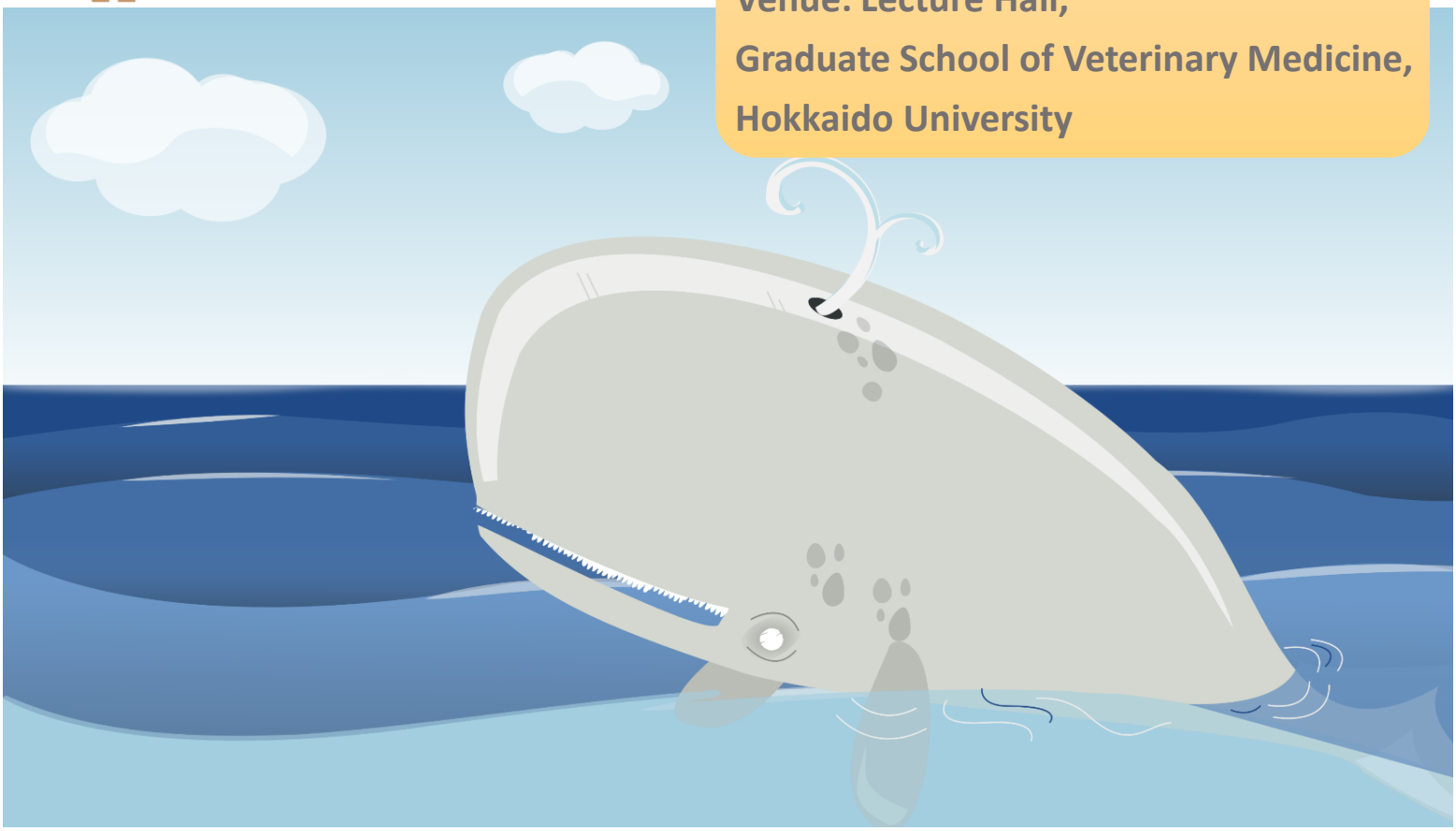
Leading Symposium on Molecular targets of environmental chemicals

