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Tsinghua Scholars Virtual Poster Session Abstract Book

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Tsinghua Poster Session #1

Cancer

Scholar Presenters:

Shikai Hu

Simeng Liu

Zongyou Cai

Xiang Li

Yang Wu

Moderator: Peter Drain, PhD

Impaired MALT1 Protease Activity Inhibits Tumor Growth and Enhances the Antitumor Immune Microenvironment

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Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), a cellular signaling protein that possesses protease activity targetable by small-molecule drugs, is a critical mediator of NF-κB signaling. MALT1-dependent NF-kB activation plays a key role in mediating lymphocyte proliferation in response to antigen. In our study, we found that genetically or pharmacologically disabling MALT1 protease activity in mice can inhibit tumor growth and enhance host antitumor immunity.

Recent reports suggest that MALT1 is required for the proper functioning of regulatory T cells (Tregs) and that mice with inactive MALT1 protease demonstrate autoimmune symptoms. We hypothesize that disrupting MALT1 protease activity will enhance antitumor immunity by impairing immunosuppressive Tregs, therefore making the tumor immune microenvironment (TME) more inflammatory. Using two syngeneic orthotopic mouse models of breast cancer (Eo771 and pB3), we compared the TME of wild-type and MALT1$^{PD/PD}$ mice implanted with breast cancer cells. In addition, we also compared the TME of mice implanted with breast cancer cells and then treated without or with MALT1 protease inhibitor. We found that breast tumors grow more slowly in MALT1$^{PD/PD}$ mice and this reduced tumor growth is associated with increased effector T cell infiltration, reduced PD-1 expression of CD8 T cells, and an increased inflammatory cytokine level in TME. The results highlight the critical impact of MALT1 on antitumor immunity and indicate that pharmaceutically inhibiting MALT1 protease activity represents a potential approach to enhancing antitumor immunity.

NOTCH-YAP1/TEAD-DNMT1 Axis Drives Hepatocyte Reprogramming into Intrahepatic Cholangiocarcinoma

Shikai Hu[1,2,#], Laura Molina[2,#], Junyan Tao[2], Silvia Liu[2,3], Mohammed Hassan[4], Sucha Singh[2], Minakshi Poddar[2], Aaron Bell[2], Daniela Sia[5], Michael Oertel[2,3], Reben Raeman[2,3], Kari Nejak-Bowen[2,3], Aatur Singhi[3,6], Jianhua Luo[2,3], Satdarshan P. Monga[2,3,4*], Sungjin Ko[2,3*]

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Intrahepatic cholangiocarcinoma (ICC), a disease of poor prognosis, has increased in incidence. It is challenging to treat due to intra- and inter-tumoral heterogeneity, which is in part attributed to diverse cellular origin, including hepatocytes (HCs). Indeed, co-expression of AKT along with NICD/YAP1 in HC yielded ICC, which we show resembled proliferative, Notch-activated, and stem cell-like subclasses of clinical ICC. NICD induced SOX9 and YAP1 in HC-driven ICC and deletion of either significantly delays ICC development. Yap1 deletion or TEAD inhibition impaired HC-to-biliary epithelial cell (BEC) reprogramming, while Sox9 was dispensable for this conversion. DNMT1 was discovered as a novel downstream effector of YAP1-TEAD complex that directed HC-to-BEC/ICC fate-switch. DNMT1 loss prevented Notch-dependent HC-driven cholangiocarcinogenesis, and DNMT1 re-expression restored ICC development following TEAD repression. Co-expression of DNMT1 with AKT was sufficient to induce hepatic tumor development, including ICC. Thus, we identify a novel NOTCH-YAP1/TEAD-DNMT1 axis essential for HC-to-BEC/ICC conversion, which may be relevant in cholestasis-to-ICC pathogenesis in the clinic.

