ T-cell responses and accelerates HBV clearance. J Gastroenterol. 56:769-785.
3. Wei Liu, Hua Song, Xiaojing Li, Deshan Ren, Shuai Ding, Yan Li (2021). Lipid Metabolism in Tumor-associated Myeloid Derived Suppressor Cells. Lipid Metabolism in Tumor Immunity (Book), Springer.
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Group members

Group leader

Yan LI

Postdoctoral Fellow

Deshan Ren

Graduate student

Siqi Li
Wei Liu
Shujie Zhang
Chunyu Cheng
Shuang Liu
Haiqiao Sun
Xiang Duan
Zijian Zhang

Trainees in collaboration

Rujie Zhu
Mihribangul Alip

Technical Assistant

Wei Zhao
Xiaohong Yu

Zhaoyu Lin, Ph.D.

Zhaoyu Lin received his Ph.D degree in 2012 from Nanjing University under the mentoring of Dr.Gao Xiang. He has been a visiting scholar in Medical School of Washington University in St. Louis for three years. In 2014, he joined the Model Animal Research Center (MARC) of Nanjing University as research associated professor. In 2019, he became associated professor and a principle investigator in MARC.

Contact Information

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

Immune and metabolic regulation of physical homeostasis

Immune and metabolism is the key factors to maintain the physical homeostasis. The disruption of immune or metabolic regulation of physical homeostasis will lead to the occurrence of complex diseases, like autoimmune disease, obesity, cancer, cardiovascular disease and Alzheimer's disease. In our laboratory, we are interest in analysis of functions and the underlying molecular mechanisms of the disease related genes in immune or metabolic homeostasis.

Recently, we focus on a new discovered immunoregulatory protein family-Gasdermin. Our lab analyzed the roles of Gasdermin family in physical status and autoimmune diseases. Gsdmd and Gsdme are demonstrated to be the executors of pyroptosis, which is a type of pro-inflammatory programmed cell death. We discovered that Gasdermin directly trigger cell death and inflammation in 2015. Our recent works are mainly about the regulation of Gsdmd in pyroptosis (Figure 1). We found that inhibition of ROS reduces the cleavage of Gsdmd in canonical pyroptosis and inhibition of GSDMB reduces the cleavage of GSDMD in non-canonical pyroptosis. We developed several methods to block pyroptosis in autoimmune diseases. Magnesium could block the membrane translocation of Gsdmd-N-terminals and greatly enhance the survival rate of sepsis mice model. We also found that nitrosonisoldipine is a selective inhibitor of inflammatory caspases and protects against pyroptosis and related septic shock

We are also interesting with the relationship between obesogenic memory and immunity. Obesity, as a rapidly emerging public health problem, are associated with many severe diseases/complications, resulting in significantly compromised life quality of the patients. These patients can be greatly benefited by weight management. However, weight is very often regained during and after the treatments for obesity. This phenomenon is named obesogenic memory, leading to the failure of weight management and more importantly, of controlling the obesity-associated health problems including diabetes. Therefore, understanding the mechanisms regulating obesogenic memory, is especially beneficial for the patients with obesity. In the previous work, we has demonstrated that among immune cells, CD4+ T cells are the direct carrier, which is necessary and sufficient to induce and maintain obesogenic memory in mice. Recently, we found that obesogenic memory related CD4+ T cells are a subpopulation of central memory T cells with high expression of CD300C, which is a receptor of phosphatidylethanolamine (PE), an essential group of phospholipids in the cell membrane (Figure 2).

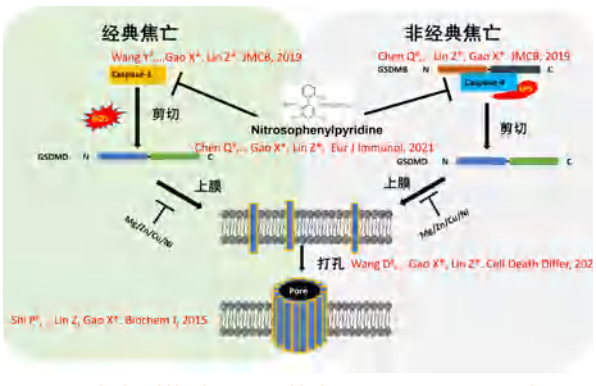

Figure 1. Gsdmd could be the target to block pyroptosis in autoimmune diseases

Figure 2. The role of immune cells in obesogenic memory

Selected Publications

1. Gao, L., X. Dong, W. Gong, W. Huang, J. Xue, Q. Zhu, N. Ma, W. Chen, X. Fu, X. Gao, Z. Lin, Y. Ding, J. Shi, Z. Tong, T. Liu, R. Mukherjee, R. Sutton, G. Lu, and W. Li*, Acinar cell NLRP3 inflammasome and GSDMD activation mediates pyroptosis and systemic inflammation in acute pancreatitis. Br J Pharmacol, 2021.
2. Chen, Q., J. Zheng, D. Wang, Q. Liu, L. Kang, X. Gao*, and Z. Lin*, Nitrosonisoldipine is a selective inhibitor of inflammatory caspases and protects against pyroptosis and related septic shock. Eur J Immunol, 2021. 51(5): p. 1234-1245.
3. Zou, D., J. Pei, J. Lan, H. Sang, H. Chen, H. Yuan, D. Wu, Y. Zhang, Y. Wang, D. Wang, Y. Zou, D. Chen, J. Ren*, X. Gao*, and Z. Lin*, A SNP of bacterial blc disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption. EBioMedicine, 2020. 52: p. 102652.
4. Wang, D., J. Zheng, Q. Hu, C. Zhao, Q. Chen, P. Shi, Q. Chen, Y. Zou, D. Zou, Q. Liu, J. Pei, X. Wu, X. Gao*, J. Ren*, and Z. Lin*, Magnesium protects against sepsis by blocking gasdermin D N-terminal-induced pyroptosis. Cell Death Differ, 2020. 27(2): p. 466-481.
5. Sun, M., S. Zheng, X. Gao*, and Z. Lin*, The role of immune cells in obesogenic memory. Cell Mol Immunol, 2020.
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9. Zou, J., B. Lai, M. Zheng, Q. Chen, S. Jiang, A. Song, Z. Huang, P. Shi, X. Tu, D. Wang, L. Lu, Z. Lin*, and X. Gao*, CD4+ T cells memorize obesity and promote weight regain. Cell Mol Immunol, 2018. 15(6): p. 630-639.
10. Lin, Z. *, F. Liu, P. Shi, A. Song, Z. Huang, D. Zou, Q. Chen, J. Li, and X. Gao, Fatty acid oxidation promotes reprogramming by enhancing oxidative phosphorylation and inhibiting protein kinase C. Stem Cell Res Ther, 2018. 9(1): p. 47.

Group members

Group Leader	Graduate Student	Technicians
Zhaoyu Lin	Ruyue Xin	Jiafeng Zou
	Tianxun Zhang	Jiaxiang Zou
	Yanqi Sun	

Cancer and Stem Cell Biology

Geng Liu, Ph.D.

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

Contact Information

Tel: +86-25-58641515 (Office) +86-25-58641519 (Lab)
Fax:+86-25-58641500
Email:liug53@nju.edu.cn liugeng@nicemice.cn

Cellular metabolism and stress response on cell behavior and function

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

p53 stress response pathway influences cell behaviors in distinctive manners

p53 is extremely important for stress response and tumor suppression as exemplified by its mutations found in over 50% of human cancers. p53 protein is undetectable in normal tissues. With the 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 presence of stress, p53 is activated to exert its role in influencing the cell fate. Various degree of stresses results 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 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 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 (Zhang, et al, 2017). These results indicate that p53 signaling pathway critically and delicately influence cell behaviors and functions in distinctive manners.

Probing, manipulating and understanding cellular metabolisms and their links with cell behaviors in vivo

To study the influence of cellular metabolism on cell behaviors and function in a multitude of in vivo contexts, we have established mouse models in imaging and probing the metabolic heterogeneity within the tissues to study their diverse cellular functional and regulatory attributes in physiological and pathological contexts (Fig.1, manuscript under revision). Extending from the in vivo observations, we focused on further elucidating the regulatory network of mitochondrial oxidative metabolism and redox homeostasis using various approaches including drug screening and expression profiling (in progress).

In addition, we have established a series of mouse models involved in promoting specific metabolic pathways in a controlled manner. Our results showed that cellular metabolisms could be manipulated in vivo and may have great impact on either cell behavior or systemic homeostasis. By genetically manipulating glucose metabolism in the mouse skeletal muscle, a glucose sensing mechanism was triggered in the skeletal muscle and as a consequence, lipid metabolism in the adipose tissue was dramatically elevated resulting from the crosstalk of the two tissues, maintaining metabolic homeostasis (Fig.2). Aiming to discover new strategies to boost cancer immune therapy, we found that specific manipulation and alteration of T cell metabolism could potently stimulate the anti-tumor immune response, revealing interesting insights for the intrinsic regulatory roles of the specific metabolic route on T cell differentiation and function (in progress).

The above attempts will help to impact on our abilities in the understanding and fighting against a variety of diseases, especially those linked to cancer and ageing, in the perspectives of cellular metabolism.

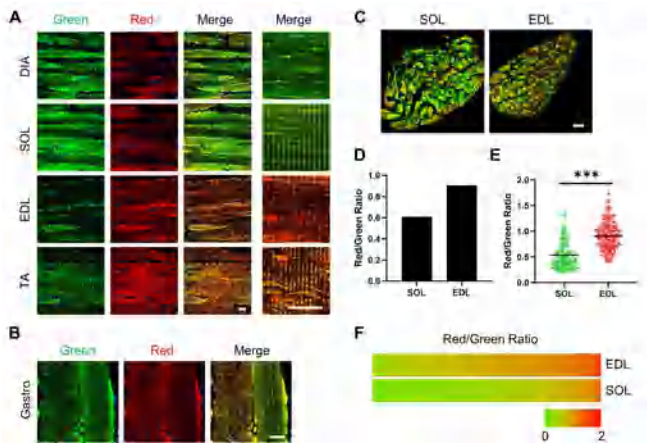

Figure 1. Heterogeneity of MT fluorescence spectrum was directly linked to metabolically distinctive skeletal muscle fibers.

(A) Representative MT fluorescence images of muscles from an individual mouse following doxycycline induction from 1 month to 3 months of age. Scale bar, 50μm in the left panels and 20μm in the right panels. (B) Heterogeneity of MT fluorescence spectrum in the two neighboring fibers from gastrocnemius (Gastro) muscle. Scale bar, 30μm. (C) Images of MT fluorescence in cross section of muscle fibers from soleus and EDL muscles. Scale bar, 200μm. (D) Quantification of the ratio of the red/green fluorescence intensities in (C). (E) Quantification of the ratio of the red/green fluorescence intensities in single fibers from the soleus and EDL muscles in (C). (F) Schematic representations of the ratio of the red/green fluorescence intensities in single muscle fiber in (E).