Hokkaido University
Leading Graduate School
Veterinary Science for One Health

Date: FEB 10, 13:30-16:00
Venue: Lecture Hall,
Graduate School of Veterinary Medicine,
Hokkaido University



Dr. Akira Kubota, Obihiro University of Agriculture and Veterinary
Regulation of CYP genes by AHR and PXR in zebrafish

Prof. Hiroki Teraoka, Rakuno Gakuen University
Involvement of prostaglandins in TCDD-induced circulation failure in developing zebrafish

Dr. Wageh Sobhy Darwish, Hokkaido University and Zagazig University, Egypt
Cross-talks between heavy metals and AhR gene battery, with special reference to protection by some phytochemicals

Prof. Alvaro Puga, University of Cincinnati, College of Medicine, Environmental Health, USA
Perinatal Lead Exposure in Mice Causes Long-Term Changes in Brain DNA Methylation

Regulation of CYP genes by AHR and PXR in zebrafish

Akira Kubota

Diagnostic Center for Animal Health and Food Safety, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan

Early life stages of organisms are in general more susceptible than adult following exposure to chemicals. Severe developmental abnormalities, including effects on developing cardiovascular and neural tissues, occur in vertebrates exposed to a wide range of chemicals. Understanding chemical effects during development requires explicit knowledge of the regulatory networks involved in xenobiotic bioactivation and metabolism. Nuclear receptor NR1I2, the pregnane X receptor (PXR; also known as the steroid and xenobiotic receptor, SXR) and the related NR1I3, the constitutive androstane receptor (CAR), are ligand-activated transcription factors often referred to as “xenobiotic sensors”. The aryl hydrocarbon receptor (AHR) also is a ligand-dependent transcription factor that in vertebrates is activated by xenobiotics such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. Together, these receptors act to protect organisms from exogenous and endogenous toxic chemicals by regulating genes involved in xenobiotic metabolism and elimination, including cytochrome P450 (CYP) genes.

Zebrafish (*Danio rerio*), the vertebrate model in developmental biology, has been used also in the field of toxicology. Expanding our knowledge of the role of Pxr and Ahr in regulating the expression of genes *in vivo* is critical to establishing a mechanistic foundation for understanding and screening for chemical effects in this premiere toxicological model. The topics of this talk will include our recent findings on chemical effects in early life stages of zebrafish, with particular emphasis on molecular mechanisms of alteration of Pxr/Ahr signaling and the relevance to developmental toxicity. First the role of zebrafish Pxr and Ahr2 in regulation of target *CYP* genes *in vivo* in developing zebrafish is addressed. Pxr/Ahr2 involvement in response to agonists was established using morpholino antisense oligonucleotides to knock down translation of *pxr/ahr2*, coupled with a search for putative Pxr/Ahr response elements in proximal promoters of target genes. Developmental effect of a dysfunctional auto-regulatory feedback loop of Ahr/CYP1A signaling is also addressed.

Involvement of prostaglandins in TCDD-induced circulation failure in developing zebrafish

Hiroki Teraoka

School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

Knowledge on the mechanism of dioxin toxicity after activation of aryl hydrocarbon receptor (AHR) and its partner molecule, AHR nuclear translocator (ARNT) is still limited. Pericardial edema is a typical of developmental toxicity by dioxin and many chemicals in zebrafish, a model organism. Recently, we found that small cavity between heart and body wall was markedly increased by TCDD (precardiac edema) even in early larvae (55 hpf) using high-speed camera. By measuring precardiac edema, we addressed the involvement of prostaglandins in the TCDD-induced edema formation. Precardiac edema (edema) caused by TCDD was reduced by morpholino knockdown of AHR2 and ARNT1, as well as antioxidant. Selective inhibitor of COX2, NS398 and a thromboxyane receptor (TP) antagonist, ICI-192,605 markedly inhibited TCDD-induced edema. Knockdown of COX2b, TBX A synthase 1 but not COX2a also showed preventive effects against TCDD-induced edema formation. While TP agonist, U46619 deformed yolk sac to affect pericardial area, short exposure of U46619 caused edema by itself. On the other hand, a prostacyclin receptor (IP) agonist, beraprost inhibited the response by TCDD and U46619 in the sensitive manner to IP antagonist, CAY10441 and IP knockdown. Knockdowns of IP or of prostacyclin synthase caused significant edema by themselves. Additionally, TCDD-induced edema was potentiated in IP morphant. These results suggest the counteracting roles of thromboxane and prostacyclin in edema by TCDD formation after stimulation of AHR2/ARNT1 pathway in developing zebrafish.