Interrogating B Cell and Tertiary Lymphoid Structure Heterogeneity in Head and Neck Squamous Cell Cancer (HNSCC)

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Head and neck squamous cell carcinomas (HNSCC) are driven by exposure to environmental carcinogens or infection by human papillomavirus (HPV). Immunotherapies like anti-PD1 are now standard of care for HNSCC, but only 20% of patients garner therapeutic benefit. Further, current immunotherapies have brought little attention to the humoral immunity compared with T cells. HPV^+ HNSCC patients have improved prognosis compared to HPV^- patients; this survival benefit has been linked to increased tumor infiltrating B cells (TIL-B) and tertiary lymphoid structures (TLS). Our preliminary data have demonstrated that HPV^+ HNSCC patients have increased germinal center (GC) B cells and mature TLS, which correlate with improved survival. However, recurrent/metastatic (R/M) HNSCC patients actively receive new immunotherapeutic regimens. Thus, interrogating differences in TIL-Bs and TLS in locally advanced (LA) patients versus R/M HNSCC patients will influence B cell-centric immunotherapies.

In our study, we performed multispectral immunofluorescence (IF, Vectra Polaris) on 13 patients and evaluated immune infiltration and TLS formation. The total number of TLS in R/M patients was not significantly different from LA patients, but the $CD20^+$ TIL-Bs were increased within TLS in LA HNSCC patients. Further, most TLS in R/M HNSCC were non-mature. TLS and TLS maturation (formation of a GC) in R/M patients was decreased compared to LA HNSCC patients. We also evaluated the effect of various HPV viral proteins on immune infiltration and TLS formation. Analyses of 499 patients in the TCGA database elucidated that 90 leukocyte-specific genes were enhanced in HPV^+ patients with all four viral proteins compared to E6-E7 expression alone. The most differential signatures were B cell-specific genes. Thus, we also performed multispectral IF on 7 $E4\text{-}E5^+$ (with E6-E7) patients, 15 E6-E7 only patients, and 26 HPV^- patients. We observed increased TLS number and maturation, such as CD20, CXCR5, and CD4 in $E4\text{-}E5^+$ HNSCC patients. Our findings will be instrumental in developing B cell-focused immunotherapies for HNSCC patients.

RET as a Novel Therapeutic Target in Breast Cancer Brain Metastases

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Current therapeutic strategies for breast cancer brain metastases (BrM) are mostly limited to surgery, chemotherapy, and radiotherapy, making the study of novel druggable targets an urgent clinical need. RET, a receptor tyrosine kinase, is upregulated in BrM compared with the patient-matched primary tumors. The ligand for RET is glial cell-derived neurotrophic factor (GDNF), which is highly expressed in brain. Therefore, we hypothesize that the high level of brain-derived GDNF provides a permissive microenvironment for RET overexpressing breast cancer cells to form brain metastases and will evaluate the therapeutic potential of RET-targeted therapies for breast cancer BrM patients.

We demonstrated that RET expression and signaling were increased in breast cancer BrM compared with primary tumors in clinical samples. We determined that activation of GDNF-RET mediated downstream targets increased in RET overexpressing cell models, which was downregulated by shRNA-mediated RET knockdown. RET overexpression promoted breast cancer cell migration and sphere formation with GDNF stimulation *in vitro* and enhanced cell colonization in brain organotypic culture compared with liver slice co-culture in *ex vivo* study. Further, breast cancer cell line xenografts are developed through mammary fat pad and intracardiac injections to study brain metastases *in vivo*.

Neomorphic Cell-Cell Adhesome Reprogramming Facilitates Metastasis of ESR1 Mutant Breast Cancer

Yang Wu[1,2], Zheqi Li[1,3], Amir Bahreini[1,4], Jian Chen[1], Ye Qin[1,3], Kevin M. Levine[1,5], Nilgun Tasdemir[1,3], Nolan M. Priedigkeit[1,3], Li Zhu[6], George C. Tseng[6], Yu Jiang[3], Benjamin Troness[7], Laki Buluwela[8], Simak Ali[8], Spencer Arnesen[9,10], Jason Gertz[9,10], Ben Ho Park[11], Jennifer M. Atkinson[1,3], Dorraya El-Ashry[7], Adrian V. Lee[1,3,4], Steffi Oesterreich[1,3,4,5]

