The 1st NUS-FU-KU Joint Symposium on Biochemistry in FUKUOKA
-National University of Singapore & Fukuoka University & Kyushu University-

Symposium title:

"Natural products with biochemical and pharmacological functions."

Date : 31st October, 2019 (Thursday)
Time : 13:30～18:20
The venue : All-purpose room, Central Library of Fukuoka University (1st Floor)

Time schedule:

13:30～13:35	Introductory remarks	(Narumi Aoki-Shioi)
13:35～13:45	Opening speech	(Masatoshi Yamaguchi, President of Fukuoka University)
13:45～14:00	Introduction of universities	(Prof. Hamase [KU], Prof. Kini [NUS])

Session 1: "Physiologically active compound; TOXIN" (Chairman: Isao Kuraoka [FU])

14:00～14:40	Koh Cho Yeow (NUS) "Drug discovery for cardiovascular diseases: venomous and haematophagous animals as sources"
14:40～15:20	Hiroki Shibata (KU) "Diversity and evolution of venom protein genes of a Japanese endemic pit viper, habu, *Protobothrops flavoviridis* reveals accelerated"
15:20～15:50	Narumi Aoki-Shioi (FU) "Regulation system of snake toxin proteins"
15:50～16:10	Coffee break

Session 2: "Drug discovery seeds" (Chairman: Yoshito Abe [KU])

16:10～16:40	Masaki Nagao (FU) "The effects of fermented ginseng on symptoms in dementia - Behavioral and pharmacological studies using animal models"
16:40～17:20	Brandon Morinaka (NUS) "Posttranslational modifications in natural product biosynthesis"
17:20～18:00	Kenji Hamase (KU) "Chiral amino acid analysis for new drug discovery and diagnosis"
18:00～18:15	Concluding remarks (R. Manjunatha Kini [NUS])
18:15～18:20	Closing remarks (Narumi Aoki-Shioi [FU])
18:30～20:30	Dinner and Discussion (need a reservation for dinner)

Information of speakers:
・Koh Cho Yeow https://sg.linkedin.com/in/cho-yeow-koh-a4649785
・Hiroki Shibata http://hyoka.ofc.kyushuu.ac.jp/search/details/K000942/english.html
・Narumi Aoki-Shioi https://www.researchgate.net/scientific-contributions/
・Masaki Nagao https://www.researchgate.net/scientific-contributions/71270846_Masaki_Nagao
・Morinaka, Brandon Isamu http://pharmacy.nus.edu.sg/profile_brandonimorinaka/
・Kenji Hamase http://soyaku.phar.kyushu-u.ac.jp/gyoseki.html
Chairman:
・Isao Kuraoka https://www.researchgate.net/profile/Isao_Kuraoka
・Yoshito Abe http://hyoka.ofc.kyushu-u.ac.jp/search/details/K000939/english.html
Supervisor and Organizer:
・R. Manjunatha Kini http://www.dbs.nus.edu.sg/staff/kini.htm
・Narumi Aoki-Shioi https://www.researchgate.net/profile/Narumi_Aoki

Session 1

“Physiologically active compound; TOXIN”

Chairman: Isao Kuraoka [Fukuoka University]

Koh Cho Yeow (NUS）

“Drug discovery for cardiovascular diseases: venomous and haematophagous animals as sources”

Hiroki Shibata (KU）

“Diversity and evolution of venom protein genes of a Japanese endemic pit viper, habu, *Protobothrops flavoviridis* reveals accelerated”

Narumi Aoki-Shioi (FU）

“Regulation system of snake toxin proteins”

Drug discovery for cardiovascular diseases: venomous and haematophagous animals as sources of inspiration

Cho Yeow Koh

Research Assistant Professor, Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore

