The 4th International Chemical Biology Frontier Symposium

第4回ケミカルバイオロジーフロンティア国際シンポジウム

主催：東京医科歯科大学
大学院生命情報科学教育部・疾患生命科学研究部
難治疾患研究所・生体材料工学研究所
共催：大学院教育改革支援プログラム「国際産学リンケージプログラム」

1st session:

Frontier of Chemical Biology for Drug Discovery

Michael Marletta (University of California, Berkeley, USA)
Masaru Taniguchi (Riken RCAI, Japan)
Laurent Meijer(CNRS, France)

2nd session:

International Cooperation of
Academic and Industrial Researchers for Drug Discovery

Hiroyuki Kagechika (Tokyo Medical and Dental University, Japan)
Takashi Owa (Eisai Corp., Ltd, Japan)

日時：2007年12月5日（水）　午前10時より
場所：東京医科歯科大学５号館４階講堂

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The 4th International Chemical Biology Frontier Symposium
Program

10:00-10:10 Opening Address

1st Session：Frontier of Chemical Biology for Drug Discovery
（創薬を目指すケミカルバイオロジー研究のフロンティア）

10:10-10:50 Michael Marletta (University of California, Berkeley)
Drug Design in the Nitric Oxide Signaling Pathway

10:50-11:30 Masaru Taniguchi (Riken RCAI, Japan)
Chemical Biology of the NKT Cell Ligand

11:30-12:10 Laurent Meijer (CNRS)
Cyclin-Dependent Kinases (CDKs) in Human Disease:
the Potential of Pharmacological Inhibitors

2nd Session：International Cooperation of Academic and Industrial Researchers for Drug Discovery
（国際産学連携による創薬研究の推進）

13:30-14:10 Hiroyuki Kagechika (Tokyo Medical and Dental University, Japan)
Retinoids: Structural Evolution and Clinical Utilities

14:10-14:50 Takashi Owa (Eisai Corp., Ltd, Japan)
Decoding Chemical Structures by Exploiting Transcriptomic and Proteomic Technologies.

14:50-15:00 Closing Remarks

Drug Design in the Nitric Oxide Signaling Pathway

Michael A. Marletta

Aldo DeBenedictis Distinguished Professor of Chemistry, Department of Chemistry
Professor of Biochemistry and Molecular Biology, Department of Molecular & Cell Biology
University of California, Berkeley

Since the discovery of nitric oxide (NO) formation in higher animals, it has become clear that this toxic, free radical, diatomic gas plays a central role in cellular function. NO acts as a cell-to-cell signaling agent in the cardiovascular system and the central nervous system. The immune system uses NO in the host response to infection. Given the toxic properties of NO, the role in immune system for NO seems appropriate. However, given the toxicity of NO, function of NO in cell signaling requires tight control over synthesis and selective and sensitive receptors. NO is synthesized by nitric oxide synthase (NOS) and the most thoroughly characterized receptor the soluble form of guanylate cyclase, and enzyme once activated, converts GTP to cGMP. S-Nitrosation of cysteine residues in proteins can also occur and may play a role in NO signaling. Over the years efforts toward selective manipulation of the NO function in biology have emerged. Selectivity is complicated by the complex biology controlled by NO and attempts toward tissue-specific responses. Approaches toward controlling this pathway will be described.

Ligand-recognition and function of NKT cells

Masaru Taniguchi, Tomoki Chiba, Takuya Tashiro, Sayo Inoue, Ryusuke Nakagawa, Etsuko Sekine, Miyuki Omori-Miyake Takashi Tsujimoto and Kenji Mori, and Hiroshi Watarai
(Riken Research Center for Allergy and Immunology, Yokohama, Japan)

