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Abstract #1

A small heat shock protein protects against Guillain-Barré syndrome in mice

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Guillain-Barré syndrome (GBS) is a peripheral system disorder in which host immune system causes neuropathy. Molecular mimicry between the gangliosides on the peripheral nerves and the lipooligosaccharide of microorganisms initiates the autoimmune response. Several case reports confirmed the GBS symptoms after the swine influenza vaccine and Campylobacter jejuni infection. Recent reports observed Zika virus infection also induced GBS symptoms. Majority of the GBS patients experienced neurological symptoms such as paresthesia, muscle weakness, pain and areflexia. Our previous studies reported heat shock protein (Hsp) 27 accelerated the axonal regeneration in mice after peripheral nerve injury. We showed that forced overexpression of human (h) Hsp27 could overcome anti-ganglioside mediated nerve regeneration inhibition. Passive transfer of anti-ganglioside antibodies (GD1a/GT1b-2b)/IB7 into hHsp 27 transgenic (Tg) mice and littermates was done to study their functional recovery. In our chronic animal model (90 days) we observed improved sensory and motor functional recovery in hHsp27 Tg mice as compared to littermates. Our electromyography and histology data showed that hHsp27 Tg mice demonstrated marked improvement of muscle function in terms of increased compound muscle action potential (CMAP) and neuromuscular junction (NMJ) reinnervation. To further investigate we used sub acute animal model (30 days) in which CMAP and muscle mass index was improved in hHsp27 Tg mice as compared to littermates. NMJ and axon quantification showed higher number of innervated NMJ and axon number in hHsp 27 Tg mice. Our future work is to elucidate the molecular pathway of hHsp27 involved in overcoming the inhibitory effect of anti-gangliosides.

Grant support – The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No: 01122026).

Abstract #2

Novel strategy for promoting axonal regeneration and repair in the nervous systems

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Injuries to the nervous system remain major obstacles in clinical medicine resulting in poor functional recovery. Damaged central nervous system (CNS) neurons cannot regenerate their axons largely due to the limited intrinsic growth capacity of injured neurons and extrinsic inhibitory microenvironment. By contrast, peripheral nervous system (PNS) neurons can regenerate their damaged axons at extremely slow rate of axonal regrowth (1-2mm/day). The slow regeneration of axons largely limited the functional recovery in patients with brachial plexus nerve injury (i.e. proximal PN injury) in which axons require to grow a long distance for muscle reinnervation. In recent years, there is emerging evidence suggesting that low-dose of ionizing radiation (LDIR) including X-ray is beneficial to living organisms via induction of adaptive response. LDIR activates the DNA repair mechanism and enhances the innate immunity, and thus promotes the overall fitness of irradiated individuals. Here, we examined whether LDIR of X-ray exhibits hormetic effects on promoting nerve repair after injury to the nervous system. In PNS, we demonstrated that LDIR of X-ray promotes neurite outgrowth of cultured peripheral neurons (i.e. dorsal root ganglions). Moreover, LDIR of X-ray accelerates axonal regrowth after sciatic nerve crush injury in vivo. In CNS, LDIR of X-ray promotes the migration of microglia towards the injury site after stab wound injury in postnatal cortex, which facilitate rapid degradation of CSPG. Further studies on the underlying molecular mechanism of LDIR on nervous system repair will shed new light on understanding the intrinsic machinery essential for successful regeneration after nervous system injuries.

Abstract #3

Cholecystokinin entorhinal neurons release glutamate

BAIBADO Joewel Tarra

The entorhinal cortex acts as a portal between the hippocampus and the neocortex. Its task on encoding and retrieval of memories has been postulated to involve Cholecystokinin-8(CCK-8). However, the synaptic architecture between axon terminals of the CCK glutamatergic entorhinal neurons and neighboring neurons is not yet elucidated. Hence, we are interested to determine anatomically the distribution of CCK neurons associated with vesicular glutamate transporter-1(VGlut1). In order to confirm this, we first labelled the CCK entorhinal neurons of CCK-cre mice using AAV5-EF1α-DIO-Chr2-mCherry and performed anterograde viral tracing. Then, we elucidated at the electron microscopic level the CCK-Vglut-1 co-expression using double immunogold labeling. Our findings reveal that the axon terminals of CCK positive entorhinal neurons are VGlut (+). Besides, on-going co-release study of CCK-8 and glutamate has been done using Multiple Ion Monitoring LC-TOF-MS/MS. Hence, both CCK and glutamate are co-released performing one of the key roles in long term potentiation.

Abstract #4

Targeting vagal afferent in learning and memory impairment associated with chronic visceral pain: the role of cholecystokinin.

CAO Bing

Cholecystokinin-octapeptide (CCK), which is a gastrointestinal hormone released during feeding, has been shown to enhance memory retention. Our series of published observations have shown CCK acting on vagal afferent fibers mediates various physiological functions (Ref). In this study vagal afferent neural responses to CCK-8 were examined by electrophysiological recorded single-neuronal firings in the nodose ganglia following neurobiotin labeling. We showed that CCK facilitated spatial learning and reference memory of control rats in the Morris water maze. Moreover, CCK enables to rescue the pain-induced deficits in spatial memory. Multi-electrophysiological recording were performed to clarify that the CCK enables to restore the reduced coherences, and repair theta-phase-locking of ACC neurons and further maintain the synchrony in MT-ACC pathway in VH rats. These results reinforce the view that gut-brain axis play an essential role in learning and memory, and may help to exploit synaptic reinforcement as a means for ameliorating the impact of visceral pain on learning and memory dysfunction.

YL References. *Journal of Clinic Investigation 92:418-424, 1993; Journal of Clinic Investigation 97:1463-70, 1996; Journal of Clinic Investigation 105:351-359, 2000; Behavioral Brain Research 236 8-15 2013; Molecular Brain 5:19 2012;*

Abstract #5

Vagus nerve stimulation modulates neuronal synchronization and phase-locking of anterior cingulate cortex associated with facilitation of decision-making in rats

CAO Bing, YANG Xiangwei

Vagal nerve stimulation (VNS) has been shown to enhance memory and cognitive functions in rats and humans (Ref). Employing a conscious rat model equipped with vagus nerve cuff electrode, we showed that daily VNS, administered immediately following the training sessions of rat gabling task (RGT), resulted in increase in 'good decision makers' rats. Simultaneity multiple-channel electrode recording showed that neural spike activity in the basolateral amygdala (BLA) becomes synchronized with ongoing theta oscillations of local field potential (LFP) following VNS. VNS elevated phase locking of ACC spike to theta oscillations of LFP in BLA. Moreover, cross-correlation analysis showed enhanced synchronization between LFPs recorded in ACC and BLA that occurred in the theta range. Our results provide evidence for VNS facilitate decision making and unveil neurophysiologic biomarkers for therapeutic VNS in cognitive impairment.