Abstract

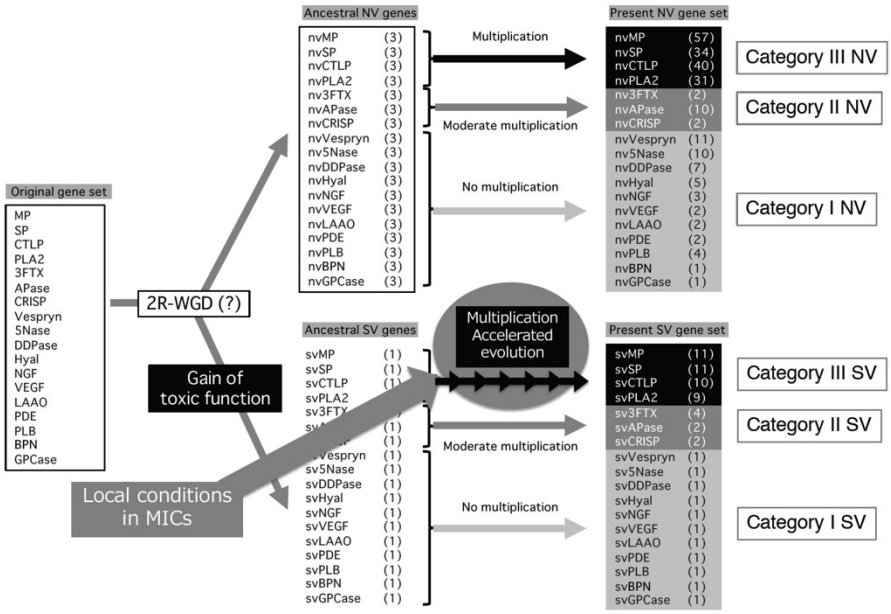
Cardiovascular diseases are the top cause of death worldwide. Ischaemic heart disease and stroke are estimated to have killed 15.2 million people in 2016 (approximately 27% of global deaths). Thrombosis is a major component in these diseases. Antiplatelet and anticoagulant therapies are widely used to prevent and treat thrombosis. The ideal antithrombotic treatment should be effective in stopping unwanted clot formation with minimum perturbation of haemostasis, which is not fully attainable using currently available therapeutic agents. Therefore, the need to discover and develop new antiplatelets and anticoagulants remained. Venomous animals such as snakes, scorpions, spiders, bees, cone snails, and sea anemones and hematophagous animals like ticks, leeches, mosquitoes, vampire bats, and horseflies, utilize their venomous or salivary secretions to facilitate predation, defence, and feeding. Refined through millions of years of evolution, these secretions contains potent, specific and stable toxins that can efficiently perturb prey or host physiology. Many of the toxins target circulatory systems for fast incapacitation and/or for directly extracting nutrients. As such, research into venomous and salivary secretions from animals have successfully resulted in life-saving therapeutic agents for cardiovascular diseases. Here we describe our discovery of novel molecules targeting various components of haemostatic system and some of our progress towards drug development. These include potent and specific inhibitors of (1) thrombin (variegin – from tick saliva); (2) factor XIa (fasxiator – from snake venom); and (3) prothrombinase complex (naniproin – from snake venom). The discovery of these anticoagulants demonstrate that venomous and haematophagous animals are enriched sources of natural libraries of molecules that can be leveraged for the development of valuable therapeutic agents, especially for the treatment of cardiovascular diseases.

Diversity and evolution of venom protein genes of a Japanese endemic pit viper, habu, *Protobothrops flavoviridis* reveals accelerated

Division of Genomics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

Abstract

To understand mechanisms involved in snake venom evolution, we decoded here the ~1.4-Gb genome of a Japanese endemic pit viper, habu, *Protobothrops flavoviridis*. We identified 60 snake venom protein genes (SV) and 246 non-venom paralogs (NV), belonging to 18 gene families. Molecular phylogeny revealed an early divergence of SV and NV gene copies, suggesting that one of the four copies generated through two rounds of whole-genome duplication was modified for use as a toxin in the venom. Among them, both SV and NV gene families of the four major venomic components, metalloproteinase (MP), serine protease (SP), C-type lectin-like protein and phospholipase A2 were extensively duplicated after their diversification. An accelerated evolution was evident in their SV genes but not in NV counterparts. On the other hand, genes for the other 14 families those were not extensively duplicated showed no evidence of accelerated evolution. RNA-sequencing revealed extensive alternative splicing in three SV gene families, MP, SP and vascular endothelial growth factors (VEGF). We also observed trans-splicing among the clustered SP genes. We further observed that venom-related SV and NV gene copies are significantly enriched in microchromosomes than in macro-chromosomes, suggesting the implementation of the genomic architecture in the multiplication and the accelerated evolution in the venom-related genes.



Regulation system of snake toxin proteins

Narumi Aoki-Shioi

Department of Chemistry, Faculty of Science, Fukuoka University, 19-1, 8-chome Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan)

Abstract

“Why are venomous snakes not killed by their own venom?”