NKT cells are characterized by the expression of a single invariant Vα14 antigen receptor paired with Vβ8.2 in mice. Human homologue is also identified as Vα24 mainly associated with Vβ11. Although the *Vα14* and *Jα281* genes are located in the TCR gene cluster, the invariant Vα14 receptor is used only by NKT cells but not by conventional T cells. An NKT cell-ligand has been identified as a glycolipid, α-galactosylceramide (α-GalCer), which is presented by a monomorphic MHC-like molecule, CD1d. Borg *et al*. have recently demonstrated the crystal structure of α-GalCer/CD1d/β2m/Vα24/Vβ11 triple complex. In contrast to TCR-peptide-MHC complexes, the Vα24 NKT cell antigen receptor docked parallel to the CD1d-binding cleft, which enables a lock-and-key type interaction with α-GalCer. The structure provides direct insight into how NKT cell antigen receptor recognizes a lipid-antigen-presenting molecule of the immune system.

NKT cells are activated by the CD1d/α-GalCer complex, immediately release both Th1 and Th2 types of cytokines, and mediate protective responses by their IFNγ production and regulatory responses by Th2 cytokines, such as IL-10. NKT cell-mediated Th1 or Th2 cytokine production has long been attempted to separate using different types of α-GalCer analogues, because Th1 and Th2 type cytokines antagonize each other. To circumvent these problems, we have been trying to develop a new type of α-GalCer analogue, α-carbaGalCer, which activates NKT cells to produce only Th1 cytokine. By BIACore analysis, α-carbaGalCer has higher affinity (*KD*) with 3.9 nM to the invariant Vα14 NKT cell receptor compared to α-GalCer with affinity (*KD*) of 60-70nM, which is 1,000 times higher than that of the TCR to MHC/peptide interaction. This suggests that the higher affinity ligand determines the cytokine production profiles of NKT cells.

Cyclin-Dependent Kinases (CDKs) in human disease: the potential of pharmacological inhibitors

Laurent MEIJER
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Phosphorylation of serine, threonine and tyrosine residues represents one of the most common post-translational mechanisms used by cells to regulate their enzymatic and structural proteins. Alterations in the phosphorylation of proteins represent a frequent feature associated with human disease. This is the reason for an exponentially growing investment in the discovery, optimization and therapeutic evaluation of small molecular weight, pharmacological inhibitors of protein kinases. 30-35% of drug discovery programs in the pharmaceutical industry currently target a protein kinase! Over 60 kinase inhibitors are undergoing clinical evaluation against diseases such as cancers, inflammation, diabetes, and neurodegeneration.

Among the 518 human kinases, cyclin-dependent kinases (CDKs) have attracted considerable interest because of their numerous essential physiological functions such as regulation of cell division cycle, apoptotic cell death, multiple neuronal functions, pain signaling, insulin release by pancreatic cells, transcription, RNA splicing. Consequently deregulations of CDKs and their regulators are observed, involved and causative in numerous and diverse human diseases. CDKs are regulated in four different ways: [1] transient association with a regulator (cyclin), [2] various post-translational modifications (phosphorylation, U-dependent degradation), [3] transient association with a natural inhibitory protein (Cip1, Kip1/2, Ink4A-D), and [4] intracellular localization. Although the human genome sequencing has allowed the detection of 20 CDKs and 25 cyclins, a small number of active CDK/cyclin complexes have been identified.

We will first present an overview of the involvement of CDKs in various human diseases. For each group of diseases, we will review the evidence in the human pathology and animal models and we will briefly assess the therapeutic potential of targeting the CDK pathway.

1) CDKs in cancers: there are numerous examples of the direct involvement of CDKs and their regulators in the development of cancers. We will select a few representative examples such as breast cancer, prostate cancer, melanoma and chronic lymphoid leukemia.

2) CDKs in chronic neurodegenerative disease: considerable data support the critical role of abnormal activation of CDK5 and CDK1 in the brains of Alzheimer's disease patients. CDK5 is also involved in Parkinson's disease and Niemann-Pick disease type C.

