Hokkaido University
Leading Graduate School
Veterinary Science for One Health

The 4th Leading Special Lecture

New Aspect in Clinical Chemistry

What is the role of "Analytical Chemistry" in Veterinary Medicine?

March 13, 2013, 16 : 00~17 : 30
Lecture Hall, Graduate School of Veterinary Medicine, Hokkaido University, JAPAN

Prof. Mitsuyoshi Takiguchi (Hokkaido Univ, JAPAN)
Dr. Kei Nomiyama (Ehime Univ, JAPAN)
Prof. Robert H Weiss (UC Davis, USA)

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Program

Chairpeson: Prof. Mitsuyoshi Takiguchi (Hokkaido Univ, JAPAN)

16:00～16:20

Dr. Kei Nomiyama (Ehime Univ, JAPAN)

Organohalogen compounds and their metabolites in the blood of pet dogs and cats, and in pet food

16:20～16:30

Prof. Mitsuyoshi Takiguchi (Hokkaido Univ, JAPAN)

Future expectation for applications of metabolomics

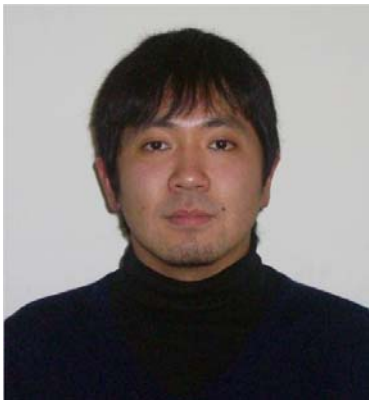
in clinical veterinary medicine

16:30～17:30

Prof. Robert H Weiss (UC Davis, USA)

Metabolomics in Kidney Cancer: From biofluids to new therapeutic targets.

Dr. Kei Nomiyama, Ph.D.



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Organohalogen compounds and their metabolites in the blood of pet dogs and cats, and in pet food

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Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) have been widely used for industrial applications since the 1960s and 1970s. As a consequence, PCB and PBDE levels have been increasing dramatically in the environment, wildlife and humans, because of their persistent properties and bioaccumulation characteristics. Toxic effects of these compounds on endocrine systems and neurodevelopment are already well known. It is suspected that the thyroid hormone homeostasis is disturbed by not only PCBs and PBDEs but also their hydroxylated metabolites. Hydroxylated PCBs (OH-PCBs) are formed by the oxidative metabolism of PCBs by cytochrome P450 (CYP) monooxygenase enzyme systems. Hydroxylated PBDEs (OH-PBDEs) are well-known metabolites of PBDEs, and also natural substances found in marine organisms.

Recently, a study found higher concentrations of PBDEs in pet cats than in human serum, and it has been hypothesized that increases of feline hyperthyroidism (HT) are related to increased PBDE exposure, with key routes of exposure being diet and ingestion of house dust. Moreover, increased thyroid hyperfunction disease may be linked to OH-PCBs and OH-PBDEs derived from metabolites of PCBs and PBDEs, diet, natural products, and/or demethylation of MeO-PBDEs. However, information on status of hydroxylated metabolites of PCBs and PBDEs in pet animals and pet food are limited.

The present study determined the concentrations and accumulation patterns of organohalogen contaminants such as PCBs and PBDEs and their metabolites (OH-PCBs, OH-PBDEs and MeO-PBDEs) in the whole blood of pet cats and pet dogs collected from veterinary hospital in Japan. Moreover, to estimate the dietary PCB and PBDE exposure levels and biotransformation to hydroxylated metabolites in pet animals, PCB, PBDE and their derivatives (OH-PCBs, OH-PBDEs, and MeO-PBDEs) in the pet foods were also analyzed.

Concentrations of OH-PCBs in the blood of dogs and cats were 220 ± 260 pg/g and 150 ± 80 pg/g, respectively. Tri- and penta-chlorinated OH-PCB congeners were predominant in cat blood. In