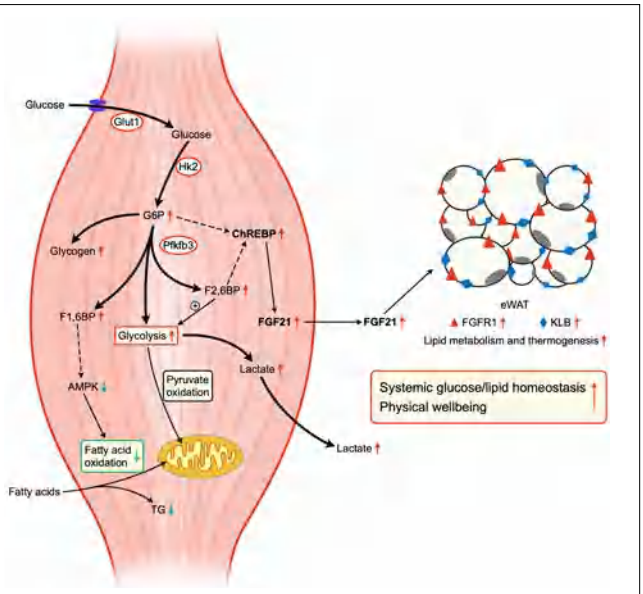

Figure 2. A working model of enhanced skeletal muscle glycolysis in systemic metabolic regulation.

The schematic depicts a proposed model for the enhanced anaerobic glycolytic metabolism in skeletal muscle engaging in two inter-connected glucose-sensing pathways to remodel the systemic metabolic network and improve the physical wellbeing.

Publications

1. Xie YY, Zhang YN, Sun AN, Peng YM, Hou WK, Xiang C, Zhang GX, Lai BB, Hou XS, Zheng FF, Wang F, Liu G*. Intrinsic coupling of mitochondrial proteolysis and oxidative metabolism underlies an active mitochondrial state. In revision.
2. Xiang C, Zhang YN, Chen QL, Sun AN, Peng YM, Zhang GX, Zhou DX, Xie YY, Hou XS, Zheng FF, Wang F, Gan Z, Chen S*, Liu G*. Increased glycolysis in skeletal muscle coordinates with adipose tissue in systemic metabolic homeostasis. J Cell Mol Med.2021 Jul 6; doi: 10.1111/jcmm.16698.
3. 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. Proc Natl Acad Sci U S A. 2017 Jan 17; 114(3):498-503.
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7. Chen L, Zhang GX, He XY, Zhang CX, Xie YY, Liu G* (2015) BAC transgenic mice provide evidence that p53 expression is highly regulated in vivo. Cell Death Dis. 2015 Sep 17;6:e1878.

Group members

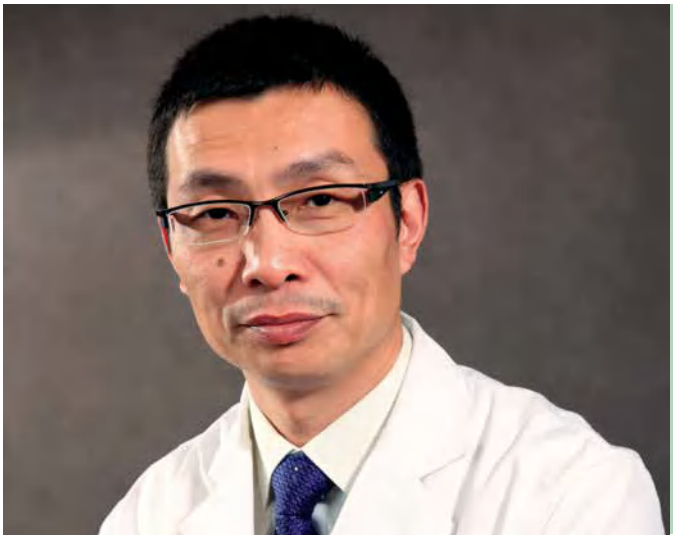
Group Leader
Geng Liu

Postdoctoral fellow
Xie Yinyin

Graduate students
Xiaoshuang Hou
Yamei Peng
Yannan Zhang
Fangfang Zheng
Fan Wang
Boxin Zhang (rotation)
Danning Chen (rotation)

Former lab members
Lai Chen (Ph.D.)
Xueyan He (Ph.D.)
Chenxi Zhang (Ph.D.)
Guoxin Zhang (Ph.D.)
Yinyin Xie (Ph.D.)
Cong Xiang (Ph.D.)
Ying Zhou (MS)
Weikang Hou (MS)
Aina Sun (MS)

Technical staff
Yan Ren



Qing Jiang Ph.D., M.D.

Chief physician / Professor / Doctoral supervisor; The head of the department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital/ The vice president of school of medicine, Nanjing University/ Director, institute of medical 3D printing, Nanjing University. Prof. Jiang, the first sports medicine clinical doctor cultivated by China, had been engaged in orthopedic and sports medicine clinical and basic research since 1989 and got the PhD degree in Beijing Medical University in 1999. In 2008, he was appointed professor in Nanjing University and Adjunct Professor in Model Animal Research Center (MARC). Prof. Jiang won the National Science Fund for Distinguished Young Scholars in 2011. The department of Sports Medicine and Adult Reconstructive Surgery is the only joint disease treatment center identified by Jiangsu Provincial Health Department, and also the training base of artificial joint and arthroscopic techniques in Jiangsu province. Jiang's team has established human gene bank of bone and joint disease including osteoarthritis (OA), developmental dysplasia of the hip (DDH), deep venous thrombosis (DVT), ankylosing spondylitis (AS) and osteoporosis (OP), and published 232 Chinese core articles, 138 SCI articles, included Nature Medicine, Nature Genetic, ACS NANO, Advanced Functional Materials, Annals of the Rheumatic Diseases, Biomaterials, Small. Prof. Jiang is the first domestic scholars who hold the post of committee member of the OARSI, who also is vice chair of China branch of ICRS, vice chairman of sports medicine branch of Chinese medical association, chairman of sports medicine branch of Jiangsu medical association, vice chairman of orthopedics branch, vice chairman of trauma branch, and next chairman orthopaedic branch of Jiangsu medical association, etc.

Contact Information

Tel: 025-83106666

E-mail: qingj@nju.edu.cn

Skeletal System Disease

Cartilage regeneration scaffold. We evaluated whether the co-culture of human umbilical cord Wharton's jelly-derived MSCs (hWJMSCs) and primary cartilage cells (pACs) in a double biomimetic acellular cartilage extracellular matrix (ACECM)-oriented scaffold could achieve a joint surface that mimics the native state as closely as possible and return the articular cartilage to its natural biomechanics and compositions (Zhang, 2020). An in vivo study was conducted on knee joint of a rabbit to verify the practicability of the proposed approach in repair of cartilage. Our approach may enhance in situ 3D bio-printing in clinical treatment (Ma, 2020).

Mechanism and treatment study of Orthopedics. A bile duct-ligated (BDL) male rat model was used to establish the features of biliary cirrhosis and the characteristics of osteoporosis. The effect of intraperitoneal injection of trehalose on bone mass in BDL male rats and the related mechanisms were investigated (Figure 1) (Xu, 2020). Treatment of cartilage lesions is clinically challenging. According to our study, (1) m-HA 1 KGN treatment facilitated hyaline cartilage and subchondral bone tissue repair in a porcine model at the 12-month follow-up. (2) m-HA 1 KGN treatment demonstrated better therapeutic efficacy in defects with a diameter of 6.5 mm or full-thickness chondral-type defects. (Figure 2) (Yan, 2020).

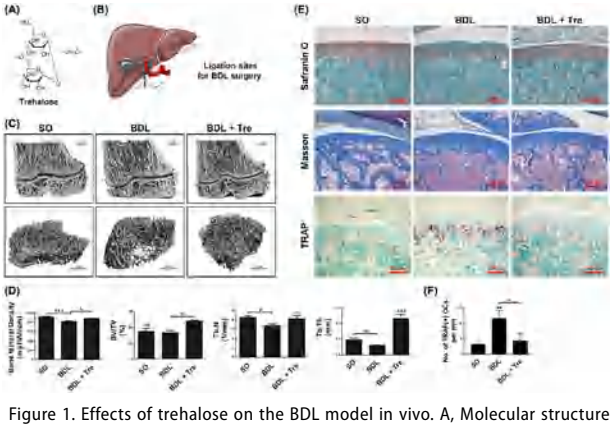


Figure 1. Effects of trehalose on the BDL model in vivo. A, Molecular structure of trehalose. B, Representative anatomical image for common bile duct ligation surgery. C, Three-dimensional micro-CT images of distal femoral metaphyseal trabecular bone (scale bar: 1.0 mm). (First line: the coronal position; Second line: representative microstructure of ROI). BDL, bile duct ligation; SO, sham operation; Tre, trehalose. D, Trabecular bone mass and architecture parameters determined by CT in the distal femoral metaphysis. BV/TV, bone volume/total volume; Tb.N, trabecular bone number; Tb.Th, trabecular bone thickness. E, Histopathological SO, Masson, and TRAP staining of femurs in rats with BDL following trehalose treatment (scale bar: 200 μm). F, The number of TRAP-positive OCs (TRAP (+) OCs) was counted on the surface of the metaphyseal trabecular bone (n = 3).

Identification and functional characterization of novel inheritable disease-causing genes. We have performed GWAS study of Developmental dysplasia of the hip (DDH), and detected novel genes and signaling pathways. A total of 406 DDH-associated genes (P< 0.001) were identified. An intronic SNP, the minor allele (rs61930502-A), tended to prevent DDH showed a dominant effect. In previous association studies, genes such as GDF5, TBX4, and ASPN was detected associate with DDH by case-control studies. We focused on and expertise in searching molecular mechnisms in human genome for genetic skeletal disesases. The DNA bank for skeletal diseases is still enlarging.

Deep vein thrombosis (DVT). Lots of retrospective and prospective experiments had conducted to study how to prevent DVT in our group. Total joint arthroplasty (TJA) is a form of high-risk postoperative venous thromboembolism (VTE). We determined the relationship between preoperative Soleal veins (SVs) diameter and postoperative DVT after TJA. A large SV diameter was significantly associated with postoperative total DVT after TJA, and also symptomatic DVT after TJA. The bank for DVT patients is still enlarging.