Cross-talks between heavy metals and AhR gene battery, with special reference to protection by some phytochemicals

Wageh Sobhy Darwish[1, 2)], Yoshinori Ikenaka[1)], Shouta Nakayama[1)] Hazuki Mizukawa[1)] and Mayumi Ishizuka[1]

[1)]*Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18, W9, Kita-ku, Sapporo 060-0818, Japan*

[2)]*Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt*

The persistent occurrence and accumulation of heavy metals, particularly lead (Pb), copper (Cu) and cadmium (Cd), and the potential human exposure from numerous sources, such as food, water, soil, and air, make them among the most hazardous and toxic substances in the environment. In humans, frequent exposure to even small concentrations of these metals may have direct biological consequences involving the xenobiotic metabolizing enzyme (XME) system, leading to disruption of xenobiotic metabolic pathways. As a result, a broad range of biochemical, physiological, pathological, and behavioral changes in the body may occur.

Among the XMEs is the aryl hydrocarbon receptor (AhR)-regulated gene battery, which includes phase I enzymes such as cytochrome P450 (CYP) 1A1 and 1A2; phase II enzymes such as uridine diphosphate glucuronosyltransferase (UGT) 1A6 and NAD(P):quinone oxidoreductase 1 (NQO1).

The AhR gene battery is responsible for metabolism and detoxification of promutagens, procarcinogens, and environmental pollutants, such as polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines (HCAs), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Disruption of AhR gene battery by heavy metals in highly polluted areas may increase the mutagenicity and carcinogenicity of PAHs, HCAs and dioxins.

Micronutrients such as β-carotene (BC) and ascorbic acid (AA) are found naturally in vegetables, fruits, and green leafy plants. These substances are absorbed from the intestine and stored in the liver or adipose tissue of human and animals.

Today, we will discuss the genotoxic effects of heavy metals (Pb, Cu & Cd) on AhR gene battery. Moreover, we will highlight the protective effects of some micronutrients (BC &AA) against harmful effects of heavy metals.

Perinatal Lead Exposure in Mice Causes Long-Term Changes in Brain DNA Methylation

Prof. Alvaro Puga

University of Cincinnati, College of Medicine, Environmental Health, USA

In spite of regulatory measures that have virtually eliminated lead (Pb) from gasoline and most dietary sources, lead exposure remains a persistent environmental health problem. Blood lead levels well below the current action level of 5 µg/dl are strongly associated with neurodevelopmental toxicity. Children in densely populated urban residential centers remain at-risk due to lead in paint, dust and soil. Although regulatory measures have substantially reduced blood lead levels in the general population, lead exposure continues to be a major environmental risk for neurodevelopmental morbidities. There is good agreement that the most important cognitive, behavioral and psychiatric health effects of lead exposure are manifest long after exposure has ceased, suggestive of either a genetic (mutational) or an epigenetic component. However, the causes of the long-term morbidity associated with prenatal and postnatal exposure to lead are poorly understood. Variability in genetic or epigenetic factors as exacerbating or protective agents of human neurodevelopmental morbidity has not been adequately examined in relationship to early exposure to lead. Studies linking attention deficits, aggressive and disruptive behavior, and poor self-regulation have shown that early exposure to lead results in an increased likelihood of engaging in antisocial behavior in later life. Debate remains ongoing over which periods of development are most vulnerable to the effects of lead and what levels and durations of exposure produce adverse effects. These facts highlight the importance of identifying risk factors and biomarkers that may identify individuals at high risk for lead-associated mal-development.