YL References. *Molecular Brain 2015 8:32 2015 4.902; Behavioural Brain Research 236 8-15 2013; Neurobiology of Learning and memory 97, 156-164, 2012.*

Abstract #6

Single-cell Analysis of Population-based Phenotypic Switching of Cancer Cells

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Tumour consist of multiple subtype of cells with different phenotypes and genotypes. It has been proposed that an equilibrium exists between these sub-populations through stochastic interconversion. Investigation of this interconversion plasticity in single cell level should prove useful in studies of phenotypic complexity. We isolated CD90+ and CD90- cells by flow cytometry and then characterized their phenotypic difference and interconversion dynamics both at population and single cells level. A549 maintains a stable expression of CD90 (~50%). The sorted subpopulations eventually achieved a new equilibrium and did not converge during the bulk cell interconversion, suggesting the equilibrium did not follow a stochastic process. The distribution of CD90 expression was significantly different between CD90+ and CD90- single cells clones and both achieved a similar average as compared with the bulk cell equilibrium. We defined transition clones and committed clones with differential interconversion capacities. The later one showed no sign of interconversion and therefore prohibit the return of cells to the original equilibrium.

Abstract #7

New Insights into Circadian Clock as a Mediator of Neurodegeneration

CHANG Yu Chen Victoria

Mammalian circadian clocks, molecular oscillators intrinsic to the brain and peripheral tissues, drive a 24-hour cycle in biological and behavioral processes. This cell-autonomous rhythm is generated by a transcriptional negative feedback loop, where the transcription factor CLOCK-BMAL1 drives the expression of its own inhibitors, Period and Cryptochrome. A growing body of evidence has shown that disrupted circadian rhythms, like altered sleep/wake cycle, are presented in patients with neurodegenerative diseases like Alzheimer's and Parkinson's diseases. This indicates that circadian dysfunction is closely linked with neuropathies. However, little is known about the underlying mechanisms and the physiological functions of circadian clocks in neurons. Therefore, to elucidate this, we will identify expression profiles of clock genes and possible mechanisms controlled by clocks in neurons by RNA-sequencing and bioinformatics approaches. Then, we will test the putative mechanisms and circadian clocks in neuropathological conditions to examine the relationship between neurodegeneration and circadian clocks.

Abstract #8

Encoding and retrieval of artificial visuoauditory memory traces in the auditory cortex requires the entorhinal cortex

CHEN Xi

Damage to the medial temporal lobe impairs the encoding of new memories and the retrieval of memories acquired immediately before the damage in human. In this study, we demonstrated that artificial visuoauditory memory traces can be established in the rat auditory cortex and that their encoding and retrieval depend on the entorhinal cortex of the medial temporal lobe in the rat. We trained rats to associate a visual stimulus with electrical stimulation of the auditory cortex using a classical conditioning protocol. After conditioning, we examined the associative memory traces electrophysiologically (i.e., visual stimulus-evoked responses of auditory cortical neurons) and behaviorally (i.e., visual stimulus-induced freezing and visual stimulus-guided reward retrieval). The establishment of a visuoauditory memory trace in the auditory cortex, which was detectable by electrophysiological recordings, was achieved over 20-30 conditioning trials and was blocked by unilateral, temporary inactivation of the entorhinal cortex. Retrieval of a previously established visuoauditory memory was also affected by unilateral entorhinal cortex inactivation. These findings suggest that the entorhinal cortex is necessary for the encoding and involved in the retrieval of artificial visuoauditory memory in the auditory cortex, at least during the early stages of memory consolidation.

Abstract #9

Protection against paclitaxel-induced peripheral neuropathy by targeted overexpression of human heat shock protein 27 in neurons

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Cancer patients withdraw their lifesaving chemotherapy due to chemotherapy induced peripheral neuropathy (CIPN). Paclitaxel is widely used to treat breast, ovarian and lung cancers. Our previous studies revealed that Paclitaxel disrupted microtubule organization in mouse primary dorsal root ganglion neuron assessed by atomic force microscopy and confocal imaging. In addition, development of allodynia with loss of intra-epidermal nerve fibers (IENFs), axonal degeneration and demyelination with reduced sensory nerve action potential (SNAP), nerve conduction velocity (NCV) and compound muscle action potential (CMAP) have been reported in animal model of CIPN. Heat shock protein 27 (Hsp27) is a chaperone protein which demonstrated anti-apoptotic and anti-oxidative activities. We have generated transgenic mouse lines which highly express human Hsp27 (hHsp27 Tg) in both sensory and motor neurons. Paclitaxel was injected intraperitoneally to hHsp27 Tg and their littermate (LM) mouse to induce peripheral neuropathy and Cremophore/Ethanol was injected as vehicle (VH) control. Assessment of mechanical and cold allodynia showed that hHsp27 overexpression significantly protects against paclitaxel induced peripheral neuropathy compared to VH control. Similarly, hHsp27 Tg group showed significant protection in IENF density, myelin basic protein and neuromuscular junctions analysis than in LM. Moreover SNAP, NCV and CMAP recordings showed better electrophysiological properties in hHsp27 Tg mice than LM indicating better axonal transport due to less mitochondrial damage. qPCR analysis for mitochondrial proteins showed significant downregulation in LM but not in hHsp27 Tg. Overall, overexpression of hHsp27 showed marked protection against Paclitaxel induced peripheral neuropathy and this may open new therapeutic approach to treat CIPN.
Grant Support: This work is supported by a GRF grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (CityU 160813).