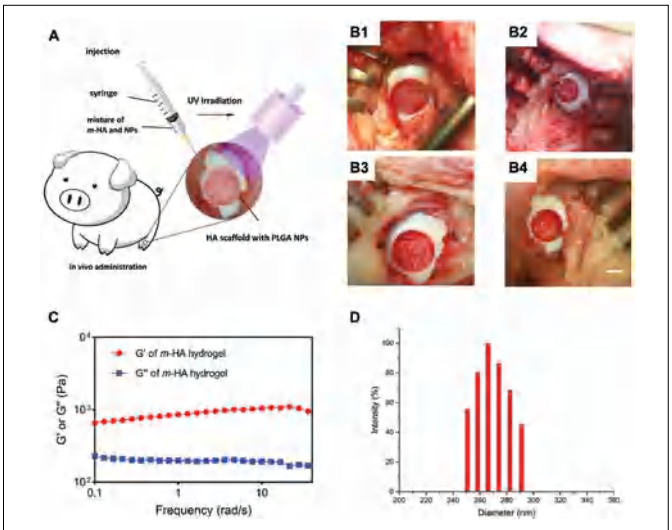


Figure 2. Schematic illustration of the operation, chondral and osteochondral defect model preparation, and characterization of the m-HA hydrogel and PLGA NPs. (A) Schematic illustration of the surgical procedure for cartilage defect repair and hydrogel solidification. (B) Full-thickness cartilage defect model and osteochondral defect model in the medial femoral condyle: fullthickness cartilage defect, F [diameter] 8.5 mm (1); full-thickness cartilage defect, F6.5 mm (2); osteochondral defect, F8.5 mm, 5 mm in depth (3); osteochondral defect, F6.5 mm, 5 mm in depth (4). Scale bar: 3 mm. (C) The rheological properties of the m-HA hydrogel. (D) Diameter distribution of PLGA NPs determined by DLS.

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

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

2. (Major Projects of NSFC) Study on the role and regulatory mechanisms of bone-derived factors in maintaining homeostasis of the body--Manipulating bone derived factors to develop therapeutic strategies for extra-bone diseases

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

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

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

6. (NSFC 81871832) Effect and mechanism of exosomes in circulating blood on venous thrombosis during perioperative orthopedics.

7. (NSFC 81702151) Exosomes derived from tendon stem cells facilitate repair of damaged tendons by delivering microRNA.

8. (NSFC 8180090535) A study on the application of hollow porous magnetic nanoparticles to target aggregation and control of the release of icariin to promote fracture healing in mice

9. (NSFC 81972124) The Effect and Mechanism of Low Magnitude High Frequency Mechanical Stimulation on Promoting the Transformation of Tissue Engineering Polylactic-hydroxyapatite Scaffold into Normal Tissue.

10. (NSFC 81902174) The study of LRP1 regulating the pathogenesis of developmental hip dysplasia (DDH) through the Wnt/ β-catenin signaling pathway.

11. (NSFC 82002370) Mechanism of effective therapy for Rheumatoid arthritis by Engineered neutrophil-derived exosomes

12. (Six Talent Peaks Project of Jiangsu ProvinceWSW-061) Peptide hydrogel repair of sustained-release small molecule organic compound BIO

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

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

15. (Natural Science Foundation of Jiangsu Province, China BK2020040424) Study on the mechanism of Engineered neutrophil-derived exosomes in effective therapy for Rheumatoid arthritis

Selected publications

1. Yan W, Xu X, Xu Q, et al. (2020) An Injectable Hydrogel Scaffold With Kartogenin-Encapsulated Nanoparticles for Porcine Cartilage Regeneration: A 12-Month Follow-up Study. American Journal of Sports Medicine. 2020 Nov;48(13):3233-3244. (IF=5.810)
2. Xu X, Wang R, Wu R, et al. (2020) Trehalose reduces bone loss in experimental biliary cirrhosis rats via ERK phosphorylation regulation by enhancing autophagosome formation. FASEB J. 34(6):8402-8415. (IF= 5.595)
3. Zhang Y, Hao C , Guo W, et al. (2020). Co-culture of hWJMSCs and pACs in double biomimetic ACECM oriented scaffold enhances mechanical properties and accelerates articular cartilage regeneration in a caprine model. Stem Cell Research & Therapy. 19;11(1):180. (IF= 5.116)
4. Kaiwei Ma, Tianzheng Zhao, Longfei Yang, Peng Wang, Jing Jin, Huajian Teng, Dan Xia, Liya Zhu, Lan Li*, Qing Jiang*, Xingsong Wang*. Application of robotic-assisted in situ 3D printing in cartilage regeneration with HAMA hydrogel: An in vivo study. Journal of Advanced Research. 23 (2020) 123–132. (IF= 6.992)
5. Zhu X, Chen F, Lu K, et al. (2019) PPARγ preservation via promoter demethylation alleviates osteoarthritis in mice. (2019) Annals of the Rheumatic Diseases. 78(10): 1420-1429. (IF= 14.299).
6. Li Y, Zhao S, Li S, et al. (2019) Surface engineering of biodegradable magnesium alloys for enhanced orthopedic implants. Small. 2019, 15, 1904486 (IF= 10.856)
7. Zhang L, Shan X, Meng X, et al. (2019) The first integrin β3-mediated cellular and nuclear targeting therapeutics for prostate cancer. Biomaterials. 2019,223: 119471. (IF=10.273)
8. Zhou S, Li Y, Qiao L, et al. (2019) Inactivation of Lkb1 in postnatal chondrocytes leads to epiphyseal growth plate abnormalities and promotes enchondroma-like formation. FASEB J. 33(8): 9476-9488. (IF= 5.595)

Group members

Graduated Doctors

Yao Yao Kaijia Zhang

Graduated Masters

Wenqiang Yan
Zixu Wang
Jianxiang Wu
Ping Pan

Technicians

Sheng Shen Peng Wang
Lan Li Qiting Ge
Wenjin Yan Jing Jin

Postdoctoral

Lei Zhang
Yixuan Li
Bin Wang
Jinxing Hou
Huixin Liang

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 (o): 86-25-58641599
FAX: 86-25-58641500
Email: liujianghuai@nicemice.cn

Immune cell plasticity and rewiring

Our immune system is programmed to exert very diverse functions that range, for instance, from defending against foreign pathogens to regulation of metabolic balance. Such diversity is mediated by the populational and functional heterogeneity of different interacting immune cells. Indeed, tracing back to the very early days of immunology, the great Ilya Metchnikoff (1845-1916) had put forward the conceptual synthesis that the primitive phagocytes (macrophages) might serve a profound biological purpose to maintain the overall organismal "harmony". This vision is validated by overwhelming current evidence, where macrophages have been demonstrated to assume a spectrum of roles in immunity, tissue homeostasis and many diseases.

The complex interface of tumor and immunity presents a highly dynamic system that is key to disease progression and cancer treatment. By establishing novel genetic tools to operate in a condition- or subset-specific manner, we are dedicated to tackling several unanswered questions about the cellular heterogeneity and plasticity in the tumor microenvironment. Another major interest of the lab is the development of cutting-edge genome engineering technologies for genetic rewiring, which shall potentially impact multiple disciplines.

Some ongoing projects in the lab are described in the following section:

1. A monocyte-intrinsic type I IFN-IL-4 cytokine cascade drives an M2-skewed phenotype in tumor-associated macrophages (TAMs):

Harnessing the nucleic acid-engaged innate immunity has strong implications for cancer therapies. Various signaling pathways activated by nucleic acids converge on the induction of type I IFNs (IFN-I), which often exert immune-stimulatory anti-tumor functions. On the other hand, our previous work showed an opposite action by IFN-I to potently promote an arginase (ARG1)-dependent immunosuppressive axis in tumor-associated monocytes/macrophages (Tong Y et al., 2019). Such an unexpected pathway may operate as a "checkpoint" under various IFN-engaging treatment strategies.

Aided by a newly generated Arg1-YFP reporter mice (Guo P et al, 2021), we established that IFN-I-induced arginase expression was localized in TAMs (Fig. 1A, B). We further demonstrated a surprising monocyte-specific "cytokine cascade" induced by IFN-I, leading to the release of IL-4, which in turn switches the co-existing, mature macrophages towards an immunosuppressive, pro-tumoral phenotype (Fig. 1C, D, E, F, G). Our work highlights an under-appreciated role by the monocytes in coordination of inflammation and repair which warrants future investigations.

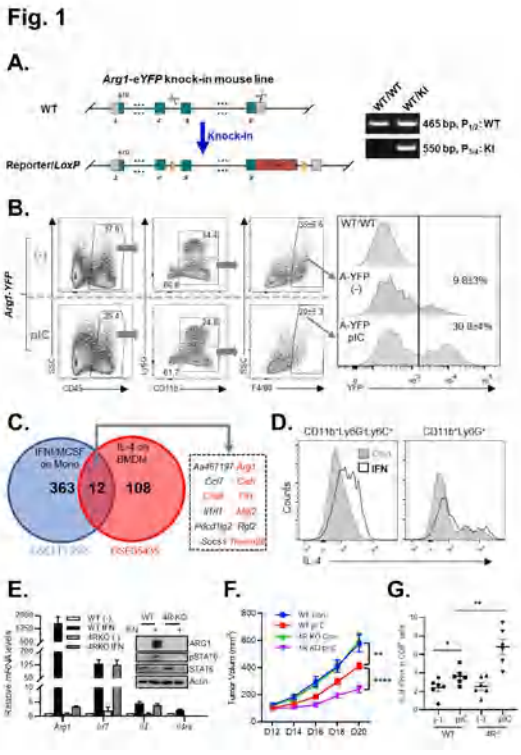


Figure 1: Mechanistic characterization of the IFN-I-ARG1 axis in TAMs. (A)

Construction of an Arg1-YFP mouse line. (B) Poly(I:C)/IFN-mediated induction of Arg1 is restricted in TAMs. (C) IFN treatment of M-CSF-influenced monocytes leads to induction of genes known to be downstream of IL-4. (D) IFN treatment of BM mononuclear cells leads to induction of IL-4 selectively in the monocyte compartment. (E) Inactivation of IL-4 receptor abrogates IFN-ARG1 axis in differentiating monocytes. (F and G) IL-4 receptor deficiency in mice led to improved anti-tumor effects by poly(I:C) (F), which is associated with higher abundance of $IFN\gamma^{+}CD8^{+}$ T cells (G).

2. Precise tumor immune rewiring via synthetic circuits gated by concurrent gain/loss of transcription factors:

Reinvigoration of antitumor immunity has recently become the central theme for the development of cancer therapies. Nevertheless, the precise delivery of immunotherapeutic activities to the tumors remains challenging. Here, we explored a synthetic gene circuit-based strategy to specifically identify tumors by their concurrent gain/loss of transcription factor (TF) activities, and to subsequently rewire them toward immune activation (Fig. 2A, B). We have provided evidence that this synthetic gene circuit enabled specific activation of a reporter gene ONLY in p53-deficient, but not -sufficient, tumor cells (Fig. 2C). Additionally, the logic circuit empowered a highly specific and effective tumor recognition/immune rewiring axis (Fig. 2D, E). This work has presented an adaptable strategy for the development of precisely delivered immunotherapy (In revision).