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Hotspot estrogen receptor-α (ER/ERα/ESR1) mutations occur in 30-40% of endocrine-resistant ER+ breast cancer and are associated with worse clinical outcome, but how these mutations facilitate metastasis remains ambiguous. In this study, we show the presence of ESR1 mutations exclusively in distant metastases, but not local recurrences. Under the guidance of transcriptomic changes in clinical samples, we find a reprogrammed cell adhesive gene network in genome-edited ESR1 mutant cell models via alterations in desmosome/gap junction genes, which confer enhanced cell-cell adhesion and can be alleviated by pharmacological blockade of desmosome/gap junction. ER ChIP-seq revealed no gained mutant ER binding sites at proximity of those target gene loci, suggesting indirect regulation by mutant ER, which was exemplified by a secondary regulation from cFos/AP1 signaling of GJA1 expression. These findings provide insights to the development of drugs targeting gap junction in ER mutant tumors.

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Tsinghua Poster Session #2

Immunology, Inflammation, and Sepsis

Scholar Presenters:

Lu Fan
Haiyue Li
Ting Jiang
Qixing Liu

Moderator: Tim Billiar, MD

Regulatory T Cell-Derived Amphiregulin Promotes Fibrosis of Heart Allografts Via Activating Fibroblasts

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Chronic rejection after organ transplantation manifests as function-limiting allograft fibrosis and vasculopathy. The development of fibrosis during chronic rejection is similar to that observed in failed tissue repair. Amphiregulin (Areg) is a growth factor secreted by multiple immune cells in response to injury and initiates repair processes. Regulatory T cell (Treg)-secreted amphiregulin has recently been implicated in muscle, epithelium, and nerve repair. Herein, we aim to study the role that Treg-secreted Areg plays in chronic rejection development. C57BL/6J (B6; (*H2-b*) Foxp3-YFP-Cre, and Foxp3-YFP-Cre x Areg$^{fl/fl}$ mice received heterotopic Bm12 (*H2-Ab1*^{bm12}) heart transplants. At Day 100 post- transplantation, isolated grafts were examined by histology and immunofluorescence staining. Primary fibroblasts were co-cultured with Tregs isolated from B6 Foxp3-YFP-Cre mice or Foxp3-YFP-Cre x Areg$^{fl/fl}$ mice to study the impact of Treg-secreted Areg on proliferation, migration, and extracellular matrix production of fibroblasts. Surprisingly, the specific deletion of Areg from Treg provided protection against fibrosis. This finding was consistent with our observation that Areg sufficient Tregs drove the proliferation and migration of fibroblasts *in vitro*. Our study established the function of Treg-secreted Areg in the progress of chronic rejection after heart transplantation, which may provide new insights into treatment of cardiac fibrosis.

TLR9 Modulates Peritoneal Immunity via Regulating the Biology of Fibroblastic Reticular Cells

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Fibroblastic reticular cells (FRC) are highly heterogeneous. Distinct FRC subsets play unique roles in the formation of secondary lymphoid organs and immune responses. We have previously shown that TLR9 signaling plays a critical role in regulating peritoneal immunity via suppressing chemokine production in FRC in the fat associated lymphoid clusters (FALCs). However, the subset-specific roles of TLR9 in FRC of FALCs remains unknown. Using single-cell RNA sequencing, we identified three distinct subsets of FRC ($CD55^{hi}$, $CD9^{hi}$, and $CD55^{lo}CD9^{lo}$) in mouse mesenteric FALCs at baseline. Based on the gene set enrichment analysis, the $CD55^{hi}$ subset was enriched in genes related to cell differentiation. The $CD9^{hi}$ subset was enriched in genes related to immune response. The $CD55^{lo}CD9^{lo}$ subset was enriched in the genes related to extracellular matrix formation. Furthermore, we found that $CD9^{hi}$ FRC from *TLR9*$^{-/-}$ mice increased expression of genes associated with inflammation. Using flow cytometry and bulk-RNA seq, we successfully isolated and validated these three subsets in mouse FALCs. Interestingly, activation of TLR9 signaling using ODN1585 significantly decreased CD9 expression in FRC *in vivo* and *in vitro*. Furthermore, treatment of ODN1585 suppressed the proliferation of distinct FRC subsets, evidenced by decreased expression of Ki67. These results indicate that $CD9^{hi}$ FRC in FALCs are immunoregulatory. TLR9 signaling may modulate peritoneal immunity via regulation of the biology of FRC. Understanding the mechanisms of how TLR9 regulates FRC may lead to discovering new therapies for inflammatory diseases.

