IS3-1

Functional validation of candidate genes for mental disorders using zebrafish

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We are conducting a systematic zebrafish knockout project to validate candidate genes for mental disorders, including autism. The Miles-Carpenter syndrome patients show microcephaly, spasticity and thoracic scoliosis. Screening of linked families and next generation sequencing identified mutations in ZC4H2 gene. Knockout (KO) zebrafish were created and zc4h2 KO mutant exhibited abnormal swimming, defective eye movement and pectoral fin contractures. Because several of the behavioral defects were consistent with hyperactivity, we examined the underlying neuronal defects and found that sensory neurons and motoneurons appeared normal. However, we observed a striking reduction in GABAergic interneurons. Analysis of cell-type-specific markers showed a specific loss of V2 interneurons in the brain and spinal cord, likely arising from mis-specification of neural progenitors. Loss of function of ZC4H2 thus likely results in altered connectivity of neuronal circuits, infantile spasm and intellectual disability. The second case is related to autism spectrum disorders (ASDs). ASDs comprise a wide range of neurodevelopmental disorders, characterized by deficits in social behavior, along with repetitive behaviors and impaired communication. Though the exact causes for ASD remain poorly understood, genetic mutations resulting in altered gene function have been implicated causally in ASD. Intragenic mutations in DYRK1A, which have been shown previously to be associated with clinical aspects of Down syndrome, have been associated recently with microcephaly and ASD-like symptoms. We will provide a case study of an individual with a 21kb microdeletion within the DYRK1A locus, who has both microcephaly and ASD. We show that dyrk1aa KO fish have microcephaly and impaired social interactions through two newly developed behavioral tests: social interaction and shoaling assays. Also, we confirmed that behavior analysis for ASD through our dyrk1aa KO zebrafish is experimentally tractable, and propose these social behavioral assay methods in zebrafish as a tool for the widespread study of ASD candidate genes.

IS3-2

Development of Foodborne Virus Concentration and Detection Methods

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Norovirus (NoV), a non-enveloped single-stranded RNA virus, causes acute gastroenteritis in humans. Considerable attention has been given to develop a rapid and sensitive method for the detection of the virus. Immunomagnetic separation (IMS) removes PCR inhibitors and reduces time-consuming concentration steps for the detection of virus in food sample, however, this method requires expensive antibodies for the binding of virus. To identify an alternative for NoV-specific antibody dependent IMS, the relative binding affinities of NoV genotype GII.4 to eight lectins, Con A, DBA, HPA, PNA, PTA, UEA, and WGA were evaluated using an ELISA, and confirmed the affinities of different subtype of NoV using surface plasmon resonance. Con A from jack-bean showed significantly higher (relative binding: >2-fold of control) relative binding affinity to NoV than other lectins tested. The SPR analysis showed that the equilibrium dissociation constants of Con A to NoV genotypes GII.4, GI.4, and GII.3 were 2.19, 4.47, 238 nM, respectively, indicating that NoV genotype, GII.4 bound Con A with higher affinity than other genotypes and con A was capable to substitute the antibodies required for NoV attachment. A new Con A-linked magnetic bead combined with quantitative reverse transcription (RT)-PCR assay detected NoV RNA level of 10^1to10^6copies/ml.

IS3-3

Biosynthesis of noscapine in opium poppy

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Noscapine is the second most abundant alkaloid after morphine in opium poppy (*Papaver somniferum*) and has been used medicinally as a cough suppressant. Unlike morphine, noscapine has no narcotic or analgesic properties. However, noscapine and several semi-synthetic derivatives have recently attracted considerable attention owing to their potential as anticancer drugs. The noscapine biosynthetic pathway in opium poppy has mostly been established at the genetic and biochemical levels. As interesting feature of the noscapine pathway in opium poppy is the occurrence of a genomic cluster containing most of the biosynthetic genes. More recently, noscapine was successfully produced in engineered yeast from norlaudanosoline in 14 steps.

Noscapine biosynthesis from the key branch-point intermediate (*S*)-reticuline involves 11 reactions responsible for converting the 1-benzylisoquinoline backbone of (*S*)-reticuline to the phthalideisoquinoline scaffold of noscapine, via protoberberine and *seco*-berberine structural intermediates, and introducing several functional groups. The remaining uncharacterized conversion involves *O*-methylation of the hydroxyl group in a protoberberine or *seco*-berberine skeletal intermediate that becomes the 4′ methoxy moiety in noscapine. A heterodimer consisting of two different *O*-methyltransferases has been proposed as the enzyme responsible for this *O*-methylation reaction. An *in vitro* biochemical and *in planta* characterization of heterodimeric *O*-methyltransferases has been performed to investigate their role in noscapine biosynthesis.