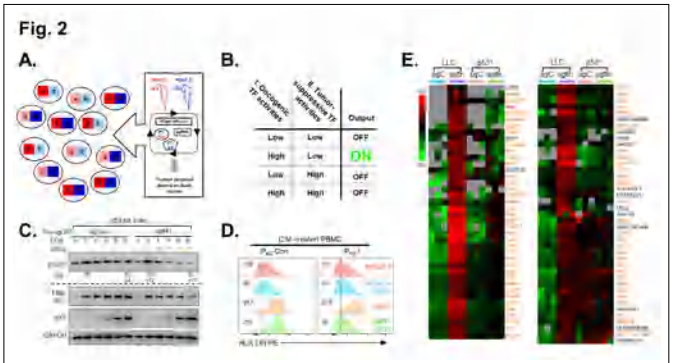


Figure 2: Synthetic gene circuit for improved sensing of the malignant state. (A) A scheme shows a synthetic cell classifier that can sense multi-parameter cell states and drive functional outputs accordingly. (B) Our goal for a tumor-targeting logic circuit gated by the simultaneous gain and loss of two TF activities, respectively. (C and D) Such a circuit enabled specific activation of reporter (EGFP), or endogenous IFNγ in p53-null H1299 cells, compared to those reconstituted with p53. (E) The synthetic circuit empowered a p53-deficiency-dependent immune activation program in LLC tumor cells.

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Group members

Graduate students:

Ya-feng Wang

Li-min Yang

Gui-quan Zhang

Yu-yan Zhang

Pei Wang (on rotation)

Lab alumni:

Dr. Hui Jiang (Molecular Infection Medicine Sweden)

Dr. Yi Lu (in Biotech industry)

Dr. Yuan-yuan Tong (in Biotech industry)

Dr. Qingzhou Meng (ShanghaiTech University)

Dr. Panpan Guo (Gulou Hospital, Nanjing)

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 (Office) +86-25-58641524 (Lab)
Fax:+86-25-58641504
Email: qinjz@nicemice.cn

Roles of the polycomb group proteins in stem cells & early development

1. Mga safeguards embryonic stem cells from acquiring extraembryonic endoderm fates.

Polycomb group (PcG) proteins form multiprotein complexes that affect stem cell identity and fate decisions by still largely unexplored mechanisms. Here, by performing a CRISPR-based loss-of-function screen in embryonic stem cells (ESCs), we identify PcG gene Mga involved in the repression of endodermal transcription factor Gata6. We report that deletion of Mga results in peri-implantation embryonic lethality in mice. We further demonstrate that Mga-null ESCs exhibit impaired self-renewal and spontaneous differentiation to primitive endoderm (PE). Our data support a model in which Mga might serve as a scaffold for PRC1.6 assembly and guide this multimeric complex to specific genomic targets including genes that encode endodermal factors Gata4, Gata6, and Sox17. Our findings uncover an unexpected function of Mga in ESCs, where it functions as a gatekeeper to prevent ESCs from entering into the PE lineage by directly repressing expression of a set of endoderm differentiation master genes.

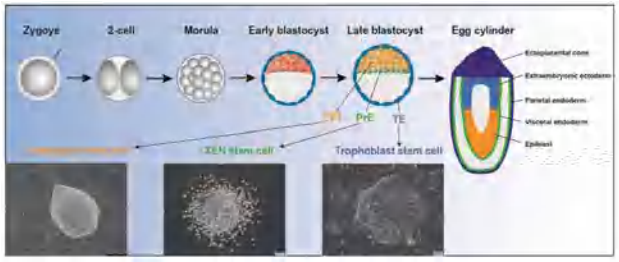

Figure 1. A process of progressive cell fate specification during mouse preimplantation development. Three distinct cell lineages are present in the implanting blastocyst: the extraembryonic trophectoderm (TE), the primitive endoderm (PE, also called the hypoblast), and the embryonic epiblast (EPI).

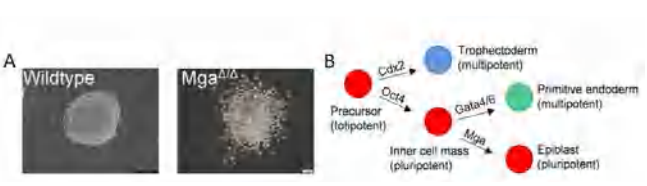

Figure 2. Mga is required for maintenance of the pluripotent state in ESCs.

(A) Mga deficiency results in dynamic changes in cell morphology and gene expression characteristic of Xen cells. (B) Mga blocks XEN lineage formation by repressing the expression of Gata4/6.

2. Rbbp4 suppresses premature differentiation of embryonic stem cells.

Polycomb group (PcG) proteins exist in distinct multi-protein complexes and play a central role in silencing developmental genes, yet the underlying mechanisms remain elusive. Here, we show that defificiency of retinoblastoma binding protein 4 (RBBP4), a component of the Polycomb repressive complex 2 (PRC2), in embryonic stem cells (ESCs) leads to spontaneous differentiation into mesendodermal lineages. We further show that Rbbp4 and core PRC2 share an important number of common genomic targets, encoding regulators involved in early germ layer specifification. Moreover, we fifind that Rbbp4 is absolutely essential for genomic targeting of PRC2 to a subset of developmental genes. Interestingly, we demonstrate that Rbbp4 is necessary for sustaining the expression of Oct4 and Sox2 and that the forced co-expression of Oct4 and Sox2 fully rescues the pluripotency of Rbbp4-null ESCs. Therefore, our study indicates that Rbbp4 links maintenance of the pluripotency regulatory network with repression of mesendoderm lineages.

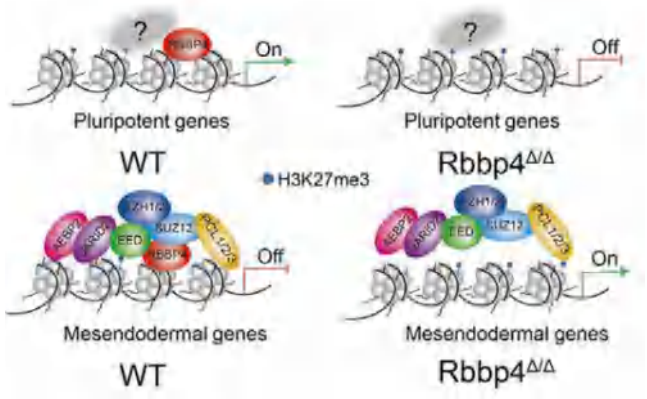

Figure 2-1. Research overview. RBBP4 as an essential chromatin factor for the maintenance of ESC pluripotency and it functions within the PRC2 complex to repress mesendoderm specififfication.

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Group members

Group Leader
Jinzhong Qin

Graduate Students
Congcong Wang
Yaru Zhu
Lixia Dong
Kunying Hao
Jingnan Wang
Linchun Zhao

Former Graduate Students
Wukui Zhao (Ph.D.)
Yun Yan (MS)
Yikai Huang (Ph.D.)
Mengjie Liu (MS)
Ting Su (MS)

Technical Assistants
Lijun Xu

Pingping Shen , Ph.D.

Pingping Shen received her Ph.D. degree at Nanjing University in 2003. From 2002 to 2003, she studied at University of California at San Diego as a visiting professor. In 2005, she was appointed as a professor in Nanjing University. Research in Pingping Shen's Lab is mainly focused on two fields: (1) the functional regulation of macrophages, adipocytes in chronic inflammatory diseases such as cancer, metabolic disturbance etc.; (2) the development of novel immunotherapeutic techniques for disease treatment. (3) the development of clinical diagnostic techniques for certain immune disorders.

Contact Information

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

CDK5-triggered G6PD Phosphorylation at Threonine 91 Facilitating Redox Homeostasis Reveals a Novel Vulnerability in Breast Cancer

Glucose-6-phosphoate dehydrogenase (G6PD) is aberrantly activated in multiple types of human cancers, in which it governs the adaptation and progression of tumor cells, as well as the efficacy of anticancer therapy. Here, we revealed that CDK5 rewired glucose metabolism from glycolysis to PPP by activating G6PD in breast cancer (BC) cells. CDK5-induced G6PD activation increased intracellular levels of NADPH and GSH, which are responsible for reactive oxygen species (ROS) detoxification, and thus promoted BC cell survival under oxidative stress conditions. Mechanistically, CDK5 phosphorylated G6PD at Thr-91, which enhanced G6PD activity by facilitating the assembly of inactive monomers into active dimers. Most importantly, CDK5-mediated G6PD phosphorylation was specifically observed in tumor cells and closely correlated with malignant development in human BC patients. Pharmacological inhibition of CDK5 significantly abrogated G6PD phosphorylation and significantly attenuated tumor growth and metastasis. Furthermore, inhibition of G6PD phosphorylation synergistically enhanced the efficacy of ROS-based treatments, such as PARP inhibitors, in a xenograft mouse model. Taken together, our results establish the crucial roles of CDK5-mediated phosphorylation of G6PD in BC growth and metastasis, while also providing a novel therapeutic target for new anticancer therapies.

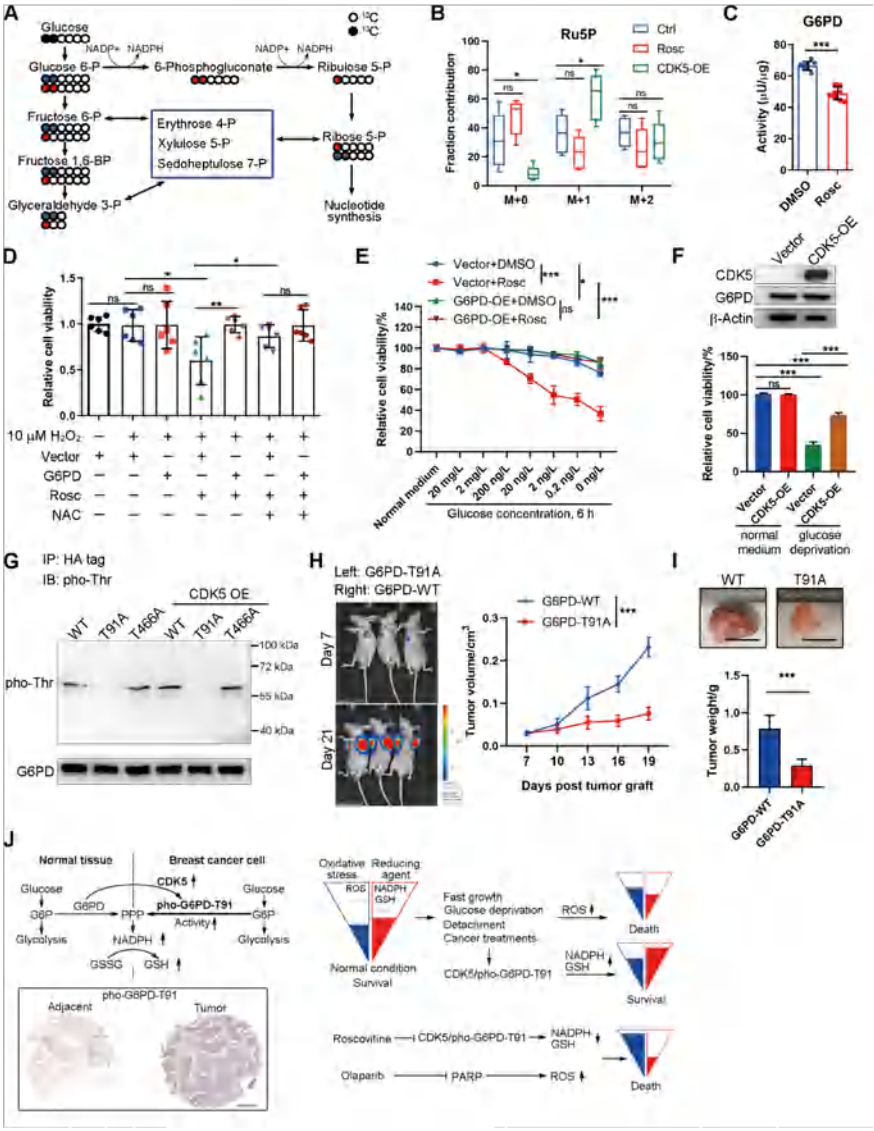

Figure 1. CDK5 activates G6PD by Thr-91 phosphorylation protecting BC cells from oxidative stress.