contrast,accumulation of hexa- through octa-chlorinated OH-PCBs has been found in dog blood. These results were similar to pattern of previous terrestrial mammal study. In contrast, OH-PCB levels in pet food (range: 0.52-1.3 pg/g) were negligible. These results indicate that the OH-PCBs detected in pet dogs and cats are metabolites of PCBs because pet food did not contain OH-PCBs. PBDE levels in pet bloods were similar to those in dry pet food, and BDE209 was the dominant congeners in both bloods and food. The residual levels of 6OH-BDE47 and 2′MeO-BDE68 in the blood of pet cats were similar. MeO-PBDEs levels in seafood rich (wet) cat diet were one order higher than those in cat bloods, while OH-PBDEs levels in cat food were one to two orders lower than the cat bloods. Among the OH-/MeO-PBDE isomers, only 6OH-/MeO-BDE47 and 2′OH-/MeO-BDE68 were detected in the blood of dogs and cats and pet food. These two abundant isomers are produced naturally by marine organisms. These results suggested that pet cats are exposed to MeO-PBDEs through cat food such as fish flavor. On the other hand, low concentrations of OH-/MeO-PBDEs in dog blood and dog food were found. This result suggests that uptake of from naturally produced OH-/MeO-PBDEs within the dog foods are very low. When *in vitro* demethylation experiment of MeO-PBDEs using dogs and cats liver microsomes was conducted, the demethylation of MeO-PBDEs in dogs and cats microsomes have been confirmed. This result shows large percentage of OH-PBDEs found in the cat blood might be direct intake from food and biotransformation of MeO-PBDEs. On the other hand, it can be suggested that dogs may have high metabolic capacities for OH-/MeO-PBDEs through phase II reaction, or low TTR binding potencies of OH-PBDEs. These results suggested that induction of thyroid dysfunction in pet cats (which are originally carnivorous) might be due to forced fish eating through seafood.

Metabolic capacities of dogs and cats were compared by the *in vitro* metabolism test of PCBs using hepatic microsomes. After exposure to 62 PCB mixtures, 4'OH-CB18, 3'OH-CB28, 4'OH-CB79, 4OH-CB107, and 13 unknown peaks (3-5Cl OH-PCBs) were found in cat liver microsomes. The homolog pattern identified in this experiment was similar to those in the pet cat blood. In contrast, 13 identified OH-PCBs and 22 unknown OH-PCBs (3-8Cl OH-PCB) were detected in the dog liver microsomes, after exposed to PCB mixtures. Different homolog pattern between this experiment and pet dog blood was observed. It suggests lower chlorinated OH-PCBs were more easily eliminated through phase II conjugation reaction in dog body.

These results indicate possibility of induction of adverse health effects, especially feline HT, due to the consumption of cat feed made from fishery product and specific metabolic capacities. In future studies, we need to investigate the metabolic capacities to halogenated compounds including phase II conjugation reactions, and assess the toxicological risk posed by these hydroxylated metabolites.

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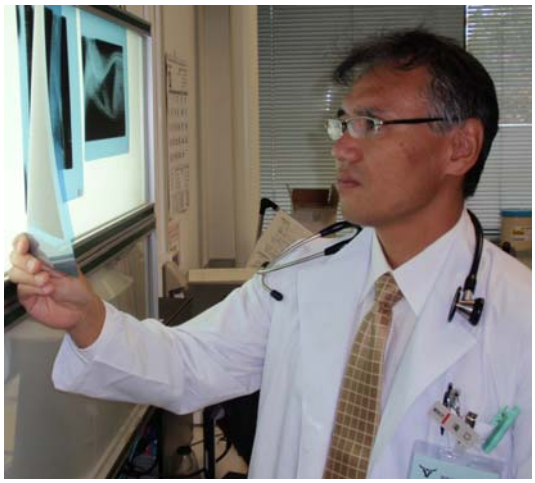
Prof. Mitsuyoshi Takiguchi



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Future expectation for applications of metabolomics in clinical veterinary medicine

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The field of metabolomics continues to grow rapidly over the last decade and has been proven to be a powerful technology in predicting and explaining complex phenotypes in diverse biological systems. Application of metabolomics has been increasing in the medical field. In this short talk, I would like to talk about future expectation for applications of metabolomics in clinical veterinary medicine.

Prof. Robert H. Weiss, MD

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Metabolomics in Kidney Cancer:
From biofluids to new therapeutic targets

Robert H. Weiss, MD
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Of the omics sciences, metabolomics is one of the newer methodologies. In this technique, all of the small molecule substances produced during bodily processes are measured. This includes endogenous metabolites as well as small peptides, amino acids, and (usually) body flora and drug metabolites. Rather than being participators in these processes, as are genes and proteins, the metabolites produced are sentinels of "what is actually happening" in an organism. The so-called "cancer metabolome" is loosely defined as the entire suite of relatively low molecular weight (~<1500 daltons) metabolites germane to cancer and includes their changes relative to a control non-cancerous group of patients or tissues[1]. Using this data, metabolic pathways altered in a specific cancer can be identified, and once a metabolic pathway is determined to be altered in a specific tumor or in the systemic response to it, and a full validation (*in vitro* and/or *in vivo*) of the role of this metabolite takes place, pharmacologic inhibitors or activators of such pathways can be either designed or re-purposed to be used as potential new chemotherapeutics[2].