Cognate Antigen Rechallenge Expands the Numerical Size of Resident Memory T Cells in Epidermis and Triggers Their Migration into Circulation

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Tissue-resident memory T cells (T_{RM}) are a subset of memory T cells that reside in nonlymphoid tissues and provide frontline protection against infections. The carrying capacity of T_{RM} cells in peripheral tissues and the migratory ability of T_{RM} are two controversial topics in T_{RM} research. By seeding OT-I cells as T_{RM} in mouse epidermis with VV-OVA and re-stimulating them with $OVA_{257-264}$, we demonstrated that the capacity of T_{RM} pool in epidermis was flexible and could be expanded by antigen rechallenge. Multiple rechallenge could further elevate the homeostatic T_{RM} number and a cell-intrinsic change might happen during this process. Using tamoxifen inducible Cre recombinase system to specifically label epidermal T_{RM} cells, we confirmed the phenomenon that T_{RM} cells undergo retrograde migration during recall response, which was shown in recent studies. Our further experiments revealed that TCR activation was indispensable to trigger the migration. We also assessed the phenotype of the ex-T_{RM} population in circulation and found they broadly kept T_{RM} properties. In conclusion, we uncovered two mechanisms by which cognate antigen rechallenge expanded the epidermal T_{RM} population quantitatively and spatially, respectively: elevating the carrying capacity of T_{RM} in epidermis and pushing epidermal T_{RM} cells to migrate into circulation.

The Role of IL17-IκBζ Signaling in Stromal Cells and DSS Colitis

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Background:
IL17 is an inflammatory cytokine best known for driving autoimmune disease. However, IL-17 also serves a protective role in the intestinal epithelium by promoting barrier function during injury, while the role of IL-17 signaling in gut stromal cells is still unclear. IκBζ is a member of the atypical IκB protein family, which is induced by proinflammatory stimulation, including IL-17. It can act as a transcriptional regulator to fine-tune downstream gene targets of NF-κB signaling pathway, particularly IL-6.

Methods:
RNA-seq and ChIPseq were performed on ST2 cells (a bone marrow-derived murine stromal cell line) pre-treated with scramble or IκBζ siRNA and stimulated with media or IL17. Col1a2CreERT2 IL17RAfl/fl and WT mice were subjected to DSS colitis. Colon tissues were harvested for analyses, including flow cytometry, qPCR, H&E staining, and immunofluorescence staining.

Results:
Based on sequencing and GSEA, genes regulated by IL17-IκBζ signaling in stromal cells show enrichment, mainly in inflammatory response. During DSS colitis, IL17RA deletion in stromal cells resulted in less inflammation, indicated by H&E staining, less inflammatory cytokines expression, and less Th1 cells. On the contrary, these mice have shorter colon length and more body weight loss, suggesting a protective role of IL17 signaling in stromal cells.

Conclusion:
IL17-IκBζ signaling is typically associated with inflammatory responses and, indeed, our findings support a pro-inflammatory function for IL-17 signaling through induction of IκBζ and IL-6. However, these data further identify a protective role for IL-17 signaling in stromal cells during intestinal damage and colitis.