To determine whether an association exists between DNA methylation patterns and the morbidity induced by exposure to lead, and, to ascertain if perinatal lead exposure caused persistent DNA methylation changes in target tissues, we designed a study in mice using advanced neuroimaging methods in combination with DNA methylation analyses. We hypothesized that animals exposed to various doses of lead during gestation and early postnatal time points would have smaller brain volumes, indications of decreased myelination, and lower N-acetyl aspartate and elevated glutamate levels compared with control animals and that these effects would correlate with DNA methylation changes in the corresponding areas of the brain of exposed mice. We exposed mouse dams to 0, 3 or 30 ppm of lead acetate in drinking water for a period extending from 2 months prior to mating, through gestation, until weaning of pups at postnatal day-21, and analyzed whole-genome DNA methylation in brain cortex and hippocampus of 2-month old exposed and unexposed progeny. Exposure to 3 or 30 ppm

resulted in neuroanatomical effects in cortex, hippocampus and thalamus and the hypermethylation of three differentially methylated regions (DMRs) in the hippocampus of females, but not males. These DMRs mapped to *Rn4.5s*, *Sf1*, and *Rn45s* loci in mouse chromosomes 2, 11 and 17, respectively. *Sfi1* was also hypermethylated in the cortex of exposed females, but not males. At a conservative false *d*iscovery rate *fdr*<0.001, 1,623 CpG sites were differentially methylated in female hippocampus, corresponding to 117 unique genes. No statistically significant methylome changes were detected in male hippocampus or in cortex of either sex. In agreement with these observations, when we tested half of these genes for gene expression changes we observed a good correlation between DNA hypermethylation and gene repression in females, but not in males. Exposure to lead during embryonic life, a time when the organism is most sensitive to environmental cues, appears to have a sex- and tissue-specific stochastic DNA methylation effect that may produce pathological or physiological deviations from the epigenetic plasticity operative in unexposed mice. Developmental lead exposure causes neuroanatomical lead effects areas of the brain some of which also present global DNA methylation patterns significantly different from those in control animals.

In humans, epigenetic responses to lead have only been described in the form of DNA methylation of interspersed repeats, but mechanistic insights and developmental windows of vulnerability are difficult to discern from associations between lead and methylation at interspersed repetitive DNA elements. We asked whether perinatal lead exposure would alter differentially methylated regions (DMRs) that control parent-of-origin, monoallelic expression of imprinted genes. Questionnaire data, serial lead measurements from the neonatal period to 78 months post-natally, and peripheral blood specimens were obtained from 104 participants of the Cincinnati Lead Study birth cohort. The level of DNA methylation at the DMRs regulating the expression of 22 human imprinted genes was quantified using a Sequenome EpiTYPER assays. Statistical analyses were conducted using linear regression models. Of the 22 imprinted domains investigated, significant associations between mean childhood lead exposure and DMR methylation were observed for *PEG3 (p=0.0016), IGF2/H19 (p=0.05),* and *PLAGL1/HYMAI (p=0.0018).* Elevated lead levels were associated with higher *PLAGL1/HYMAI* CpG methylation, regardless of sex (p=0.002) only if exposure was during the neonatal period. In contrast, elevated lead levels during early childhood were associated with lower *PEG3* and the association was most apparent in males (p=0.0018), while the association between mean lead levels and *IGF2/H19* was most pronounced in females (p=0.01). Because methylation at these DMRs is established in the gametes and early embryo after which it is stable through the life course, these findings demonstrate, for the first time, that lead exposure during early childhood results in persistent changes in the epigenetic regulation of specific imprinted genes.