Most of the metabolic processes in the body, such as those involving energetics and amino acid catabolism, are common to all living cells. However, it is logical that in cells which are highly proliferative or which have deranged apoptosis, such as cancer cells, there would be at least some biochemical pathways that are enhanced or diminished. For example, in order for a cell to grow rapidly, there exists the necessity for high energy requirements as well as for the provision of membrane and other cellular building blocks. In addition, there are likely to be profound changes in metabolic pathways such as glycolysis (e.g. the Warburg effect), amino acid metabolism, and fatty acid oxidation. Due to its appetite for transforming many metabolic pathways to its own advantage (which ultimately leads to its causing mortality), cancer is ideally suited for metabolomics analysis. Since cancer, especially in the later stages, so profoundly usurps normal metabolic processes, it can be considered the ultimate metabolic disease.

As applied to RCC, metabolomics evaluations can be performed on urine, blood, and tissue to identify altered metabolic pathways and thereby point to new targets for therapeutic intervention[3]. In addition, such signatures, if they are found to be specific to the cancer in question, can be used either singly or as multiplexed as biomarkers. Even though it is likely that most RCCs are "walled off" from the urinary space, the use of urinary metabolite determination has been especially fruitful and has yielded signatures of

tryptophan metabolism as well as energetic derangements. Similar analyses could potentially be accomplished with cyst fluid. While to date there have been no proven specific metabolic biomarkers for ccRCC, this technique has yielded novel targets, such as PPARα, CPT-1, and IDO[2], which can be evaluated using available pharmaceuticals.

In summary, metabolomics is a powerful new technique which can be utilized in cancer biology to identify both markers and new therapeutic targets, and in the form of pharmacometabolomics[4], can lead to the repurposing of heretofore discarded drugs and thus lead to therapeutic advances.

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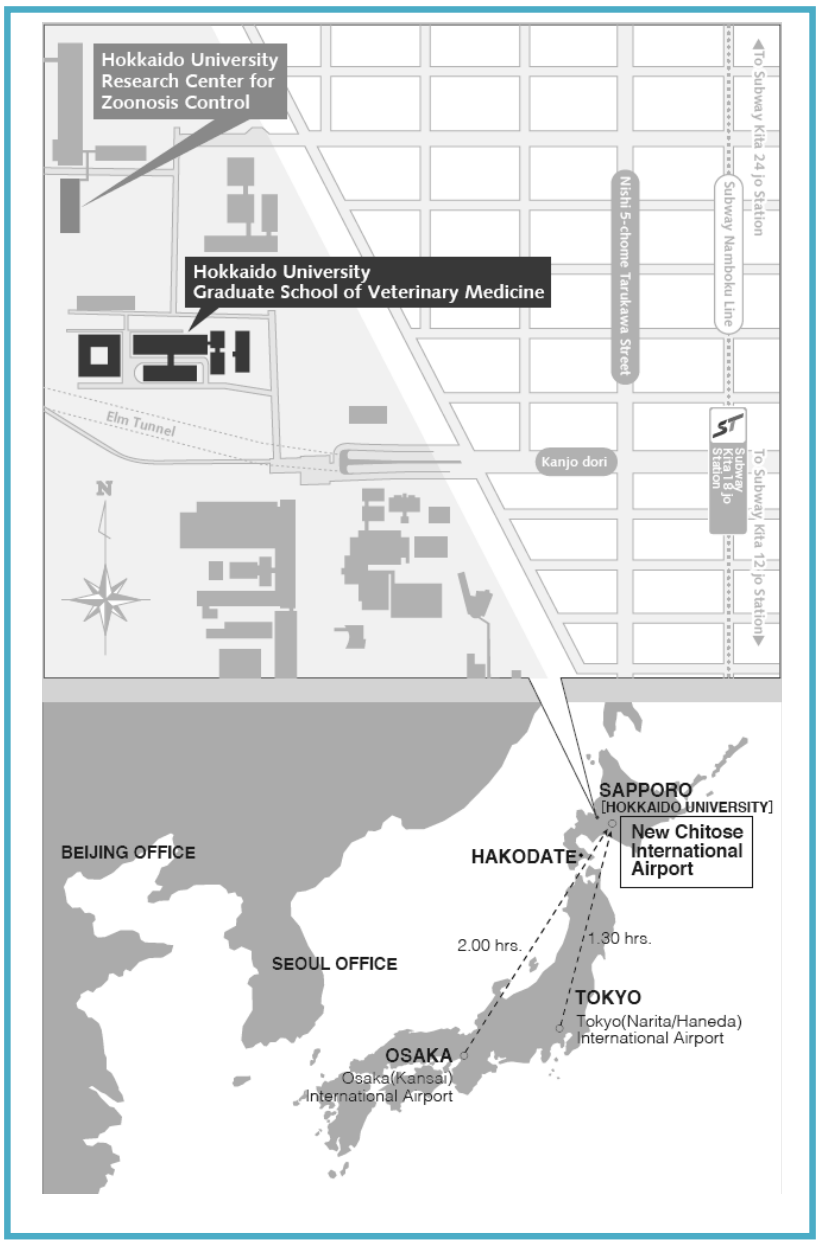

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