IS3-4

Seed Germination and Biochemical Changes during Accelerated Aging and Osmopriming Processes in Sweet Pepper (*Capsicum annuum* Linn.) Seed

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Seed deterioration during storage is a complex physiological and biochemical process leading to loss of germination ability. On the other hand, seed priming offers an effective means to improve seed quality. This study focuses on seed germination and biochemical change during an artificially accelerated aging process in sweet pepper seed (*Capsicum annuum* Linn.) and the improvement of those seed germination and biochemical change after osmopriming process of aging seed.

The sweet pepper seeds were accelerated aging by incubated at 42℃ and 100% relative humidity for 0, 5, 10, 15, 20, 25 and 30 days. The germination ability in terms of percentage of emergence radical (%ER) was decreased in all different accelerated aging times at $p<0.01$. The critical period for rapid decrease of %ER is 20 days of aging time. The electrolytes leaked which were K^+, Na^+, Ca^{2+} and Mg^{2+} including electrical conductivity (EC) in 24 h soaked seed solution with distilled water were increased during 10-30 days of aging time. The decrease in germination ability exhibited well correlation with increase in electrical conductivity and electrolyte leakage in soaked seed implied membrane deterioration. Malondialdehyde (MDA) was the major product of lipid peroxidation which its concentration was also rapidly increased in sweet pepper seed from 0 to 75 mg g^{-1} within 10 days of accelerated aging time. This phenomenon was associated with an increase in total antioxidant activity (TAA) when aging was carried on 0-10 days. The peroxidation reaction of fatty acid in the cell impacts in germination ability and some biochemical parameters related to membrane deterioration and loss of membrane integrity.

The seven accelerated seed treatments from different aging times of 0, 5, 10, 15, 20, 25 and 30 days were primed in a polyethylene glycol (PEG 6000) solution with the osmotic potential of -1.5 MPa for 6 days. Seed germination and biochemical changes of primed seeds were compared with aged seed. Seed germination was improved in primed seeds. MDA and total peroxide concentration in primed seeds were decreased. The accumulation of TAA, total ascorbate, dehydroascobate and catalase (CAT) activity in primed seeds enhanced the defense mechanism in protecting the cell membrane damage from reactive oxygen species. The enhancement of seed germination possibly was resulted due to the accumulation of antioxidants and the improvement of cell membrane integrity. These results pave the way to gain insight into how seed quality was improved by the priming process.

IS3-5

Bioinformatics Approaches in Natural Product-omics

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Genomes sequences have been extensively released due to the advancement of sequencing technologies. For example, the 1,000 Fungal Genomes Project was launched to sequence the species especially focused on rarely sequenced taxa, hence providing foundation for deciphering underpinnings of fungal evolution and diversity. The emerging technologies and an array of omics data would provide an opportunity towards digital biology. In order to fully take advantage of such an opportunity, comprehensive and systematic genomics solution have an immediate need. Interdisciplinary nature of bioinformatics enables all disciplines among the biological science to be applicable. Fungal plant pathology would be a good example exhibiting synergistic advances with bioinformatics approaches, such as i) user-friendly analysis platforms with standardized data, ii) specialized databases focused in gene families/functional groups of biological importance, iii) established interconnection between phenome and genome, and iv) platforms supporting community research. In natural product researches, biosynthetic gene clusters have been highlighted for discovery of useful secondary metabolites. There are a number of bioinformatics tools for natural product researches, yet an integrated and standardized platform for utilizing them at one place. The abovementioned bioinformatics approaches would vitalize natural product-omics researches and suggest new possibilities.

S3-1

Hydroxy Fatty Acid : Efficient Substrate for Production of a Novel Antimicrobial Agent against Multidug-resistant *Staphylococcus aureua*

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Structural modification of natural lipids via chemical reaction or microbial bioconversion can change their properties or even create novel functionalities. Enzymatic oxidation of lipids leading to formation of oxylipin is one of those modifications. Hydroxy fatty acids, one of those oxylipins have gained important attentions because of their structural and functional properties compared to other non-hydroxy fatty acids. Recently 7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD) was produced from vegetable oils by microbial bioconversion, and further study confirmed that DOD contained strong antimicrobial activities against broad range of microorganisms. In this study we tried to modify DOD molecules by enzymatic or physical reaction to create new functionality or to enhance the antimicrobial activity of DOD. After modification of DOD molecules by different ways, we confirmed that the antimicrobial activity of DOD was highly enhanced. In addition a novel furan fatty acid, a derivative of DOD, showed strong antibacterial activity against multidrug-resistant *Staphylococcus aureus* suggesting that DOD and its derivatives could be used as an efficient antimicrobial agent for industrial and medicinal applications.