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Tsinghua Poster Session #3

Immunology, Inflammation, and Sepsis

Scholar Presenters:

Jinyi Zhang
Haopu Yang
Yupeng Wang
Zhongli Xu
Yanlin Zeng

Moderator: Steffi Oesterrich, PhD

Metabolic Stress in the Tumor Interstitial Fluid Drives Long-Term T Cell Dysfunction

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With the rapid rise and success of cancer immunotherapies, it's generally appreciated that the immune system shapes cancer patient outcomes. While T cells are the primary effector cells of antitumor immunity, they are rendered dysfunctional and, thus, incompetent for tumor clearance in the tumor microenvironment (TME). The TME is characterized by severe metabolic restrictions, including nutrient depletion and metabolic waste accumulation, posing great challenges on the tumor infiltrating T lymphocytes (TILs). Using the tumor interstitial fluid media (TIFM), formulated based on the absolute concentration of more than 118 metabolites in the tumor interstitial fluid of pancreatic ductal adenocarcinoma, we found that T cells have less cytokine production in the TIFM and develop dysfunction in the TIFM culture, which is characterized by decreased proliferation, increased PD-1 expression, and decreased cytokine production. Dysfunctional T cells also cannot fully restore their cytokine production after resting in stress-free RPMI media. By manipulating the level of the metabolites that are depleted or enriched in the TIFM, we found that arginine depletion in the TIFM causes the halt of proliferation and that phosphorylethanolamine (PEtn) enrichment leads to increased PD-1 expression and decreased cytokine production. Our study reveals the key metabolic drivers of T cell dysfunction in the TME and sheds light on potential metabolic targets for T cell reinvigoration in the TME.

Blimp-1-Expressing ILC2s Are a Distinct Cell Population in Allergic Asthma

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Blimp-1 is a transcription factor known to have critical roles in multiple cell types, including B, T, and NK cells. However, its expression and function in non-NK innate lymphoid cell populations have not been well studied. To examine the expression and function of Blimp-1 in group 2 innate lymphoid cells, we analyzed several models of type 2 immunity. We found that Blimp-1 is not highly expressed in ILC2s at steady-state; however, it is induced under specific stimulation conditions. Especially, Blimp-1 expression is substantially increased in house dust mite (HDM) activated mouse lung over time. *In vivo* and *in vitro* ILC2 stimulation with cytokines show that both IL-33 and IL-10 can induce Blimp-1 expression, indicating Blimp-1 is driven by multiple signals. Interestingly, HDM-driven Blimp-1 expressing ILC2s have a distinct transcriptional program compared to classically-activated ILC2s treated with IL-25 and IL-33. ILC2 that express Blimp-1 predominantly lack type 2 cytokine expression, suggesting Blimp-1 plays a role independent of cytokine production and may represent a unique functional state of ILC2 cells in settings of chronic antigen, including allergic disease. Our findings, therefore, demonstrate a previously unappreciated Blimp-1 expressing ILC2 population with distinct features that may be important for type 2-mediated disease.

Integrative Analysis of Spatial Transcriptome with Single-cell Transcriptome and Single-cell Epigenome in Mice Lungs after Immunization

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RATIONALE: *Klebsiella pneumoniae* is an important cause of community-acquired pneumonia (CAP) and has developed antibiotic resistance. Immunization with whole bacterial lysate could induce *K. pneumoniae*-specific Th17, which are both required and sufficient to provide protection. Diversity and dynamic changes in T cells during immunization remain unclear. Multi-omics at the single-cell level provide unprecedented opportunity to unravel the puzzles.

METHODS: Mice were immunized with *K. pneumoniae* twice on Day 0 and Day 7 (“immunized mice”). A subgroup of them was then re-challenged with *K. pneumoniae* on Day 14 (“re-challenged mice”). Lung tissue was collected from a re-challenged mouse and sent for single-cell RNA sequencing (scRNA-seq) and single-cell sequencing assay for transposase-accessible chromatin (scATAC-seq) to obtain transcriptome and epigenome at the single-cell level. Four lung sections were collected from immunized and re-challenged mice and sent for imaging, then spatial transcriptome (ST) was obtained from the sections. scRNA-seq and scATAC-seq data were integrated via label transfer. scRNA-seq data were also used as a reference to decompose each spot (typically contains 1-10 cells) in the spatial transcriptome.