Abstract #10

Oblongifolin M, an active compound isolated from a Chinese medical herb Garcinia oblongifolia, potently inhibits enterovirus 71 reproduction through downregulation of ERp57

Qi DONG, Ying CHEN, Ming-Liang HE

There is no effective drug to treat EV71 infection yet. Traditional Chinese herbs are great resources for novel antiviral compounds. Here we showed that Oblongifolin M (OM), an active compound isolated from Garcinia oblongifolia, potently inhibited EV71 infection in a dose dependent manner. To identify its potential effectors in the host cells, we successfully identified 18 proteins from 52 differentially expressed spots by comparative proteomics studies. Further studies showed that knockdown of ERp57 inhibited viral replication through downregulating viral IRES (internal ribosome entry site) activities, whereas ectopic expression of ERp57 increased IRES activity and partly rescued the inhibitory effects of OM on viral replication. We demonstrated that OM is an effective antiviral agent; and that ERp57 is one of its cellular effectors against EV71 infection.

Abstract #11

HBx-upregulated lncRNA UCA1 promotes cell growth and tumorigenesis by recruiting EZH2 and repressing p27Kip1/CDK2 signaling

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It is well accepted that HBx plays the major role in hepatocarcinogenesis associated with hepatitis B virus (HBV) infections. However, little was known about its role in regulating long noncoding RNAs (lncRNAs), a large group of transcripts regulating a variety of biological processes including carcinogenesis in mammalian cells. Here we report that HBx upregulates UCA1 genes and downregulates p27 genes in hepatic LO2 cells. Further studies show that the upregulated UCA1 promotes cell growth by facilitating G1/S transition through CDK2 in both hepatic and hepatoma cells. Knock down of UCA1 in HBx-expressing hepatic and hepatoma cells resulted in markedly increased apoptotic cells by elevating the cleaved caspase-3 and caspase-8. More importantly, UCA1 is found to be physically associated with enhancer of zeste homolog 2 (EZH2), which suppresses p27Kip1 through histone methylation (H3K27me3) on p27Kip1 promoter. We also show that knockdown of UCA1 in hepatoma cells inhibits tumorigenesis in nude mice. In a clinic study, UCA1 is found to be frequently up-regulated in HBx positive group tissues in comparison with the HBx negative group, and exhibits an inverse correlation between UCA1 and p27Kip1 levels. Our findings demonstrate an important mechanism of hepatocarcinogenesis through the signaling of HBx-UCA1/EZH2-p27Kip1 axis, and a potential target of HCC.

Abstract #12

Inter organ communication in zebrafish

FALLAH Samane

There are studies supporting the idea that two organs communicate to maintain their homeostasis. One of these studies is in Drosophila intestine which shows how inflammatory signaling emanated from neighboring or distant tissues can accelerate intestinal epithelial renewal.

While Drosophila intestine is really close to human gastrointestinal tract, zebrafish is a good model organism to study regeneration in different tissues such as spinal cord, retina, fin and heart.
We looked at how to two regenerating organs, namely caudal fin and heart, can affect each other's regeneration rate. We observed that fin and heart amputation cannot accelerate heart and fin regeneration respectively. One possible explanation can be the difference in tissue types. While fin is a cartilage, heart is a muscular tissue. Another explanation may be the distant between these two organs.

Abstract #13

The role of entorhinal cholecystokinin in long term potentiation induction as well as associative memory formation in auditory cortex.

FENG Hemin

According to our previous study, serving as an abundantly distributed neuropeptide in central nervous system, cholecystokinin (CCK) from entorhinal cortex can enable the neural plasticity in auditory cortex. As a kind of most widely studied neural plasticity, long term potentiation (LTP) is regarded as the base of learning and memory. However, there is few evidence to show the link between CCK and LTP. Now we try to find out the role of CCK plays in LTP by a series of experiments. With injection of CCK antagonist into auditory cortex of mice, TBS-induced LTP can be blocked. And by injecting CCK-8 alone, we can induce LTP without TBS. Furthermore, LTP can no longer be induced in auditory cortex of transgenic CCK-KO mice. That means CCK is not only necessary, but also sufficient for LTP induction in auditory cortex. In addition, we design two behavioral experiments to show the significance of CCK in associative memory. Firstly, we can establish a two-tones associative memory in wild type mice, while cannot in CCK-KO mice. Secondly, we use laser to specifically activate the CCK-positive terminals in auditory cortex, to establish an associative memory between electrical stimulus and tone. Taken all above, we can conclude that CCK plays an essential role in LTP induction as well as associative memory formation in auditory cortex.

Abstract #14

Prefrontal cortex helps maintain short-term associative memory in the auditory cortex through galanin

Xi CHEN, Jingyu FENG, Jia TANG, Wenjian SUN, Zicong ZHANG, Xiao LI, Jufang HE

In the present study, we have established a rat model of patient H.M. with permanent lesion of bilateral entorhinal cortices. No responses to the visual stimulus in the auditory cortex could be established for longer than 20 min after conditioning the light stimulus and the electrical stimulation of the auditory cortex with foot shock. However, the auditory cortex neurons responded to the light stimulus immediately after the conditioning for about 5min. We defined this short-term visual response, as short-term associative memory trace. Inactivation of the medial frontal cortex (mPFC) with infusing of DNQX reversibly suppressed this short-term association. Electrical activation of the mPFC on the anesthetized rat induced a transient increase in the neuronal response to the auditory cortex. This increase abolished after an infusion of galanin antagonist in the auditory cortex, while a direct local infusion of galanin induced an increase in the neuronal responses. Back to the behaving rat with bilateral entorhinal cortex lesion, local infusion of galanin antagonists suppressed the conditioning-induced short-term association, similarly to that of the mPFC inactivated rat. We concluded that the mPFC was involved in short-term memory in the auditory cortex, and the maintenance of the short-term memory was through the neuropeptide, galanin.

Abstract #15

DeepCC: a deep learning-based framework for cancer classification

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Major malignancies such as breast, colorectal and lung cancers are known to be heterogeneous both molecularly and clinically, which hampers the selection of right patients for optimized therapy. Molecular subtyping of cancer provides biological insights into cancer heterogeneity, and is a critical step towards more individualized therapy. Although signature gene-based classification has been widely demonstrated as an efficient approach, the performance has long been limited by platform differences, batch effects, etc. Here, we propose a versatile cancer subtyping framework, DeepCC, based on functional gene sets and deep learning, which can overcome the limitations of the current signature gene-based classification strategy. As case studies, we demonstrated the performance of DeepCC on colorectal cancer and breast cancer, by comparing with signature gene-based approach, our DeepCC showed much better performance on accuracy, robustness, and clinical relevance. Furthermore, we also propose a new approach to interpret cancer heterogeneity, which improves our understanding of underlying biological mechanism.