Establishment of a novel mesenchymal stem cell-based regimen for chronic myeloid leukemia differentiation therapy

Chronic myeloid leukemia (CML) is a myeloproliferative disorder, characterized by the blocked differentiation process of hematopoiesis. Despite the fact that current tyrosine kinase inhibitors (TKIs) are capable of achieving the complete remission by reducing the tumor burden, severe adverse effects often occur in CML patients treated with TKIs. The differentiation therapy exhibits therapeutic potential to improve cure rates in leukemia. However, there is still a lack of efficient differentiation therapy strategy in CML. Here we showed that MPL gene decreased along with the progression of CML. MPL signaling blockade impeded the megakaryocytic differentiation and contributed to the progression of CML. While allogeneic human umbilical cord-derived mesenchymal stem cells (UC-MSCs) treatment efficiently promoted megakaryocytic lineage differentiation of CML cells through restoring the MPL expression and activating MPL signaling. UC-MSCs in combination with eltrombopag, a non-peptide MPL agonist, further activated JAK/STAT and MAPK signaling pathway through MPL and exerted a synergetic effect on enhancing CML cells differentiation. The established combinational treatment not only markedly reduced the CML burden but also significantly eliminated CML cells in a xenograft CML model. We provided a new molecular insight of MPL signaling in MSCs-mediated megakaryocytic differentiation of CML cells. Furthermore, a novel anti-CML treatment regimen that uses the combination of UC-MSCs and eltrombopag shows therapeutic potential to overcome the differentiation blockade in CML.

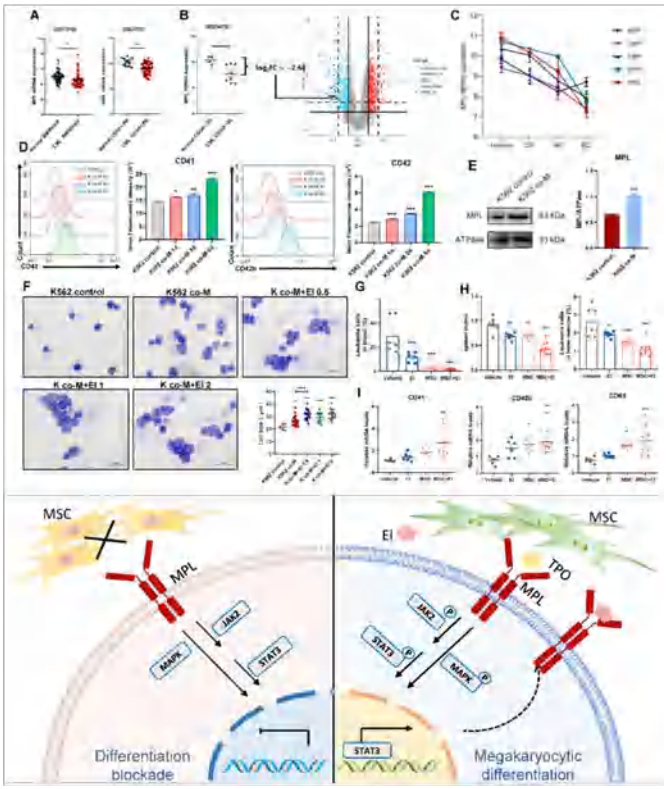

Figure 2. The mechanism of MPL-mediated differentiation in chronic myeloid leukemia and establishment of mesenchymal stem cell-based differentiation therapy

Selected publications

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Group members

Group leader

Pingping Shen

Former Graduate students

Jun Cui
Ying Liu
Nanfei yang
Nan Cheng
Yuxin Shu
Wenlong Zhang
Qian Shi
Ting Chen
Xiaofeng Bao
Yuanyuan Su
Yuanyuan Wu
Jiafa Xu
Yinna Wei
Haocheng Wu
Tingzhe Sun
Xiaojuan Pang
Xiujing Feng
Yongfang Yao
Yuncheng Bei
Pei Liu
Jingfa Zhao
Hanren Dai et al

Graduate students

Luchen Sun
Xiang Wu
Xuhui Dong
Qiang Tian
Yuxin Wang
Sijie Wang
Shiman Zuo et al.

Teachers

Yan Lu
Yahong Huang

Technician

Wei Zheng

Yohei Niikura, Ph.D.

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

Contact Information

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

Mitotic regulators in cancer and brain development

Our lab is interested in the molecular mechanism involved in both cell division and human diseases, currently focusing on cancer and brain development using human cells and animal models (zebrafish and mouse).

During cell division, proper chromosomes segregation must be achieved otherwise it can result in unequal distribution of chromosomes to daughter cells. Spindle microtubules must attach to a single region of each chromosome, termed the "centromere" in most eukaryotes. The kinetochore is a complex of proteins that is located at the centromere (Figure 1). Defects in the centromere-kinetochore function as well as the spindle check point function, lead to aneuploidy, cancer, and abnormal brain development, and are often associated with a poor prognosis. Therefore, it is highly important to study the temporal-special regulation and the structure of centromere and kinetochore protein(s) to understand chromosome instability (CIN) in cancer and brain development.

MYCN-MAD2 pathway in children's cancer, rhabdomyosarcoma (RMS)

The chromosomes in rhabdomyosarcoma (RMS) are very instable that high percentage cells are tetraploid. It has been found recently that most RMS contain chromosome 2p24 amplification, in which MYCN is located, but the consequence of 2p24 amplification in the tumorigenesis and if it contributes to the chromosomal instability of RMS remain unknown. Mitotic arrest deficiency 2 (MAD2), a critical component of the spindle assembly checkpoint (SAC), is overexpressed in many cancer cells (Figure 1). It has been observed upon inactivation of two major tumor suppressor (Rb and p53) pathways. However, molecular mechanism is not yet clear to explain the relationship among oncogene activation, tumor suppressor activation, and chromosome instability. Recently, our lab found that MYCN and MAD2 are co-overexpressed but not with the other SAC proteins in mouse xenograft model implanted with RMS cell lines (data not shown).

Myc responsive element E-box motifs reside with E2F-binding sites within promoter region of mitotic regulator genes

N-myc is a member of myc family (c-myc, n-myc and l-myc). MYC proteins are basic helix-loop-helix leucine zipper transcription factors and share similar cellular functions. MYC/MAX heterodimers binds to DNA with CAC(G/A)TG (E-box) sequence and drive the expression of numerous genes important for cell proliferation. Schwartzman et al. performed an analysis of the MAD2 promoter and found that the presence of a cell cycle gene homology region (CHR) and a cell cycle-dependent element (CDE) close to the transcription start site of multiple mitotic regulator genes including MAD2 as well as E2F binding sites. In their model, an unknown protein(s) (X) stabilizes the interaction of the repressor E2F/p107 or p130 (p107/p130) complex through the CHR element, and activator E2Fs serve as transcriptional activators at the upstream E2F binding site. Our preliminary results also showed that Myc responsive element E-box motifs reside with E2F-binding sites within promoter region of these mitotic regulator genes shown by Schwartzman et al. (Figure 2). These data suggest that MYCN is a candidate of CHR-binding protein, an unknown protein X, that control the MAD2 promotor in exclusive manner with E2F (Figure 3).

Therefore, we will perform ChIP-qPCR using Flag-MYCN precipitants and/or endogenous E2F4 precipitants using E-box and CHR-CDE elements to validate if MYCN is a candidate of CHR-CDE-binding protein that controls the MAD2 promotor in an exclusive manner with E2F. In the future, we will also perform qPCR using endogenous MYCN precipitants immunoprecipitated from HSkMCs or Rh30 cells combining with MYCN CRISPRi or CRISPR KO technique. We will also address how MYCN-induced transcription is mis-regulated in RMS development through these experiments combining with zebrafish xenograft.

Figure Legends

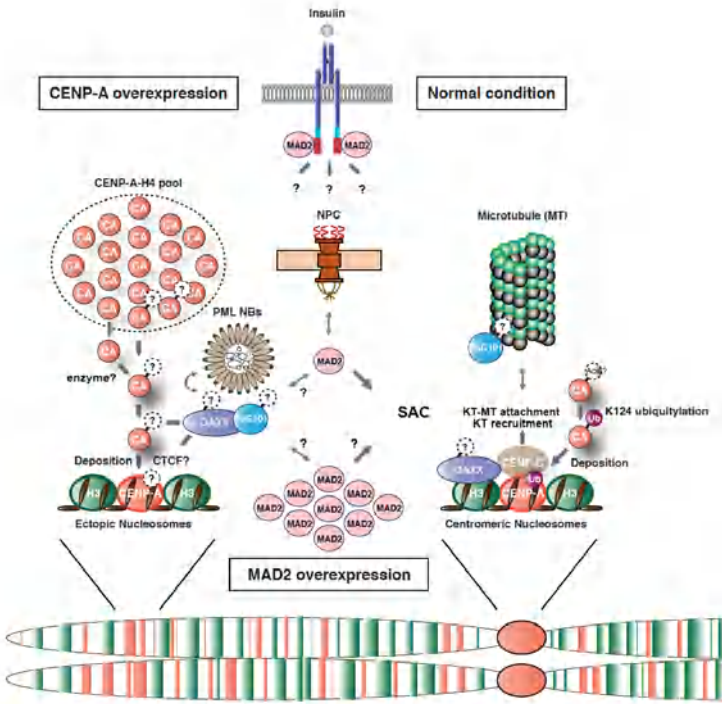

Figure 1. Inter-organelle signaling of MAD2 involved in MAD2 and CENP-A overexpressions.

MAD1 (not shown) and MAD2 associate with nuclear pore complexes (NPCs) in interphase, and nuclear pores and kinetochores (KT) both emit spindle checkpoint signals. Regulation of downstream factors of insulin receptor (IR) and its link to MAD2 overexpressing cancer is not yet clear. CENP-A ubiquitylation is required for CENP-A deposition into centromeric nucleosomes, while CENP-A overexpression leads to CENP-A deposition into ectopic nucleosomes through DAXX.