RESULTS: We identified 12 lung cell types, including immune cells, epithelial cells, and endothelial cells, in scRNA-seq data. Spatial transcriptome can be robustly decomposed using scRNA-seq reference, with inferring proportions of cell types for each spot. By integrative analysis of scRNA-seq and scATAC-seq data, we further identified Th17 and Th1 in both data sets. Th17 and Th1 were then decomposed in the spatial transcriptome, enabling the calculation of their weighted distance to airway. We found Th17 were closer to airway than Th1 in re-challenged mice, whereas Th1 were closer to airway in immunized mice.

CONCLUSION: We successfully integrated spatial transcriptome with single-cell transcriptome and single-cell epigenome in mice lungs for the first time and revealed dynamic changes of Th17 and Th1 locations in mice lungs upon *K. pneumoniae* re-challenge. These multi-omics resources could contribute a lot to the respiratory community.

Circulating Microbial Cell-Free DNA Is Associated with Inflammatory Host-Responses in Severe Pneumonia

Haopu Yang[1,2,3,4], Ghady Haidar[5], Nameer Al-Yousif[6], Haris Zia[7], Daniel Kotok[8]; Asim A. Ahmed[9]; Lily Blair[9], Sudeb Dalai[9]; Sivan Bercovici[9], Carine Ho[9], Bryan McVerry[3,4,10], Alison Morris[3,4,10,11], Georgios D. Kitsios[3,4,10*]

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Host inflammatory responses predict worse outcome in severe pneumonia, yet little is known about what drives dysregulated inflammation. We performed metagenomic sequencing of microbial cell-free DNA (mcfDNA) in 83 mechanically-ventilated patients (26 culture-positive, 41 culture-negative, and 16 uninfected controls). Recognized respiratory pathogens were detected in plasma from 91% and 40% of culture-positive- and -negative pneumonia patients respectively. Culture-positive patients had higher levels of mcfDNA than those with culture-negative pneumonia and uninfected controls ($p<0.005$). We found significant differences between clinical groups in mcfDNA from respiratory pathogens ($p<0.05$), but not from other microbes, with unclear clinical importance. Plasma levels of inflammatory biomarkers (fractalkine, procalcitonin, pentraxin-3, and suppression of tumorigenicity-2) were independently associated with mcfDNA levels (adjusted $p<0.05$) among all patients with pneumonia. Such host-microbe interactions in the systemic circulation of patients with severe pneumonia warrant further large-scale investigations on biological mechanisms and clinical utility.

Protists Protect from Loss of Oral Tolerance and Development of Celiac Disease

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Oral tolerance to innocuous food antigens is established via the induction of peripheral regulatory T cells (pTreg). However, reovirus T1L can elicit T helper 1 (Th1) immunity against dietary gluten, resulting in loss of oral tolerance (LOT) and celiac disease. In this study, we aimed to find countermeasures against T1L-mediated LOT and hypothesized that certain commensal gut microbes may have the capacity to protect from T1L-mediated LOT. Here we show that the gut commensal protist *Tritrichomonas muris* (T.mu, class: *Parabasalia*) promoted oral tolerance by elevating pTreg immunity to dietary antigen. T.mu colonization was also sufficient to restore T1L-mediated pTreg suppression and to block T1L-induced Th1 immunity to dietary antigen. The protection was achieved by suppression of T1L-mediated proinflammatory activation in antigen-presenting dendritic cells. To determine the relevance of these findings to celiac disease, we tested oral tolerance to gluten in celiac disease-predisposed DQ8tg mice. We found that T.mu efficiently suppressed T1L-mediated LOT to gluten. To directly investigate a role for *Parabasalia* in celiac disease, we screened the presence of *Parabasalia* in human stool samples. We found that *Parabasalia* was underrepresented in celiac disease patients compared to healthy controls. Our study shows that *Parabasalia* has immunomodulatory properties that protect from adverse reactions to food antigens and may protect from the development of celiac disease.