Abstract #16

Study of Tiny Droplet Adhesion and Pathogen Spreading on Bio-inspired Liquid-repellent Surfaces

Jieke JIANG, Xi YAO

Exhaled microdroplets containing pathogenic bacteria and virus could facilitate the spread of many infectious diseases. Understanding of the interaction between microdroplets and solid surfaces may help design antifouling materials and surfaces that can prevent the transmission of microdroplets and hence reduce the spread of pathogens. Here we established a model to generate and characterize microdroplets which enable us to simulate the surface biofouling of microdroplets containing bacteria.

Abstract #17

Global transcriptome analysis of Escherichia coli J53 harboring the drug resistance incFII plasmid pHK01

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Background
The dissemination of CTX-M gene is commonly achieved through plasmid conjugation. Narrow host-range incFII plasmids play important roles in the dissemination of CTX-M-14. CTX-M-carrying plasmid pHK01 and pHK01-like plasmids belong to incFII group and are widely disseminated in Hong Kong. Comprehensive characterization pHK01 is critical to understand and combat the emerging drug resistance plasmids globally. However, most studies focused on drug resistance genes and little is known of other aspects such as the bacterial host fitness changed upon pHK01 acquisition.

Methods
We sequenced the transcriptome of transconjugant strain Escherichia coli J53 at log and stationary phases using J53 as control. qRT-PCR analysis on genes were used to confirm the gene expression levels identified using RNA-Seq.

Results
The transcriptional profiling of Escherichia coli J53 shows that the host genes were altered upon the acquisition of pHK01. Further studies reveal that key pathways involving in metabolism and motility were significantly changed, which suggests that pHK01 could potentially induce diverse fitness changes in bacterial host.

Conclusions
In this work, we systematically studied the pHK01-induced transcriptome change in bacterial host. Significantly altered pathways in transconjugant suggest diverse fitness changes such as metabolism and motility. Our work could provide new insights in plasmid-host interaction and brings new directions in therapeutic researches.

Abstract #18

Single-cell analysis of EMT-related gene expression heterogeneity in metastatic tumor cell clusters isolated from ovarian cancer ascites

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Metastases account for over 90% of cancer-related deaths, as the malignant metastatic seeds, tumor cell clusters are found in both peripheral blood and effusions from cancer patients. These cell clusters occurring in the blood are also well known as circulating tumor cell (CTC) clusters and have been proved to be more aggressive than single CTCs, however, the rare appearance make them hard to be applied in large-scale intra-cluster single-cell study to reveal the molecular mechanism they played during metastasis. So here we are using metastatic tumor cell clusters isolated from ovarian cancer ascites for intra-cluster single-cell EMT-related transcriptional heterogeneity analysis. Our results showed a clear cell-to-cell molecular regulatory heterogeneity and identified a huge subgroup of tumor cells with the up-regulation of most EMT-related genes and the loss of typical epithelial tumor cell marker – EpCAM. Thus, our novel molecular evidences revealed the reason why these metastatic tumor cell clusters are greatly contribute to the metastatic spread of cancer.

Abstract #19

Bacteria factory for 'recombinant' siRNAs

KAUR Guneet, Linfeng HUANG

Small interfering RNAs (siRNAs) cause posttranscriptional silencing of genes in a sequence specific manner via RNA interference (RNAi). Recent years have witnessed an upsurge in interest in RNAi therapeutics, especially for cancers, rare genetic diseases, and viral infections. However their availability for use as RNAi therapeutics is critically dependent on easy and cost-effective manufacture process, which still remains a challenge for conventional chemically synthesized siRNAs. Here we report a robust, efficient and renewable method for large scale production of highly potent and specific siRNAs in E. coli. The bioprocess has been engineered by understanding key determinants in pro-siRNA biogenesis including the activity of RNase III, production of dsRNAs and abundance of p19 protein in E. coli. Process kinetics, design of novel pro-siRNA expression plasmids, selection of optimal expression strains and culture conditions were carefully explored to achieve maximum pro-siRNA production efficiency. Our method can generate pro-siRNAs at milligram per litre scale very quickly and reproducibly. We also demonstrated high gene knockdown efficiency for multiple disease associated genes. Hence our bio-based siRNA production method, which is adaptable to industrial scale production, holds great promises for RNAi therapeutics.

Abstract #20

Identification of plasmid-encoded sRNAs in a multidrug-resistant plasmid pNDM-HK carrying blaNDM-1

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Small RNAs (sRNAs) regulated gene expression is one of the emerging post-transcriptional regulatory mechanisms in bacteria and it plays a key role in response to physiological stimulation in bacteria. The first plasmid-encoded sRNA was identified from Escherichia coli plasmid and found to modulate plasmid replication and segregational stability.

In this project, we set out to identify novel sRNA in an emerging multidrug resistance IncL/M plasmid pNDM-HK through high throughput sequencing. Numerous antibiotic resistance genes are found in pNDM-HK, including New Delhi metallo-β-lactamase 1 (NDM-1), a carbapenemase that causes worldwide health threat in recent years. Intriguingly, six novel sRNAs located at different regions of plasmid such as replication, stability and variable regions, were identified from pNDM-HK. The sRNA-phylogenetic tree that was built based on these novel sRNAs provided unexpected but significant information about the evolutionary pathway of pNDM-HK plasmid including the possible genes acquisition and insertion from the relevant plasmids. In addition, the sRNA-phylogenetic tree can specifically cluster the IncM2 type and distinguish from other IncL/M plasmids. Moreover, we also characterized one of the plasmid-encoded sRNAs, pNDM-sR3 down-regulate genes for DNA replication in an Hfq-dependent manner, facilitating the fitness and circulation of drug resistance plasmid in bacteria. In conclusion, this is the first study to systematically identify and characterize sRNAs in the clinically-isolated multidrug resistance plasmid, pNDM-HK. We believe these novel sRNAs could provide further information on dissemination and maintenance of the multidrug resistance plasmid, which can be adopted as therapeutic targets in the future.