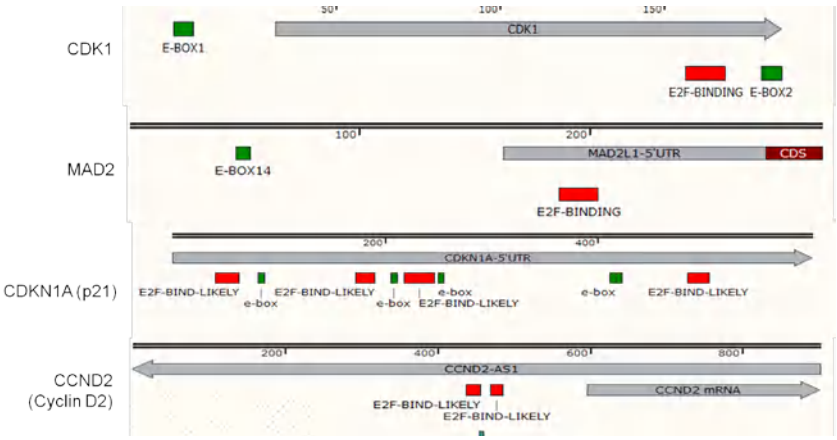

Figure 2. Myc responsive element E-box motifs reside with E2F-binding sites within promoter region of multiple mitotic regulator genes.

E-box is frequently present in human promoter sequences of various cell cycle genes whose expression is controlled by CDE (cell cycle-dependent element) and CHR (cell cycle homology region) elements reported by Schwartzman et al.. E-box is shown with green.

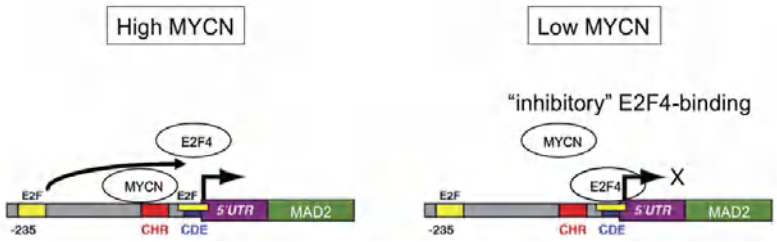

Figure 3. Hypothetical working model representing that MYCN is a candidate of CHR-binding protein, an unknown protein X, that control the MAD2 promotor in exclusive manner with E2F.

Selected publications (*Co-corresponding author)

1. Niikura Y#*, Kitagawa K#. E3 ligases for CENP-A. In: Catala A, editor. London, UK: IntechOpen [two book chapters]; 2021, In Press.
2. Niikura Y#*, Fang L#, Kitagawa R#, Li P, Xi Y, You J, Gao Y, Kitagawa K*. Mass Spectrometry Analysis to Identify Ubiquitylation of EYFP-tagged CENP-A (EYFP-CENP-A). J. Vis. Exp. 2020(160). Epub 2020/07/01. doi: 10.3791/61138. PubMed PMID: 32597847. (# Equal contribution)
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Group members

Graduate students

Yao Xi

Yidan Zhang

Rui Xu

Zhifei He

Jiezhu Fang

NJU-MARC Core Facilities

After five years in operation, the Core Facilities of MARC have begun to take shape. We have been equipped with more than 24 state of the art instruments and provide over 19000 hours service within or outside MARC research community in 2021.

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

Imaging

► Services

- Live cell imaging
- Optical sectioning of thick biological samples
- 3D reconstruction of images
- 3-D mosaic imaging
- Multi-area time-laps and spectral scanning
- Super-resolution imaging

► Equipment

- Olympus FluoView 1000 confocal
- Zeiss LSM880 with Airyscan
- Leica TCS II sp5 confocal
- GE Healthcare DeltaVision Imaging System
- GE Healthcare DeltaVision OMX 3D-SIM

Mass Spectrometry

► Services

- Quantitative analysis of small molecules
- Identification of unknown metabolites
- Able to analyze various kinds of samples
- Metabolomics study

► Equipment

- Agilent 6550 iFunnel Q-TOF LC/MS System

High Resolution Ultrasound

► Services

- Cardiovascular research
- Oncology study
- Drug metabolism study
- ……

► Equipment

- FUJIFILM Vevo® 3100 LAZR-X system
- FUJIFILM Vevo® 770

Flow Cytometry

► Services

- Cell sorting
- Able to analyze multiple fluorescent probes simultaneously

► Equipment

- BD LSRFortessa™ Flow Cytometer
- BD FACSCalibur Flow Cytometer
- BD FACSAria™ III Cell Sorter

Cellular Metabolism

► Services

- Live cell energy metabolism

► Equipment

- Agilent Seahorse Xfe24 Extracellular Flux Analyzer

Biomolecule Analysis

► Services

- SPR based molecular interaction

► Equipment

- GE Biacore T200 SPR system

Real time qPCR

► Services

- Gene expression detection

► Equipment

- ABI StepOne Plus
- Roche LightCycler 96

Others

► Equipment

- BioTek synergy H1 plate reader
- Beckman OPTIMA XPN-100 centrifuge

National Resource Center for Mutant Mice of China

The National Resource Center for Mutant Mice (NRCMM) is one of the 51 designated national platforms by Ministry of Science and Technology of China for resource preservation and sharing. The goal of NRCMM is generating, collecting, preserving and providing laboratory mouse models for research and biotech communities.

Currently the NRCMM is co-managed by Nanjing University and Gempharmatech company, with more than 10000 strains of mice. In addition to Nanjing site, NRCMM has established regional center in Guangdong and Sichuan. Mouse models from NRCMM have served thousands of research projects by universities, hospitals, research institutes, biotech and pharmaceutical companies.

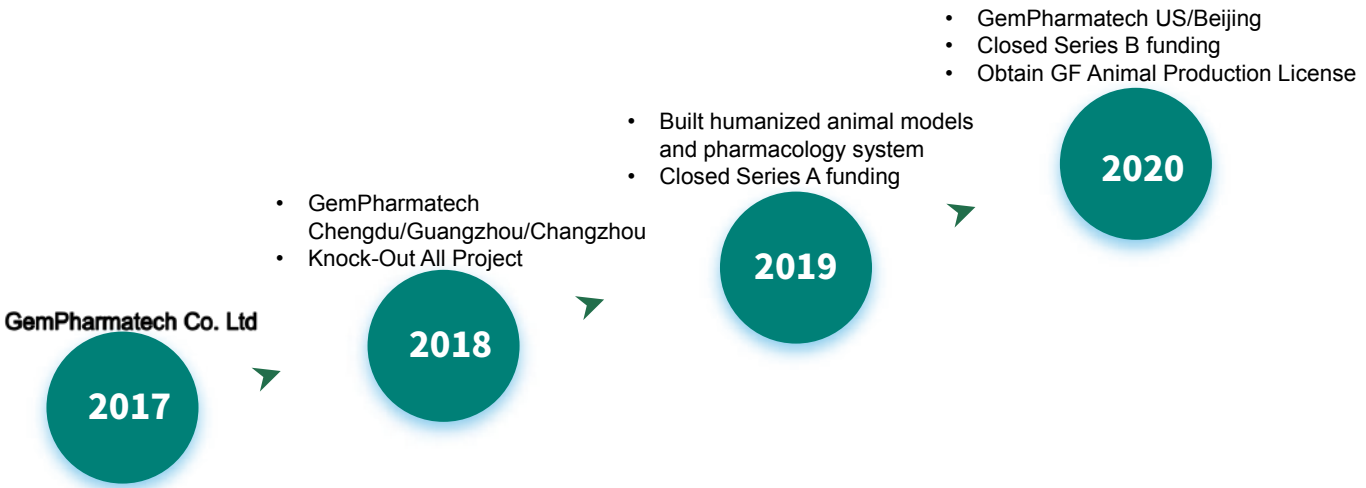
In 2021, NRCMM launched the "Mouse Models for Rare Disease" project and developed 124 new mouse disease models for supporting the studies for rare diseases and the related drug development.

NRCMM organized the "2nd Symposium on Development and Cooperation of Laboratory Animal Models Industry" in Beijing in July, 2021. More than 400 people attended the meeting. The Symposium focused on many key issues related to laboratory animal production, and quality control.

On August 26, 2021, the NRCMM Science Advisory Board meeting was held in Nanjing University. The meeting confirmed the progresses of NRCMM in the past two years and planned out the future direction of NRCMM.

Co-construction unit of NRCMM

GemPharmatech is a global biotech company dedicated to providing a one-stop-shop solution for in vivo biomedical research using genetically engineered mouse models, it is also a co-construction unit of the National Resource Center for Mutant Mice of China (NRCMM). The GPT team has 20 years of experience in generating and breeding disease models and conducting preclinical efficacy studies for both academic and industrial clients.

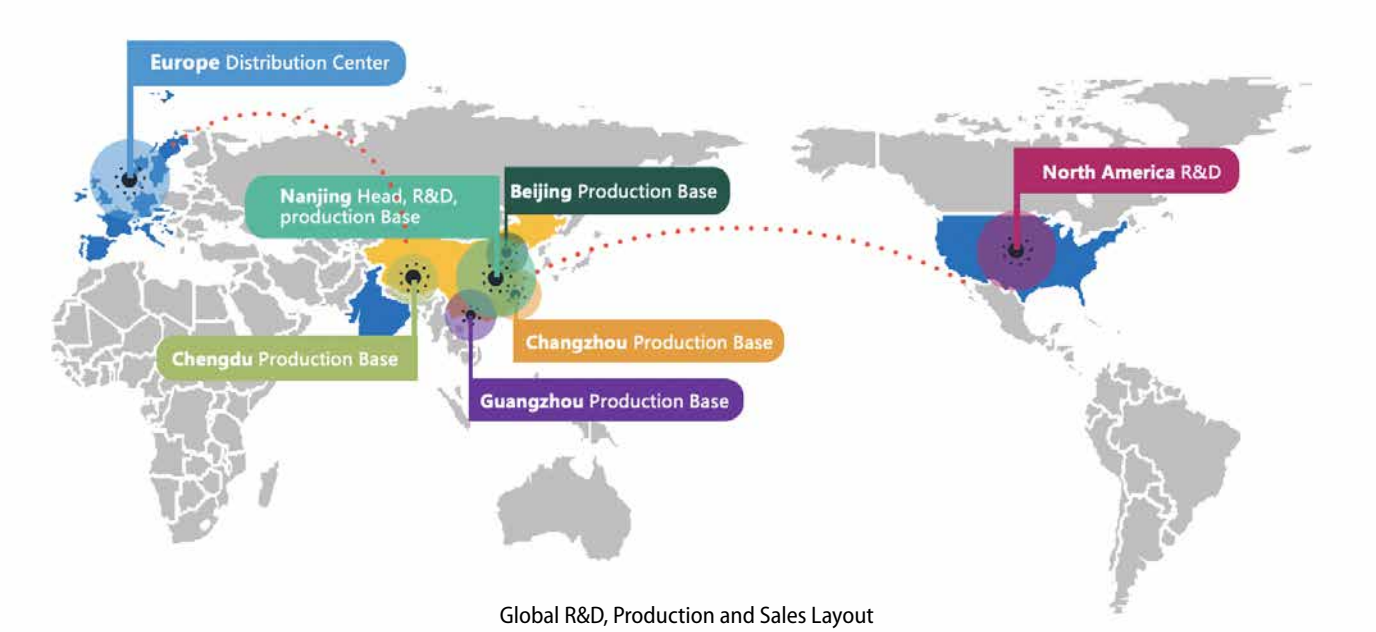


OUR VISION

We are dedicated to our long-term mission of providing world class mouse models and pre-clinical services to facilitate an efficient and rapid advancement of science to treat human diseases. We believe that the next 20 years will bring forth amazing medical breakthroughs.