3) CDKs in "acute" neuronal disorders and neurodegeneration: we will review the evidence for CDK5 implication in stroke, traumatic brain injury, pain signaling. There is also some evidence for the involvement of CDKs in preventing axonal regeneration.

4) CDKs in kidney diseases: CDKs play a key role in the development of several kidney diseases. Examples comprise glomerulonephritis, lupus nephritis, collapsing glomerulopathy, polycystic kidney disease, cisplatin-induced nephrotoxicity.

5) CDKs in inflammation: pleural inflammation and arthritis represent the best illustrated examples.

6) CDKs in type 2 diabetes: CDK5 prevents insulin secretion by pancreatic cells, and its inhibition favors glucose-dependent secretion. CDK inhibition reduces loss of β cell function under glucotoxic conditions.

7) CDKs in viral infections: some viruses (HSV, HCMV, HPV, HIV) use cellular CDKs (CDK2, CDK9) for their transcription.

8) CDKs in unicellular parasites (*Plasmodium*, *Leishmania*, etc…). These organisms, responsible for devastating diseases such as malaria and leishmaniosis, have CDK homologs involved in their proliferation and differentiation.

Clinical implications

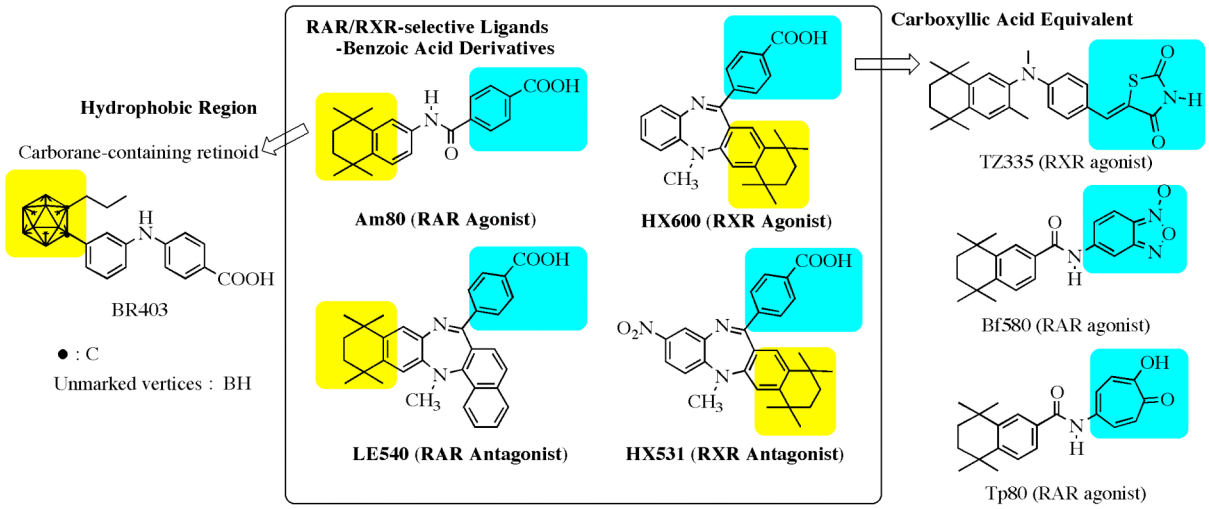
All these links between CDKs and human disease have encouraged an intensive search for potent and selective pharmacological inhibitors of these kinases. Over 120 small molecular weight inhibitors of CDKs have been characterized, most of which appear to act by direct competition with ATP for binding to the catalytic site of the kinase. Over 30 of these compounds have been co-crystallized with CDK2 and/or CDK5, demonstrating their binding in the ATP-binding pocket of CDKs. Seven CDK inhibitors are currently undergoing clinical trials. To illustrate the properties of these CDK inhibitors, and their effects on cellular and animal models o various human diseases, we will describe our own compound, (R)-roscovitine and second generation analogues. The family of 2,6,9-trisubstituted purines encompasses some of the first CDK inhibitors which have been described. Among these purines, the (*R*)-stereoisomer of roscovitine is one of the most frequently studied and used CDK inhibitors. Also referred to as CYC202 or Seliciclib, (*R*)-roscovitine is developed by Cyclacel Pharmaceuticals, Inc. (http: www.cyclacel.com). It has now reached phase 2b clinical trial for non small cell lung cancer, phase 2 against nasopharyngal cancer, phase 1 trials for glomerulonephritis, and phase 2 trials in IgA nephropathy. It is undergoing pre-clinical animal evaluation against Alzheimer's disease, Parkinson's disease, stroke, and polycystic kidney disease. The selectivity and intracellular mechanism of action of roscovitine have been extensively studied and will be presented as a representative example of the multiple effects of CDK inhibitors in cells, tissues and organisms. Other recently discovered CDK inhibitors will be briefly reviewed.