Abstract #21

Development of Transparent and Gas-permeable Liquid Marbles for Culture and Drug Sensitivity Test of Tumor Cell Spheroids

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A novel approach based on liquid marble is demonstrated to realize in situ tumor spheroid culture, which can be used to understand how tumor spheroids response to external environment in three dimensions. The sizes of coating particles impact the transmissivity of liquid marble and liquid marble coated by silica nanoparticle showed high transparency then could substitute for cuvette in absorbance measurement. Furthermore, culture process of tumor spheroids from bulk cells or single cell in liquid marble could be recorded clearly due to high transparence of liquid marble. Tumor spheroids cultured in liquid marbles were placed in 384-well plate or liquid marbles, and the viability change of spheroids in continuous culture under simulated hypoxic conditions revealed that liquid marbles are gas-sensitive. This platform could be applied in analyzing the effect of anti-cancer drugs and anti-cancer siRNAs (small interfering RNA) at different concentration on tumor spheroids, which indicated that the liquid marble had the feasibility to work as a bioreactor for drug screening.

Abstract #22

Lipid Nanoparticles functioned as vehicle for efficient siRNA delivery

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Gene silencing therapy is one of the most promising treatments against diseases like cancer and genetic deficiency. However, efficient delivery and release still remain the greatest obstacles to clinical trials. Here we report a modified platform of synthesizing lipid nanoparticles (LNPs), which is capable of delivering siRNA into tumor cell lines. These LNPs possess a core of siRNA and a shell of three types of lipids (DOTAP, DOPE and DSPE-PEG). Due to the existence of PEG chains on the outer layer, these nanoparticles show good biocompatibility and bioavailability. The LNPs are around 30 nm in size and have a slightly negative surface charge. After DEAE purification and further concentration steps, LNPs can be used as transfection reagent to silence the GFP expression in vitro. No significant cell toxicity is detected by Flow Cytometry. Further in vivo study is needed to exploit the therapeutic potential of LNP on nude mice.

Abstract #23

High-frequency stimulation induces cholecystokinin releasing from their terminals that switches long-term potentiation in the auditory cortex

LI Xiao

In our previous studies, we found that the medial temporal lobe influences neocortical plasticity via CCK-positive cortical projection neurons in the entorhinal cortex. In the rat auditory cortex, long-term potentiation (LTP) could be induced either by high-frequency (HF) stimulation or by only low-frequency stimulation in the presence of CCK. We hypothesized that, with two low-impedance electrodes, LTP of the connection A→B could be induced by stimulating electrode A with HF, or by stimulating electrode B with HF. We then implanted two electrodes in two hemispheres of the auditory cortex, which showed connectivity physiologically. After recording the baseline field potential responses in one hemisphere (electrode B) to the electrical stimulation in the other (electrode A), we applied HF burst stimulation at A. LTP was induced over the above baseline. We further applied HF burst stimulation at B, LTP (with A as the stimulation electrode and B as the recording electrode) was further enhanced. We further hypothesized that HF stimulation was to induce the release of CCK in the stimulation site. In the following experiment, we embedded electrodes and drug-injection needles bilaterally into the rat auditory cortex. After injection of CCK antagonist in the high-frequency stimulation side, rat showed no more LTP, whereas LTP could still be triggered when CCK antagonist was injected on the recording side. The results indicate that HF stimulation induced CCK releasing from their terminals and CCK strengthened the connection in the surrounding synapses. In conclusion, HF-induced LTP of the direction A □ increased input current from A.

Abstract #24

The role of bacterial RNA in host innate immunity

LI Yingxue

Antisense RNAs (asRNAs) are found to be widespread in bacterial genome. Since asRNAs are transcribed from the opposite strand of sense gene, they have perfect complementarity with the RNA transcripts of sense gene. As a result, they can form double-stranded RNAs (dsRNAs) by base-pairing with sense RNAs in bacterial cells. RNase III, a dsRNA specific endoribonuclease, encoded by the *rnc* gene, is implicated in cleaving dsRNAs. Widespread asRNAs could either be transcription noises, which are rapidly degraded by RNase III, or gene regulators, which play important roles in controlling the expression of sense genes. However, the molecular mechanism and biological functions of asRNAs in bacteria are still not well understood. We aim to investigate the roles of asRNAs and dsRNAs in bacterial growth, bacterial infection and host immune response using *E. coli* and Salmonella as the models.

Abstract #25

Multi-functional Nanoparticles for Bioimaging and Cancer Therapy

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The efficacy of conventional chemotherapy is reduced by the nonspecific distribution, rapid clearance, drug re-sistance, and the often significant toxicity of existing anti-cancer agents when administered at higher doses. This has prompted the exploration of nanomedicines that can overcome the major drawbacks of conventional cancer treatments by attacking disease-specific mechanisms and properties.

We have established a small library of monodisperse nanoparticles with different morphologies and properties, which consist of (1) iron oxide nanospheres/nanocubes ranging in size from 3 nm to 20 nm, (2) rare earth nano-sphere/nanohexagon/nanorod/nanowire) ranging in size from 10 nm to 200 nm with fluorescent properties of up- or down-conversion, (3) silica oxide nanospheres. These nanoparticles were further modified by surface engi-neering, such as silica coating (2-20 nm thickness tunable), polymer coating (PAA, PEI, PEG, PVP) or ligand free process, followed by conjugation of targeting ligands (e.g., enzymes, antibodies, peptides). These nano-materials are promising candidates for early detection and therapy of disease.

Abstract #26

Galanin Improves the performance of Rats During the Auditory Discrimination Tasks

LU Danyi Catherine

In a recent study, we have found that the neurons in the auditory cortex of anesthetized rat increased their responses to the auditory stimulus each time after direct local infusion of galanin. In the present study we investigate whether galanin application can amplify the neuronal signals in the auditory cortex produced by electrical stimulation in the cortex. Rats with bilateral implantations of electrode arrays in both hemispheres of the auditory cortex will be trained to approach the left or right hole of a behavioral apparatus to retrieve a reward depending on whether the right or left auditory cortex is electrically stimulated. A drug infusion cannula will be implanted in both hemispheres. After training, the rat is able to perform the task with the correct rate of 100%. We will then adjust the current of electrical stimulus to adjust performance to a correct rate of about 70% and examine whether infusion of galanin into each hemisphere would increase the correct rate in the performance. At a different session at which we would set the baseline performance at 80% correct rate, we will examine whether infusion of galanin antagonist (M40) would worsen the performance in the correct rate. Moreover, Artificial cerebral spinal fluid (ACSF) will be used as the vehicle control to make sure the increase or the decrease of the correct rate is correlated to galanin or M40. Our results of the present study would provide strong evidence that galanin is an attention related chemical from behavioral experiment.