S3-2

Molecular Targeting of ERKs-RSK2 Signaling Axis in Human Cancer

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Receptor tyrosine kinases (RTKs) which are activated by diverse stimuli, such as growth factors, cytokines and environmental stresses, play a key role in cell proliferation, transformation and cancer development in humans. Since constitutive active mutations in Ras and Raf are frequently observed with high percentage in many solid tumors, including colon, pancreas, ovarian, melanoma, non-small cell lung and other cancers, Ras-mediated Rafs/MEK/ERKs/RSK2 signaling axis plays a key role in the regulation of cell proliferation, transformation and cancer development. Thus, Ras/Rafs/MEKs/ERKs/RSKs signaling pathway has become an important target to develop/identify chemopreventive and therapeutic agents. Recently, our results demonstrated that RSK2, a downstream kinase of ERKs, is an important proof-of-concept on the human cancer development. Ectopic expression of RSK2 induced anchorage-independent cell transformation without stimulation of tumor promoters such as epidermal growth factor. Moreover, human skin cancer tissue array demonstrated that total- and phospho-RSK2 protein levels were higher in skin cancer tissues compared with normal skin tissues. Utilizing cutting edge molecular and computational research tools, we provided evidences that kaempferol and eriodictyol were natural compounds which target and inhibit RSK2 activity. Moreover, we found that magnolin, a natural compound abundantly found in magnolia flos, targeted ERK1 and ERK2 and inhibited ERK1 and ERK2's activities with 68 nM and 16.5 nM of IC_{50} values. Moreover, magnolin suppressed cell migration and invasion in cancer cells by inhibition of epithelial-to-mesenchymal transition of cancer cells. Taken together, our results provide strong evidences that ERKs and RSK2 are key kinases regulating cell proliferation and transformation, and are important targets to develop/identify small molecules as chemopreventive and/or therapeutic agents.

S3-3

Discovery of Proprotein Convertase Subtilisin/kexin type 9 Inhibitors from *Schisandra chinensis*

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Dyslipidemia which is apparently associated with cardiovascular disease, the leading cause of death in the western world,1 is characterized by increased triglyceride (TG) or low-density lipoprotein (LDL) levels, and also declined high-density lipoprotein (HDL) levels. Among them, the elevated LDL-cholesterol levels have been treated with statin therapy which is known to inhibit hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCoA) involved in synthesis of cholesterol. Despite the efficacy of statin therapy, some patients with familial hypercholesterolemia, an inherited autosomal dominant disorder characterized by extremely high levels of low-density lipoprotein cholesterol (LDL-C) and premature atherosclerosis, still face substantial residual risk associated with high levels of LDL-C because statin therapies did not achieve full success in lowering LDL-C levels. Recently, in order to discover new approach to control LDL-C in the patients with familial hypercholesterolemia, much attention has been paid to proprotein convertase subtilisin/kexin type 9 (PCSK9) that regulates the levels of circulating LDL-C by inducing the degradation of the hepatic low-density lipoprotein receptor (LDLR). PCSK9 is also expressed in the small intestine of mice and in human intestinal cells and is known to be involved in the regulation of lipid absorption. Thus, inhibition of PCSK9 has been emerged as an attractive target to control LDL-C levels. Two antibody drugs have been launched at the market as PCSK9 inhibitors while there are no small molecule-derived drugs so far. As part of search for PCSK9 inhibitors from natural products, *Schisandra chinensis* was chosen for the follow-up isolation work. From this plant, 30 compounds were isolated and their structures were confirmed by NMR spectroscopic data, CD and MS analysis. All the isolates were evaluated for their inhibitory activities against PCSK9 mRNA expression in liver cells in vitro. Of the tested compounds, 17 compounds seemed to inhibit PCSK9 mRNA expression. Further in high fat diet-fed mice, one of active compounds displayed its downregulation of PCSK9 in vivo.