Retinoids: Structural Evolution and Clinical Utilities

Hiroyuki Kagechika
School of Biomedical Science, Tokyo Medical and Dental University

Retinoids are natural and synthetic analogs of retinoic acid, and are specific modulators of cell proliferation, differentiation, and morphogenesis in vertebrates. The pleiotropic activities of retinoids are mediated by binding to and activating two classes of nuclear receptors, retinoic acid receptors (RAR α, β, γ) and retinoid X receptors (RXR α, β, γ). In spite of the beneficial activities of retinoids, the scope of retinoid therapy is still limited owing to high toxicity, and only a few retinoids, such as all-*trans*-retinoic acid and etretinate for the treatment of psoriasis, have been clinically used until recently.

We have developed various RAR and RXR ligands. For example, several unique retinoids bearing a functional group that is new bioisoster of the carboxyl group (or benzoic acid moiety), such as a thiazolidinedione (TZ335), benzofloxane (Bf580), and tropolone (Tp80), were found by the computer-assisted ligand search using a virtual library or available-molecule database. Carborane-containing retinoids are another unique class. Carboranes (dicarba-*closo*-dodecaboranes) are icosahedral boron clusters with remarkable thermal and chemical stability. We showed that carborane is useful hydrophobic pharmacophore and synthesized various nuclear receptor ligands bearing carborane moiety. Among synthetic retinoids, Am80 was approved as a drug for relapsed acute promyelocytic leukemia (APL) in Japan in 2005. The clinical utilities of Am80 have been further examined in the field of autoimmune and cardiovascular diseases. In this lecture, the structures, biological functions, and clinical utilities of synthetic retinoids and related nuclear receptor ligands will be discussed as a representative example of chemical biology and/or medicinal chemistry targeting nuclear receptors.



Decoding Chemical Structures by Exploiting Transcriptomic and Proteomic Technologies

Takashi Owa
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The research concept of chemical biology with biologically active small molecules is now recognized as a complement to genomics-based approach toward uncovering unknown gene functions, biological pathways, and genomic networks. In chemical biology studies, organic small molecules are utilized as stimulants or perturbagens that induce gain-of-function or loss-of-function to their protein targets. For the efficient success in this approach, high-quality small molecule libraries are essential. While substantial efforts using diversity-oriented synthesis and combinatorial synthesis have been made for improving the quality of small molecule libraries, it should be also critical for chemical biologists to decode chemical structures and interaction modes between a compound and its target protein(s).

DNA microarray analysis and stable isotope labeling methods like ICAT and SILAC have enabled us to perform quantitative differential analysis of gene and protein expression, respectively. By exploiting these new technologies, we are now trying to characterize some chemical structures and the patterns of molecular recognition with their protein targets. In this presentation, I will highlight the use of transcriptomic and proteomic analyses for the design of small molecule libraries, with particular focus on anticancer sulfonamide derivatives and naturally occurring transcription modulators.