Japanese vipers have natural resistance comportments to neutralize from accidental bite the venom injection into their blood. The serum proteins called “**endogenous inhibitors**”, but they are different than immune antibodies. They have capability to inhibit snake enzymes specifically. It is naturally to understand that poisonous (venomous) animals have equipped something against their toxins. However, an endogenous inhibitor groups remain unclear the actual inhibition mechanism due to the lack of information on the three-dimensional structures of toxin and inhibitor complexes. We isolated small serum proteins (**SSP-1** to **SSP-5**) as a new group of endogenous inhibitors from Japanese vipers. Interestingly, these inhibitors target different toxins as follows; SSP-1 and SSP-4 are suppressed apoptosis of vascular endothelial cells induced by snake venom metalloprotease, HV1. SSP-2 and SSP-5 have high affinity for a CRISP family protein, triflin found as an ion channel blocker. SSP-3 inhibits a novel P-III type snake venom metalloproteinase.

Here, we described the first crystal structure of the complex of SSP and toxin and define the structural basis of SSP-mediated inhibition of toxin activity. Some amino acid residue changes among SSPs inhibitors were significantly greater on N-terminal region. On the basis of structure, these hotspots of mutations are found on the molecular surface related to the interaction site with toxin, on the other hands, the backbone of the protein retains a highly conserved structural scaffold of the inhibitors.

Our results suggested that these molecular interactions of an endogenous inhibitor with the toxin explain the physiological role of SSPs in resistance to divergent toxins.

Session 2

“Drug discovery seeds”

Chairman: Yoshito Abe [Kyushu University]

Masaki Nagao (FU)

“The effects of fermented ginseng on symptoms in dementia-Behavioral and pharmacological studies using animal models”

Brandon Morinaka (NUS)

“Posttranslational modifications in natural product biosynthesis”

Kenji Hamase (KU)

“Chiral amino acid analysis for new drug discovery and diagnosis”

The effects of fermented ginseng on symptoms in dementia-Behavioral and pharmacological studies using animal models

Masaki Nagao[1], Takuya Watanabe[1,2], Kaori Kubota[1,2], Shutaro Katsurabayashi[2], Katsunori Iwasaki[1,2]

1) Institute for Aging and Brain Sciences, Fukuoka University

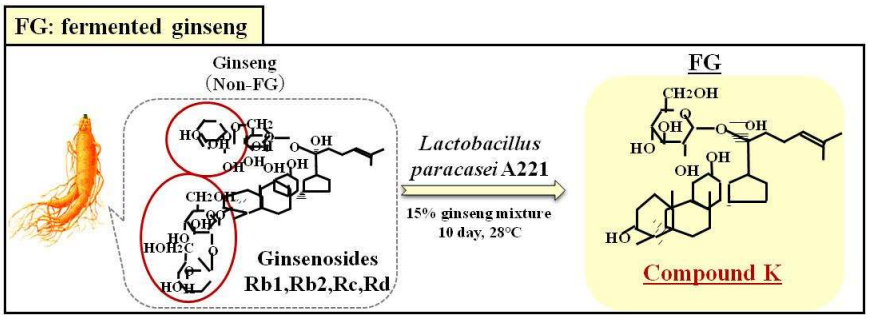
2) *Dept. Neuropharmacol. Fac. Pharm. Sci. Fukuoka Univ. Fukuoka, Japan*

Abstract

Dementia is a neurodegenerative disease characterized by marked cognitive dysfunction, and behavioral and psychological symptoms of dementia (BPSD), including sleep disturbance, anxiety, and depression. A variety of drugs have been developed as therapeutic agents for the treatment of dementia. However, these drugs are unable to fully prevent the progression of the disease. Therefore, alternative therapies, including herbal medicine or supplements, have gained attention as a strategy for preventing the development and progression of dementia.

Ginseng (Panax Ginseng, C. A. Meyer, Araliadea) is one of the most famous traditional herbal medicines in East Asia including China, Korea and Japan, and is used as a popular supplement for healthy people. Ginseng provides various pharmacological effects, such as anti-stress, anti-fatigue and anti-inflammatory. These effects were due to the main active ingredients of ginseng, called ginsenosides, such as Rb1, Rb2, Rc of the protopanaxadiol type, Rg1, and Re of the protopanaxatriol type. However, the therapeutic effects of ginseng vary among patients because absorption of ginseng's components is required for their major active metabolites, M1(compound K) and M4 by intestinal bacteria. Considering that the majority of older dementia patients have weakened gastrointestinal conditions, it is likely that the efficacy of ginseng is reduced in these patients. Thus, we focused on a fermented ginseng (FG), which contains an increased amount of compound K produced by *Lactobacillus paracasei* A221.