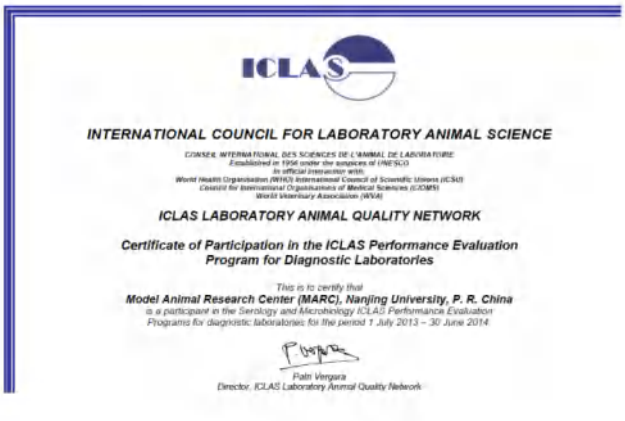
SERVICES

The company has a comprehensive service platform which includes humanized mouse models, customized model generation, germ-free mice service, breeding and husbandry and numerous disease models. Our ambitious knockout all platform (KOAP) is currently generating knockout and conditional knockout mice for all protein coding genes. In addition, we can perform phenotyping, drug development and efficacy testing.



Global R&D, Production and Sales Layout

International quality control standards


ICLAS

INTERNATIONAL COUNCIL FOR LABORATORY ANIMAL SCIENCE

ICLAS LABORATORY ANIMAL QUALITY NETWORK

Certificate of Participation in the ICLAS Performance Evaluation Program for Diagnostic Laboratories

This is to certify that
Model Animal Research Center (MARC), Nanjing University, P. R. China
is a participant in the Serology and Microbiology ICLAS Performance Evaluation Programs for diagnostic laboratories for the period 1 July 2013 – 30 June 2014

Patti Vergara
Director, ICLAS Laboratory Animal Quality Network

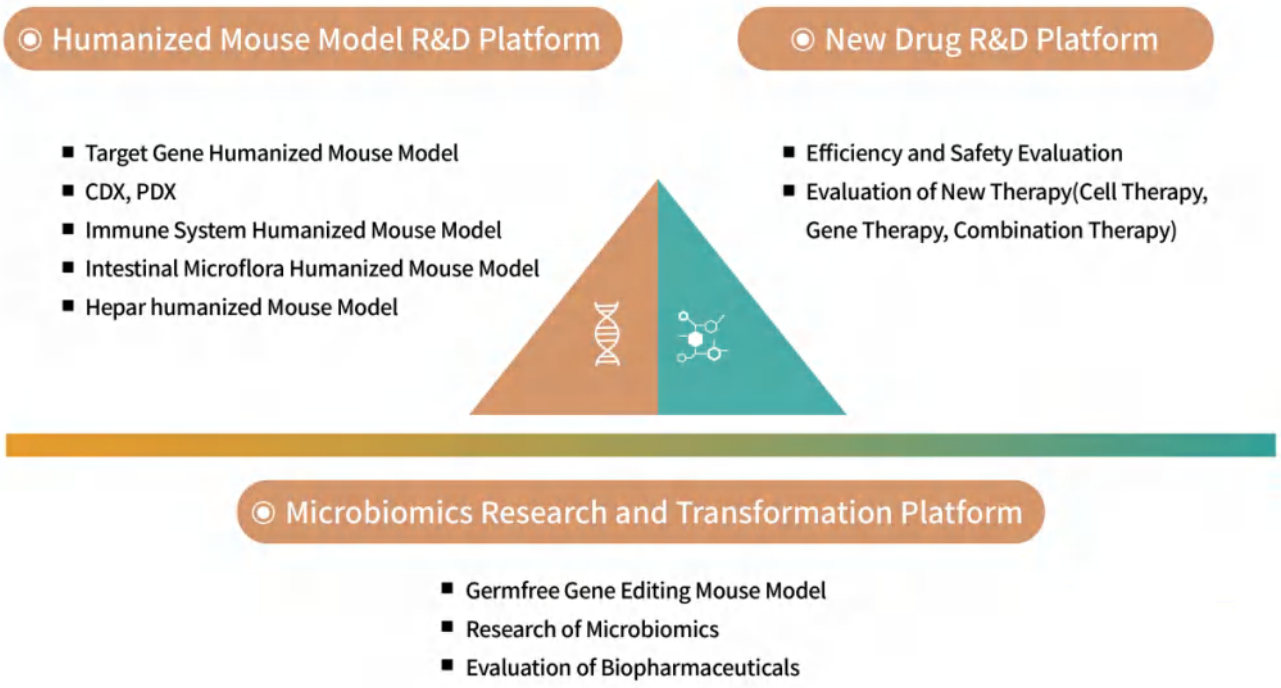
International Council for Laboratory Animal Science

Association for Assessment and Accreditation of Laboratory Animal Care

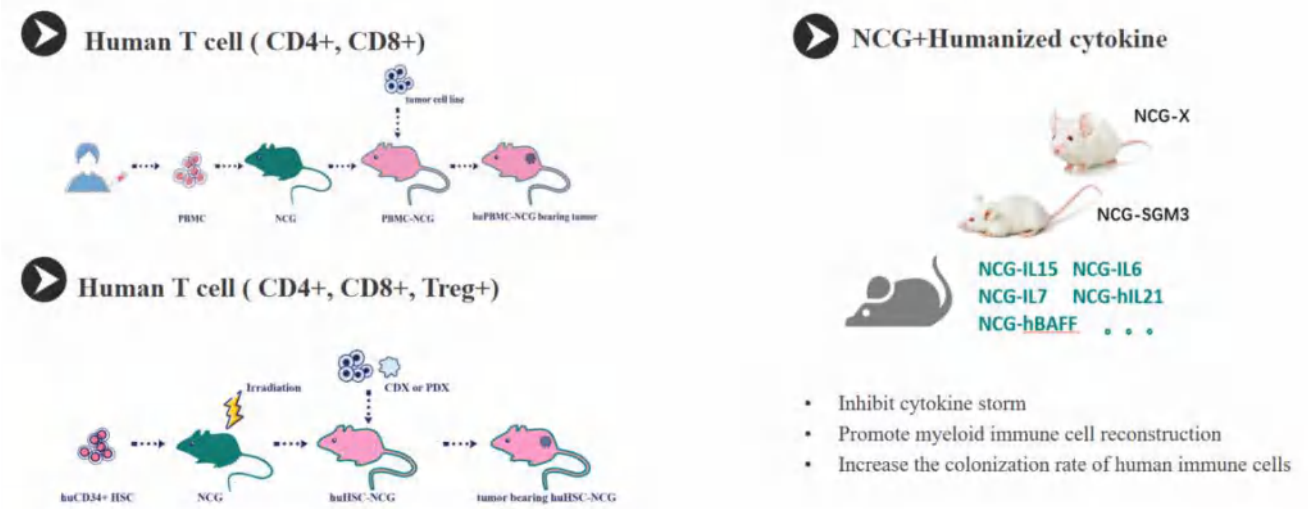
GPT has extensive experience in gene editing technology, and has established tens of thousands of gene-edited mouse models with CRISPR/Cas9, TALEN, ZFN, ES and other gene editing technologies. At present, GPT can make over 5,000 cases of transgenic, gene knockout and knock-in mouse models annually. Model Customization Platform can provide customers with model products, model customization, Joint R&D and other services.

Knockout All Mouse Project (KOAP)

In 2018, GemPharmatech launched KOAP to accelerate the delivery of cKO/KO mouse models. KOAP is aiming to generate cKO/KO strains for all 20,000 protein-coding genes in three years. Currently, GPT has successfully generated over 16,000 cKO/KO mouse models, covering genes in different research fields such as tumor, metabolism, immunity, development, DNA and protein modification. These strains can be directly ordered online (www.gempharmatech.com).



Technology Innovation Platform



Technology Platform of Immune System Humanized Mouse Model

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Seminar in 2021

	Date	Speaker	Title	Unit
1	2021/5/27	Wei Wang Ph.D.	Opportunities and challenges for biological research in the era of genome engineering	National Institute of Biological Sciences
2	2021/6/3	Hongyuan Chen Ph.D.	When chemical measurement meets life science	College of Chemistry and Chemical Engineering, Nanjing University
3	2021/6/10	Shouhong Guang Ph.D.	Small Regulatory RNA and nuclear RNA interference	University of Science and Technology of China
4	2021/7/15	Zhiqian Zhang Ph.D.	Stem regulation and targeted intervention of tumor stem cells	Peking University
5	2021/7/20	Hailong Piao Ph.D.	Cancer Metabolism: Molecular Network and Mechanisms	Dalian Institute of Chemical Physics, Chinese Academy of Sciences
6	2021/10/12	Qiutan Yang Ph.D.	Understand organ development from tissue self-organization to inter-tissue crosstalk	Friedrich Miescher institute
7	2021/10/28	Chaoqun Wang Ph.D.	Lung Stem Cell, Niche, and Lung Diseases	University of California,San Francisco (UCSF)

Courses and Lecturers

The MARC, as an institute of the Nanjing University Medical School, is home to more than 100 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 2021, the following lecture series and courses were given by MARC staff at Nanjing University (unless indicated otherwise).