Abstract #27

P19 as a siRNA carrier for transkingdom gene silencing

LU Mingxing

Over 150 years ago first attempts to use bacteria for treatment of cancer have been made. The employment of tumor-colonizing bacteria that exert anticancer effects is extremely intriguing. Today, as various possibilities exist to tailor bacteria for a particular purpose, bacteria-mediated tumor therapy undergoes a spectacular renaissance. Small interfering RNAs (siRNAs) hold great promise in gene-targeted therapies, the main obstacle is delivery into target cell cytosol. P19 was initially characterized as siRNAs binding protein is expressed in plants for suppressing RNA interference. P19 protein stabilizes RNA in a size dependent and sequence independent manner. This study aims to engineer biological properties of P19 protein from bacteria for siRNAs delivering and fine-tune natural propensity of bacteria expressing p19 to colonize solid tumors in vivo for cancer therapy. First, proof of concept was established that P19 bearing siRNAs from bacteria has gene knockdown effect on mammalian cell. Knockdown efficiency is positively related with P19 protein dosage. Thus, P19 could be a promising carrier for trans-kingdom gene silencing. Next, we would like to search tumors-specific bacterial strain can be used to safely express P19 protein bearing siRNAs directing into tumor cell in vivo.

Abstract #28

Genetic evolution of Human Enterovirus A71 subgenotype C4 in Shenzhen, China, 1998-2013

MEN Ruotin, Ming-Liang HE

BACKGROUND:
Human Enterovirus A71 (EV-A71) is one of the severest enteroviruses that causes hand, foot, and mouth disease (HFMD) among children. This study identified the mutations of EV-A71 VP1 amino acid residues over a number of years and explored the possible association of identified mutations and HFMD epidemic outbreaks in Shenzhen, China.

METHODS:
A total of 3760 stool specimens were collected from HFMD patients by Shenzhen Centers for Disease Control and Prevention (CDC) between 1998 and 2013. In total 289 VP1 strains were sequenced in this study, and amino acids mutation frequency was calculated. There were 2040 China nationwide sequences downloaded from Genebank as replication data.

RESULTS:
In our samples, 1036 subjects (27.6%) were EV-A71 infected. Three amino acid positions on VP1 protein were found to have high mutation prevalence. These are Q22H, S283T, and A289H. Site 22 showed a fast mutation fixation in the year 2008, at the time of the large scale epidemic outbreak in Shenzhen. Analysis of the nationwide data replicated the same trend of mutation prevalence of the three sites.

CONCLUSION:
The switching from Q to H on site 22 of the EV-A71 VP1 strain might be associated with the HFMD outbreak in Shenzhen in 2008. The identified amino acid sites 22, 283 and 289 provided information for developing anti-viral drugs against EV-A71 in the future.

Abstract #29

Targeted delivery of Antrodin B-loaded poly (lactic-co-glycolic acid) nanoparticles to neurons promotes functional recovery after peripheral nerve injury.

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Targeted drug delivery by using biodegradable nanoparticles showed substantial improvements in drugs with poor solubility and bioavailability *in vivo*. Our studies showed a traditional Chinese medicine, *Antrodia cinnamomoea* (Niu-Chang-Chih in Chinese) extract promoted functional recovery after peripheral nerve injury in mice. We identified antrodin B as an active ingredient from A. *cinnamomea* extract which increased neurite outgrowth from adult mouse dissociated dorsal root ganglia (DRG) neurons and from DRG explants. However, the effect of antrodin B *in vivo* is limited. We therefore developed poly (lactic-co-glycolic acid) (PLGA) based nanoparticles (NPs) delivery system for targeted delivery of antrodin B to the site of injury after a sciatic nerve crush in mice. We first examined if the antrodin B-loaded PLGA-NPs exhibit the same promoting effect as the PLGA-NP free antrodin B *in vitro*. Antrodin B-loaded PLGA-NPs showed significantly increased neurite outgrowth from dissociated DRG neurons. Local administration of antrodin B-loaded PLGA-NPs to sciatic nerve crush injury site in adult mice significantly accelerated sensory axonal regrowth (up to 40%) as assessed by nerve pinch test. Functional recovery monitoring assays, such as Pinprick assay revealed significant and earlier return of sensory function in mice treated with antrodin B-loaded PLGA-NPs. Also, Toe-spreading motor assay indicated significant improvement and earlier motor function regain in treatment group as compared with control groups. Taken together, our findings suggest antrodin B as a potential candidate for nerve repair. Further studies to reveal underlying mechanism of accelerated axonal growth by antrodin B are yet to be investigated.

Abstract #30

Molecular mechanism study using pro-siRNA screening

REN Yutian Carrie

RNA interference (RNAi) is a well elucidated gene silencing mechanism in eukaryotic species. A new kind of siRNA named as pro-siRNA is the product of a novel technology of producing siRNAs in bacteria. Our lab has established a platform for producing pro-siRNAs against disease-related genes. Meanwhile, a high-throughput RNAi screening platform using pro-siRNA Library is under development. This genome-wide RNAi screening method can be applied to study virtually any biological question. For example, Cholecystokinin (CCK) is a neurotransmitter in the brain that is involved in neural activities such as learning, memory, stress and anxiety but the molecular mechanisms of CCK functions are not well understood. In collaboration with Prof Jufang He's group, we are investigating if the pro-siRNA screening method can be applied to uncover genes involved in CCK signaling pathways.

Abstract #31

VCP is Required for G0/G1 to S phase Transition in Mammalian Cell

SHI Xianli

The Ubiquitin Proteasome System (UPS) is the protein quality control process by timely destroying the damaged or unnecessary proteins, which is fully utilized by the cancer cell to reach the accelerated cell growth. VCP/p97 is one of the chaperons in the UPS, which is over expressed in many types of cancer cells. What is the potential relationship between the over expression of VCP and the up-regulated cell proliferation in cancer cell? By VCP knockdown or inhibition, we observed less number of cell in S phase while increase in G1 phase with increase expression of p21 and p27. We hypothesize that VCP is required for the timely degradation of p21 and p27 in the G0/G1 to S process. Indeed, p21 and p27 double knockdown rescued the cell cycle defect causing by VCP inhibition. Our research unveiled one of the potential molecular relationships of VCP and unregulated proliferation of cancer cell.