We have previously evaluated the effects of various drags on cognitive dysfunction or BPSD with dementia animal models. In this study, we investigated whether FG is effective on spatial memory impairment or BPSD-like behavior in dementia animal models, and compared them with the effects of non-FG in behavioral studies.



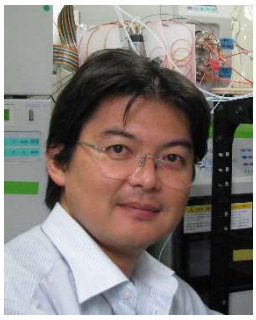
Content (%)	Non-FG	**FG**
Rb1	2.0	0 ⇓
Rb2	1.7	0.1 ⇓
Rc	2.2	0.5 ⇓
Rd	1.3	0.1 ⇓
Rg1	0.4	0.2
Compound K	0	**0.9** ⇑

Posttranslational modifications in natural product biosynthesis

Brandon I Morinaka
Department of Pharmacy, National University of Singapore

Abstract

In fundamental biochemistry, ribosomal biosynthesis is based exclusively on α-amino acids of the L-configuration. Here I will discuss the genome-guided discovery of bacterial natural product pathways that post-translationally create either D-configured or β-amino acid-containing products. These transformations are widespread in bacteria and are catalyzed by two different subfamilies of enzymes belonging to previously uncharacterized members of the radical *S*-adenosylmethionine (rSAM) superfamily. The D-configured amino acids are introduced by an rSAM epimerase while the D-amino acids are introduced by a radical mediated protein splicing process. These enzymes are encoded across a wide range of bacterial species, but the majority of their end natural products remain cryptic. These reactions can be used to incorporate diverse and multiple D- or β-amino acids into genetically encoded precursors in *E. coli*. Initial insights into these reactions and potential uses in biotechnological applications will be shown.



Chiral amino acid analysis for new drug discovery and diagnosis

Kenji Hamase

(Department of Drug Discovery and Evolution, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan)

It is long believed that all amino acids in higher animals are L-forms and the enantiomers (D-forms) are not present at least as biologically active substances. However, along with the progress in analytical technologies, several D-amino acids were found in mammals including humans both in the free form and in the protein bound form (D-amino acid residues). The distribution and regulation of these D-amino acids are clearly different from those of L-amino acids, and they are increasingly gathering attention as new drug candidates and biomarkers. However, the amounts of these D-amino acids in higher animals are usually trace, and uncountable known/unknown interfering substances are present in real biological samples.

In the present study, multi-dimensional HPLC systems combining reversed-phase, anion-exchange/mixed-mode and enantioselective columns have been designed and applied to the chiral amino acid analysis using various clinical samples, foods and beverages. For the sensitive determination, amino acids are labeled with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). Detection was carried out using their fluorescence and also by the ESI-MS (MS/MS).

In the sera of patients with chronic kidney disease, the amounts of D-Ala, D-Asn, D-Pro and D-Ser are higher than those in healthy volunteers, and their amounts are closely correlated with the kidney function. In the fermented foods/beverages, relatively high amounts of D-amino acids, such as D-Ala, D-Asp, D-Glu and D-Ser, were found and some of the D-amino acids have clear physiological functions in mammals. The present multi-dimensional HPLC platform is useful for the D-amino acid researches, and further applications are currently ongoing.

Education/Career:

1993: M. S. from Graduate School of Pharmaceutical Sciences, The University of Tokyo

1996: Ph. D. from Graduate School of Pharmaceutical Sciences, The University of Tokyo

1996-2000: Assistant Professor, Faculty of Pharmaceutical Sciences, Kyushu University

2001-2016: Associate Professor, Graduate School of Pharmaceutical Sciences, Kyushu University

2016-present: Professor, Graduate School of Pharmaceutical Sciences, Kyushu University

Research Interests:

D-Amino acids, Multi-dimensional HPLC, Chiral separation, Drug discovery, Diagnosis, Functional foods, Anti-aging