Progress in Life Sciences

All PIs at MARC

Cell Biology and Molecular Biology

Guoqiang Wan

Hongyu Wang

Min Li

Genetics

Qing Zhang

Jinzhong Qin

Di Chen

Xin Lou

Mechanism of Development

Jiong Chen

Ying Cao

Zhongzhou Yang

Xin Lou

Information Genomics:

Zhenji Gan

Yohei Niikura

Topics in Genetics I

All PIs at MARC

Topics in Developmental Biology I

All PIs at MARC

Frontier of Cell Biology

Wei Wang (National Institute of Biological Sciences)

Zhiqian Zhang (Peking University)

Shouhong Guang (University of Science and Technology of China)

Basic Concepts and Frontiers in Immunology

Jianghuai Liu

Yan Li

Huiming Gao

Zhaoyu Lin

Doctoral qualification exam I&II

All PI at MARC

MARC seminar in Genetics

All PIs at MARC

MARC seminar in Developmental Biology

All PIs at MARC

Physiology

Yun Shi

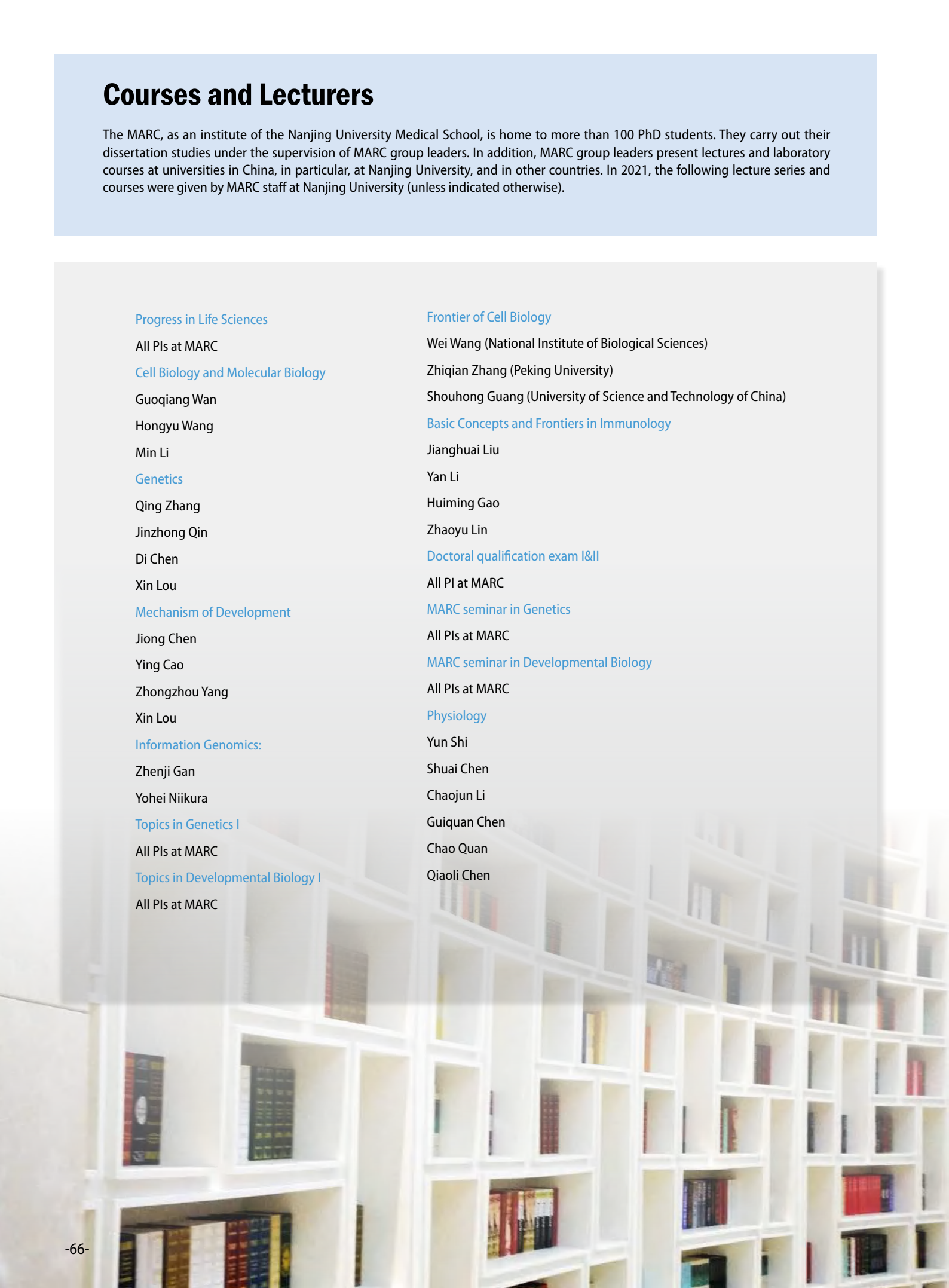
Shuai Chen

Chaojun Li

Guiquan Chen

Chao Quan

Qiaoli Chen



PhD Theses

MARC students successfully defended the following PhD thsese in 2021

PhD Theses:

Group Xiang Gao

Yujie Zou
The deficiency of SCARB2/LIMP-2 impairs metabolism via disrupted mTOR-dependent mitochondrial OXPHOS

Group Shuai Chen

Ping Rong
Obesity promotes hepatocellular carcinoma through a fatty acid responsive small GTPase Rab8a

Group Zhenji Gan

Yujing Yin
The function and mechanism of FNIP1 on thermogenic fat remodeling and systemic metabolism

Zhiheng Xu
The physiological role of mitochondrial protease LONP1 in skeletal muscle

Group Hongyang Wang

Dan Liu
Spatial multiomics study of human liver cancer stem cells

Group Jinzhong Qin

Yikai Huang
The role and molecular mechanism of RBBP4 in maintenance of mouse embryonic stem cell pluripotency

Group Chaojun Li

Jingzi Zhang
GGPP Allosterically Sensitizes FBP1 to Couple Cholesterol Biosynthetic Pathway with Glucose Metabolism

Group Xinyuan Fu

Zhongnan Ma
Knockout of Stat5 in T cells ameliorates high cholesterol and high fat diet-induced hypercholesterolemia by influencing cholesterol metabolism in the liver.

Group Guoqiang Wan

Qing Liu
High throughput screening on cochlear organoids identifies VEGFR-MEK-TGFB1 signaling promoting hair cell reprogramming

Chaorong Yu
Mechanisms of cochlear ribbon synapse pruning and maturation

GroupYun Shi

Jiahui Sun
Effects of epileptic-related Glua3 mutation on AMPA receptor channel dynamics and its mechanism

Xiaoyu Teng
LGI1 is involved in epilepsy and myelination

Group Guiquan Chen

Xiaolian Ye
Robust alleviation on neuropathology in a mouse model of Alzheimer's Disease by genetic inhibition of PDK1.

Huiru Bi
Differential roles of the Pen-2-Notch signaling in neuronal maintenance and oligodendrocyte development.

Group Geng Liu

Xiaoshuang Hou
The manipulation of glutamine metabolic reprogramming in T cell differentiation and antitumor response

Group Xiaosong Gu

Qihui Wang
The Vav1 guanine-nucleotide exchange factor promotes peripheral nerve regeneration via Klf2-Vav1-Rac

Lili Zhao
The gene expression profile and molecular regulation of axonal regeneration in dorsal root ganglion neurons by LCM-seq

Group Zhongzhou Yang

Mingjie Xu
The SRCAP chromatin remodeling complex promotes oxidative metabolism during prenatal heart developme

Xinyi Huang
Deficiency of the G4 resolvase RHAU causes left ventricular noncompaction cardiomyopathy

Yingchao Shi
Znhit1 deficiency in post-natal cardiomyocytes caused vacuolar cardiomyopathy and branched-chain amino acid catabolic disorder

Group Qingshun Zhao

Nan Li
ATRN participates in the mechanism of schizophrenia by regulating the level of testosterone

Luqingqing He
Zebrafish ventricular maturation is monitored by Foxc1a towards its direct targets wwtr1 and nkx2.5

Group Ying Cao

Lu Chen
Study on the PRMT1 maintains neural stemness in tumor cells and its underlying mechanism

Min Zhang Min
Neural stemness promotes cell tumorigenicity and differentiation potential

Xiaoli Yang
Study on the inhibition of neural properties in tumor cells by non-neural pro-differentiation factors and the underlying mechanism

2021 Summer Camp

As the primary task for MARC is to excel in scientific research and education, graduate students are the most valuable assets of our institute. To attract more outstanding students to MARC, we held the 12th Summer Camp on July 6-7th and 29-30th this summer.

This summer camp was broadcast live at Tencent conference, 136 candidates participated online, allowing productive two-way communications of both enrollments and queries. Eight excellent undergraduates were awarded "outstanding participants" from a pool of 101 applicants.

A set of wonderful programs have been organized to better facilitate the interactions between undergraduate students and our faculty members/ graduate students. Dr Shuai Chen, director of MARC, gave an opening speech and introduced the history and passion of MARC. Seventeen faculty members introduced their research areas at Model Animal Research Center, including areas of metabolism and immunology, neuroscience, developmental biology, stem cells and tumor biology.

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. In addition to scientific courses delivered by our prominent PIs, both PIs and the summer camp students who share same passion continued to change ideas and communicate after the broadcast. Overall, the online Summer Camp allowed the participants to experience the strong atmosphere of academic research at MARC and stimulate their enthusiasm for scientific research.

2021 Students Activities

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

Many students in our research center are active in various sports activities. To satisfy the interest of our students, we organized kinds of activities. We held table tennis competetion in April and fun sports meeting in May.

We rented three badminton courts, all members of MARC can enjoy the happiness of playing badminton every Friday afternoon. On the badminton fields, we all felt the charm of this sport activity, relaxed ourselves and strengthened our own bodies a lot. Also, we held a heat badminton match that attracted many badminton enthusiasts in June. All the players dived themselves in the gaming and their passion filled the courts. Both the players and spectators enjoyed a lot.

Except for the sports events, we conducted some academic activities. On one hand, we ran a Poster Exhibition in which all the students can show their research to others. All members in MARC voted for top posters in their mind. And finally, authors of the top three were selected as MARC Star Candidates of 2020. Posters ranked fourth to eighth were selected as outstanding posters. And all authors of these posters were rewarded for their excellent research work. On the other hand, we conducted scientific research exchange meeting between teachers and students monthly which can promotes communications of students and teachers from different labs.

Through all these sports and academic activities, we can savage our body and civilize our spirit, so that we can stay in a fuller state of mind for scientific research and live a happier life.

We invite two professors to report in Scientific research exchange meeting each month. Professors share their scientific research, new ideas and brain storms with students from different labs. We have a cold meal after the report and communicate in a relaxed and pleasant atmosphere.

Marc Academy

Marc Academy is a newly established teacher-student organization of MARC in 2021. Its purpose is to promote teacher-student exchanges and break the communication barriers of students in various laboratories. At present, Marc College is divided into 4 colleges. The dean of the school is elected spontaneously by students, and competitions between colleges are carried out with the college as a unit. Each college includes students and teachers in each laboratory, and ensures full communication and interaction between teachers and students through activities between each college and within the college. Each activity is sponsored by one of the 4 colleges, and activity funds are obtained through the ranking of each activity to support free activities in the college.

At present, Marc Academy has successfully hosted the first MARC Fun Games and a table tennis competition jointly organized with the Student Union.

As a traditional MARC activity, table tennis competition is an activity that teachers and students look forward to every year. This year's table tennis competition was held by the MARC Academy. It was the first time everyone won the competition for the Academy.

The early stage of the Fun Games was actively prepared by the Dean of the First Branch Xiang Duan and the students, and it was held on a sunny weekend. The students actively responded to the call of the school, and clamored for the honor of the school.

In addition, the activity funds obtained in the competition between the college and the college can fully support the free activities in the college, such as ball games, literary competitions, etc., and add a touch of fun to the students' after-school activities.

In the coming days, Marc Academy will hold singing competitions, hiking, fishing and other exciting activities to enrich the extracurricular activities of teachers and students, increase communication between teachers and students, and encourage everyone to get out of the laboratory to improve their physical and mental health. .

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