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Tsinghua Poster Session #4

Neuroscience and Infectious Diseases

Scholar Presenters:

Yuanchen Cheng

Qingya Shi

Haichao Chen

Siying Guo

Yuliang Wei

Moderator: Tim Greenamyre, MD, PhD

Divergent Function of Spinal Ascending Pathway in Nociception and Sensorimotor Integration

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Pain is conveyed from the spinal cord to the brain via an unknown number of output pathways that emanate from different laminae. Recent studies from our lab and others support the idea that lamina I neurons, which target the parabrachial nucleus, the periaqueductal gray, and the thalamus, play an essential role in aversion and suffering. In contrast, the function of projection neurons in deep laminae remains unknown and has been controversial for a long time. While some believe that layer V projection neurons encode intensity and localization of pain, others argue they are critical for sensorimotor control. By retrograde tracing from the ventral posterior thalamus, which is the major target of spinal-thalamic tract, we selectively labeled a large number of lamina V neurons, enabling visualization of these deep-layer output neurons in their entirety and manipulation of their function for the first time. Through viral tracing and behavioral approaches, we suggest that spinal parabrachial neurons (superficial dorsal horn neurons) are closely related to nociception, while spinal thalamic projection neurons (deep dorsal horn neurons) send collaterals to multiple brain areas related to motor function and mediate sensorimotor integration, rather than nociception.

Subthalamic Nucleus Activity Changes during Freezing-Like Behaviors in Parkinsonian Monkeys

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Background: “Freezing,” defined as episodes of movement breakdown, is one of the common symptoms developed in advanced Parkinson’s disease. Freezing of gait (FoG) largely affects patients’ independence and life quality, and non-gait freezing can also affect other arm and leg movements, and even speech. But the underlying mechanisms remain unclear. **Method**: Our hemisphere parkinsonian monkey, induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), exhibited a movement arrest, which was similar to freezing behavior, while performing a target-reaching task. Here we recorded single-unit activity within subthalamic nuclei (STN) during both non-freezing-like and freezing-like trials. **Results**: Firing rate in the baseline period was not changed between the two conditions, but the peri-movement activity in individual neurons was different. The size or onset time of the movement-related response showed no differences as well. However, in the increasing-type subset, the onset rate is significantly slower in freezing-like trials. **Conclusion**: Our paradigm could be useful for studying freezing. And this difference emerged even before the movement onset, so it might be used as a marker for freezing behavior.

Mice with S82A Mutation in the 4e-Bp1 Protein Are Predisposed To Develop T Cell Lymphomas after Sublethal Total Body Irradiation

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4E-BP1, a translation regulator, inhibits the formation of eIF4F cap-dependent translation initiation complex by binding to eIF4E. The inhibitory function of 4E-BP1 is regulated by multiple phosphorylation sites on the protein. When hyperphosphorylated, 4E-BP1 dissociates from eIF4E, allowing initiation of cap-dependent translation, particularly for 5' terminal oligopyridine (5'-TOP) transcripts. However, unlike other phosphorylation sites, which are under the control of mTOR, serine 83 (S83) is specifically phosphorylated by CDK1 during mitosis. To uncover the functional role of this specific phosphorylation site i*n vivo*, we generated 4e-bp1^{S82A} homozygous mice that carry a point mutation at S82 to alanine (S82A, S82 corresponds to S83 in human 4E-BP1). The S82A is functionally unable to be phosphorylated, allowing us to observe the mitotic changes from serine 82 under physiological conditions. In this study, we found that 4e-bp1$^{S82A/S82A}$ mice were prone to develop T cell lymphomas after sublethal total body irradiation. The CD4 and CD8 expression was distinct in different T cell lymphoma samples. The two derived tumor cell lines showed aberrant Pten function, represented by the absence or low expression of Pten protein and upregulated PI3K-Akt signaling. Ectopic Pten expression, however, was unable to revert the hyperactive PI3K-Akt signaling, nor could it inhibit the growth of tumor cells. Taken together, our results suggest a tumor-suppressor function of 4E-BP1 S82 phosphorylation in hematolymphoid cells and shed light on the new role of CDK1-dependent, mitosis-specific 4E-BP1 phosphorylation in maintaining cellular homeostasis after irradiation insults.