Abstract #32

Cholecystokinin from the entorhinal cortex switches high-frequency-induced long-term potentiation in the hippocampus

SU Junfeng Angus

Our earlier studies showed that activation of cholecystokinin (CCK) neurons that originated from the entorhinal cortex induces long-term potentiation and neuroplasticity in the auditory cortex. Lesions in the entorhinal cortex or the hippocampus will lead to memory deficits which have received intensive studies including temporal, spatial, episodic memory, as well as associative fear conditioning. After AAV-Dio-ChR2-eYFP into the entorhinal cortex of the CCK-Cre mouse, we discovered that entorhinal CCK neurons project to hippocampus regions. We hypothesized that CCK is involved in the high-frequency stimulation induced long-term potentiation (LTP). High-frequency stimulation in CA3 induced CA3-CA1 LTP on the wild-type mouse and CCK B-receptor knockout (CCK-BR KO) mouse, but not on the CCK peptide knockout mouse (CCK-KO). It was interesting to note that the hippocampus has many CCK A-receptors (Allen Brain Atlas), which might be the reason of why CCK-BR-KO still has HF-stimulation induced LTP. The CCK-KO mouse exhibited severe fear memory and spatial memory deficit. On the mouse with injection of AAV-DIO-eYFP-ChR2 in the entorhinal cortex of the CCK-Cre, we induced LTP with low-frequency stimulation between CA3-CA1 after HF laser stimulation of the projection terminals originated from the entorhinal cortex. We explain that HF stimulation of the CCK terminals induced CCK release in hippocampus, and LF stimulation could induce LTP in the presence of CCK.

Abstract #33

L-lactate release by optogenetic activation of astrocytes rescues decision-making deficit in visceral hypersensitive rats

WANG Jun

Visceral hypersensitivity (VH) is a key factor in the pathophysiology of gastrointestinal functional disorders. Our previous studies have revealed anterior cingulate cortex (ACC) as a key brain target for mediating visceral pain-cognitive interactions (Ref). Nonetheless, the underlying mechanisms are poorly understood. Here we showed decision-making deficit in chronic VH rats. Exogenous L-lactate could repair impairment of long-term potentiation in VH rats. Further, we established an optogenetic approach and found that activation of astrocytes induced L-lactate release, and could rescue visceral pain related decision-making deficit. Multiple-channel recordings showed that activation of astrocytes facilitates neuronal activity, theta oscillation, and spike-field relationship in the ACC of VH rats and functional connectivity in basolateral amygdala-ACC network through lactate pathway. This set of data supports the theory of 'astrocyte-neuron L-lactate shuttle', and provides a strategy targeting astrocytes for treatment of cognitive dysfunction under chronic visceral pain state.

YL References. J Physiolog (Lond). 570 (1):169, 2006; Gastroenterology. 134:535-543, 2008; Gastroenterology 136:1732, 2009; Cerebral Cortex 2013; doi: 10/cercor/bht273;

Abstract #34

Aza Boron Dipyrromethenes Based Photosensitizers for Photodynamic Therapy

Qiong WANG, Gigi LO

Photodynamic therapy (PDT) is a promising treatment modality for a variety of cancers and wet age-related macular degeneration. Owing to its potential advantages over traditional therapeutic methods, various classes of photosensitizers have been developed which exhibit a different degree of photocytotoxicity and selectivity toward a range of cancer cells. Focuses are also put on the development of photosensitizers with targeting property to the vulnerable intracellular compartments, such as the nucleus, mitochondria, and lysosomes. It is believed that photosensitizers with an organelle-targeting property can trigger specific cell death pathway and enhance the PDT efficacy. Aza boron dipyrromethenes (aza-BDPs) are promising theranostic agents because of their strong absorption in the near-infrared region, high fluorescence emission/ singlet oxygen generation efficiency, high photostability, and ease of chemical modification. In this presentation, we will report a series of aza-BDP-based fluorophores and photosensitizers that are localized in mitochondria or lysosomes. To enable localization in these subcellular organelles, triphenyl phosphonium and morpholine moieties were used respectively as the targeting groups.

Abstract #35

Incorporate molecular subtyping to better predict recurrence for stage II colorectal cancer

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Traditionally, clinical factors such as TNM staging, KRAS mutation and microsatellite instability (MSI) are used for prognosis for stage II colorectal cancer (CRC), but inadequate for risk assessment. Here we present a new method that incorporates molecular subtyping to improve CRC relapse prediction.

In total, 346 stage II CRC samples from three independent cohorts were classified into four consensus molecular subtypes (CMSs). Using one cohort as a training set, a classifier was trained to predict relapse specifically within each subtype, and then applied to the two independent test cohorts. Overall, our new approach identified high-risk patients robustly, with an HR of 11.7 and 6.7, respectively, in the two test cohorts. The prediction accuracy and recall also reach 61.5% and 48%, respectively, which are both higher than other approaches in the literature. In summary, we demonstrated the first time that molecular subtyping can be used to improve prediction of CRC recurrence.

Abstract #36

Development of nanomedicine targeting cancer stem cell

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Cancer stem cells (CSCs) have been implicated in recurrence and treatment resistance in many human cancers. Therapeutic strategy targeting CSCs is the subject of active current investigation. In this project, we designed and synthesized multifunctional silica-based nanoparticles encapsulated with magnetic cores and chemotherapeutic agent, and coated with specific antibody against surface markers of CSCs for targeted and combined thermotherapy and chemotherapy under an alternating magnetic field (AMF). The antibody specifically bound to CSCs, leading to enhanced cellular uptake in vitro and highly accumulation in the tumors in vivo. The survival rate of lung CSCs was dramatically decreased in only 30 min application of AMF by synergistic effects of heat and drug. Moreover, use of this therapy in vivo significantly suppressed tumor growth of lung CSC xenograft-bearing mice and increased the survival rate of tumor-bearing mice. The results showed significant promise of nanoparticle-based combinatorial thermotherapy and chemotherapy in cancer treatment.

Abstract #37

MMP mediate collage degradation and inflammation during zebrafish heart regeneration

XU Shisan

Unlike human beings, zebrafish displayed tremendous heart regeneration ability after heart injury. Matrix metalloproteinases (MMP) were upregulated in the scar after injury, accompanied with leucocytes infiltration into the wound. Zebrafish mutant breakdance (Bre) showed heart arrhythmia syndrome, and pool regeneration potential after heart cryoinjury. Compared with AB zebrafish, collagen deposition in Bre was lower in the scar, but the cell proliferation was no significant difference. RNA-sequencing results showed that mmp13a expression was higher in Bre than in AB zebrafish. Lots of leucocytes related genes, such as irg1, lyz, irf8, L-plastin, cxcl11.1 were overexpressed in Bre, which implied that over immune-response was occurred in the mutant. Treat zebrafish with MMP inhibitor in AB zebrafish after heart cryoinjury, neutrophil and macrophages were attenuated recruitment into the scar, which blocked the heart regeneration. Inflammation was indispensable for zebrafish heart regeneration, and mediated by MMP.

Abstract #38

Isolation of Lung Cancer Single Cells by Arc-edge-channel Monolithic Valves (AMVs) Microfluidic Chips for Target Gene Mutation Analysis

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To study gene mutation heterogeneity in lung cancers using liquid biopsy, we propose a simple, low-cost and extensible scheme, based on the combination of arc-edge-channel monolithic valves (AMVs) to isolate single cells and real-time PCR. A low-cost, efficient and simple technique to fabricate arc-edge-channel monolithic valves (AMVs) was developed to con-trol liquid in large scale and finely manipulate single cells. The features of arc-edge channels (ACs) and the performance of AMVs were systematically investigated. Furthermore, with the combination of AMVs and real-time PCR, hundreds of single lung cancer cells were isolated by AMVs microfluidic chips, one of which contains 4 AMVs and 1 capillary valve, and analyzed using Allele Specific Locked Nucleic Acid quantitative PCR (ASLNAqPCR). The detection of KRAS gene (G12S and G12D) mutations, EGFR gene (L858R, T790M and 19 del) mutations and BRAF gene (V600E) mutation of lung cancer cells at the single cell level was evaluated and the success rate of these mutation detections were more than 80%. Finally, mutation het-erogeneity in a lung cancer patient using pleural effusion from liquid biopsy was revealed. Some lung cancer cells had EGFR (L858R) mutation, some lung cancer cells had EGFR (T790M) mutation from the same sample. Additionally, in single lung cancer cells, there were two mutations. These results reflect that gene mutation heterogeneity existed in the lung cancer patient and demonstrate the feasibility of our scheme.

Abstract #39

Dynamic interconversion between CD90$^+$ and CD90$^-$ cancer cell populations in lung cancer A549 cell line

Wai-Kin YU[1], Yuen-San CHAN[1], Weimao WANG[1], Chi-chun FONG[1], Tak-Chun YIP[2], Siu-Kie AU[2], Mengsu YANG[1]*

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Functional diversity of various cancer cell populations is crucial for tumor growth and maintenance, which affects cancer therapy outcomes. The diversity can be characterized by the co-existence of different phenotypic states of cancer cells. To investigate the possible fluctuation and dynamics in phenotypic states, two subpopulations in A549 adenocarcinoma cells were identified by flow cytometry after CD90 immunostaining. FACS-enriched populations were found to interconvert to each other in subsequent culture, which the CD90$^+$ percentage of positive population reduced from 97.0% to 60.0% and that of negative population increased from 0.1% to 30.0%. This formed a new equilibrium instead of following stochastic phenotypic switching. Combined characterization results revealed higher migration ability, invasive ability and up-regulation of EMT-related gene expression in CD90 positive cells, while negative subset displayed higher colony formation ability and tumorigenicity. These results may provide clues on the correlation between the function of CD90 and its dynamic expression, which shed light on the control of phenotypic conversion for improved cancer therapy.

Abstract #40

Genome-wide expression analysis identifies two molecular markers for diagnosis of nasopharyngeal carcinoma

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Nasopharyngeal carcinoma (NPC) is rare in the Western world, but predominant in Southeast Asia, particularly in Guangdong and Hong Kong, and poses a major threat to the healthcare in these regions. Using cDNA microarray and bioinformatics analysis, we identified 281 differentially expressed genes (DEGs) between 43 primary tumor and 22 non-tumor tissues of nasopharyngeal epithelium. Gene set enrichment analysis (GSEA) showed that multi-pathway are participated in the pathogenesis of NPC. Dysregulated genes and gene sets can be grouped into 5 groups: immune response, cell cycle, virus response, extracellular matrix (ECM) and metabolism. A 68-gene classifier was subsequently built to distinguish NPC samples from normal ones with a sensitivity of 100% and specificity of 81.5%. Importantly, the performance of the classifier can be validated by two independent NPC microarray data sets. QPCR experiments showed good classification performance of our top two variable genes between 11 NPC and 9 normal patients' samples. Our study provided a better understanding of the underlying regulatory mechanisms of NPC tumorigenesis and identified two novel targets for diagnosis and treatment of NPC.

Abstract #41

Non-fouling Closed Microcirculation System as a Biomimetic Model for Thrombosis and Whole Blood Hemodynamics Analysis

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In *vitro* microcirculation system provides a quantitative and accurate potential alternative for clinical cardiac hemodynamics analysis and drug screening platform in elucidating the circulation disease mechanisms underlying morphogenetic and pathogenetic processes. In this study, we designed and fabricate a novel closed microcirculation system for non-diluted blood hemodynamic analysis. By infused with Liquid-Polydimethylsiloxane (PDMS) (M.W.4000), the microcirculation system was found to be stable, slippery and antifouling when analyzing non-diluted blood sample. Our results showed that non-diluted blood left less blood residual in the Infused PDMS-based microcirculation channels demonstrating the enhanced antifouling property. Slippery circulating channels were capable to run non-diluted blood flow smoothly and stably. Additionally, integrated micro-valves and micro-chambers worked with higher sensitivity in the system to control the blood flows. Mimicking human circulatory system and thrombus modelling, we analyzed the blood flow hemodynamic under different anticoagulants drug conditions. Such closed microcirculation system may provide a stable and important in *vitro* blood hemodynamic analytical and drug screening platform.