Systems-Level Study Reveals Anti-SARS-CoV-2 Repurposable Drugs and Compounds Targeting the Host Cell

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Understanding the mechanism of SARS-CoV-2 infection and identifying potential therapeutics are global imperatives. Using a quantitative systems pharmacology approach, we identified a set of repurposable and investigational drugs as potential therapeutics against COVID-19. These were deduced from the gene expression signature of SARS-CoV-2-infected A549 cells screened against Connectivity Map and further sorted by network proximity analysis to identify candidates relevant to disease modules. We also identified immunomodulating compounds aiming at suppressing hyperinflammatory responses in severe COVID-19 patients based on the transcriptome of ACE2-overexpressing A549 cells. Experiments with Vero-E6 cells infected by SARS-CoV-2, as well as independent syncytia formation assays to probe for ACE2/SARS-CoV-2 spike protein-mediated cell fusion using HEK293T and Calu-3 cells, showed that several predicted compounds had inhibitory activities. Among them, salmeterol, rottlerin, and mTOR inhibitors exhibited antiviral activities in Vero-E6 cells; imipramine, linsitinib, hexylresorcinol, ezetimibe, and brompheniramine impaired viral entry. These novel findings provide new paths for broadening the repertoire of compounds pursued as therapeutics against COVID-19.

Mutations in the HIV-1 3'-Polypurine Tract and Integrase Strand-Transfer Inhibitor Resistance

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Integrase strand-transfer inhibitors (INSTIs), such as dolutegravir (DTG), are routinely used for first-line treatment of HIV-1 infected individuals. INSTI resistance is typically associated with mutations in the HIV-1 viral integrase gene. However, recent studies suggested that mutations located in 3'-polypurine tract (PPT) of the HIV-1 genome, a region of the viral genome critical for initiation of plus-strand DNA synthesis, may also confer INSTI resistance. Specifically, Malet et al. reported in an *in vitro* selection experiment that mutations in the 3'-PPT confer INSTI resistance. Wijting et al. reported on the selection of mutations in the 3'-PPT in the virus from an infected individual who failed DTG maintenance monotherapy. However, no follow-up studies have ascertained whether the 3'-PPT mutations reported by Wijting et al. reduce INSTI sensitivity in phenotypic analyses of drug resistance. The primary objective of this study was to address this critical knowledge gap. Using site-directed mutagenesis, we constructed subtype B HIV-1^{LAI} infectious viruses in which the 3'-PPT sequence was changed from HIV-1^{WT} to HIV-$1^{PPT(1)}$ HIV-$1^{PPT(2)}$, in line with the mutations reported by Malet et al. and Wijting et al., respectively. We also examined the biophysical interactions between purified HIV-1 reverse transcriptase and template/primer substrates that contain the mutations. We found that HIV-$1^{PPT(2)}$ replicated as efficiently as HIV-1^{WT}. In contrast, we could barely detect replication of HIV-$1^{PPT(1)}$, suggesting that the mutations dramatically reduce replication efficiency. In light of this diminished replication efficiency, HIV-$1^{PPT(1)}$ was excluded from further analysis. Using phenotypic assays of drug sensitivity, we found that HIV-1^{WT} and HIV-$1^{PPT(2)}$ exhibited similar susceptibility to each of the INSTIs and to drugs belonging to the nonnucleoside reverse transcriptase inhibitors. Collectively, our data do not support a role for mutations in the PPT in INSTI resistance.