The 13th

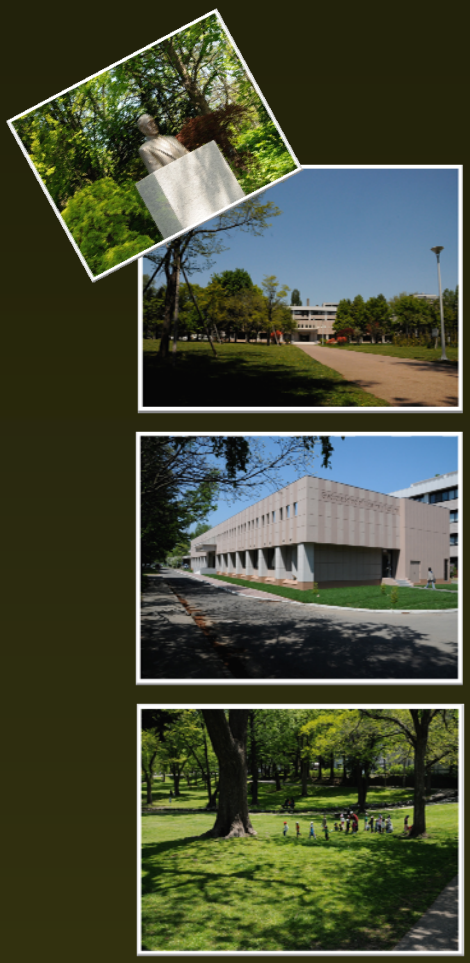
Joint Symposium

Between

Hokkaido University

and

Seoul National University



Satellite Symposium

Towards Infectious Disease Control

Time & Date: 13:30-17:05, November 26 (Friday), 2010
Venue: Conference Hall
Graduate School of Veterinary Medicine
Hokkaido University
Address/ Kita 18-jyo Nishi 9-chome, Kita-ku, Sapporo, Japan

Organized by Graduate School of Veterinary Medicine, Hokkaido University
In cooperation with College of Veterinary Medicine, Seoul National University and
Global COE program "Establishment of International Collaboration Centers for Zoonosis Control"

“Towards Infectious Disease Control”
Satellite Symposium of
The 13th Joint Symposium between Hokkaido University and Seoul National University

Program

13:30 - 13:35 **Opening remarks and welcome address**

Dr. Shigeo Ito, Dean, Graduate School of Veterinary Medicine, HU

Session 1

Chair persons

Dr. Takashi Umemura, Graduate School of Veterinary Medicine, HU
Dr. Kentaro Yoshii, Graduate School of Veterinary Medicine, HU

13:35 - 14:05 **Intrathecal vaccination as a possible therapeutic measure for rabies**
Dr. Takashi Umemura
Graduate School of Veterinary Medicine, HU 6

14:05 - 14:35 **Surveillance strategy for early detection of highly pathogenic avian influenza viruses and recent results of virus isolation in Korea**
Dr. Jae-Hong Kim
College of Veterinary Medicine, SNU 8

14:35 - 15:05 **CTL peptide vaccine against Influenza A virus infection**
Dr. Kiichi Kajino
Research Center for Zoonosis Control, HU 10

15:05 ~ 15:30 **Coffee break**

Date: **November 26 (Friday), 2010**
Venue: **Conference Hall, Graduate School of Veterinary Medicine, Hokkaido University**

Session 2

Chair persons

Dr. Satoru Konnai, Graduate School of Veterinary Medicine, HU
Dr. Rie Hasebe, Graduate School of Veterinary Medicine, HU

15:30 - 16:00 **New genetic variants of *Anaplasma phagocytophilum* and *Anaplasma bovis* from Korean water deer (*Hydropotes inermis argyropus*)**
Dr. Joon-Seok Chae
College of Veterinary Medicine, SNU 12

16:00 - 16:30 **Studies on the molecular mechanisms for Marek's disease virus to increase its virulence in the field**
Dr. Kazuhiko Ohashi
Graduate School of Veterinary Medicine, HU 14

16:30 - 17:00 **Investigation of prion disease neuropathogenesis using stable knock-down neuroblastoma cells established by chained microRNA-mediated RNA interference**
Dr. Han-Sang Yoo
College of Veterinary Medicine, SNU 16

17:00 - 17:05 **Closing remarks**

Dr. Oh Kyeong Kweon, College of Veterinary Medicine, SNU

18:30 - **Welcome party**

General Information & Guideline:

Oral presentation

- The laptops running Windows XP Professional operating system, with MS Office 2007 and Macintosh OS X operating system, with MS Office 2004 will be equipped.

- You can have your presentation in your own personal laptop if you use "moving images" or special programs included in your Power Point.

- Please keep the time for the presentation to ensure smooth proceedings.

- Please bring your presentation loaded in USB thumb drive (flash disk) or CD -ROM at the registration desk.

Accommodation in Sapporo

Sapporo Aspen Hotel
Address/ 5, Kita8-jyo Nishi4-chome, Kita-ku, Sapporo,
Hokkaido 060-0808 Japan
Phone +81 11-700-2111
FAX +81 11-700-2002
http://www.aspen-hotel.co.jp/english/frame.htm


Research Center for Zoonosis Control
Veterinary Medicine
Conference Hall
Hokkaido University Campus
convenience store
Weekly Inn
Kita 18 Jo Station
convenience store
"Dinner Bell" Supermarket
Graduate school of Medicine
Faculty House Trillium (Enreiso)
Kita 12 Jo Station
Subway
Centennial Hall
convenience store
University Conference Hall
Aspen Hotel
Loop bus runs every 15 mins between 8:30 to 18:30 in the campus. Leaving in front of Administration Bureau
Kelo Plaza Hotel
JR Sapporo Station

Takashi Umemura

Professor
Lab. of Comparative Pathology
Graduate School of Veterinary Medicine
Hokkaido University
Phone: +81-11-706-5191
Fax: +81-11-706-5194

ACADEMIC DEGREES:

DVM 1973 School of Veterinary Medicine, Hokkaido University
MVSc 1975 Graduate School of Veterinary Medicine, Hokkaido University
PhD 1982 Hokkaido University

PROFESSIIONAL APPOINTMENTS:

1979. 4 ~ 1982. 3	Chief pathologist, Hamamatsu Seigiken Research Institute
1982. 4 ~ 1984. 3	Lecturer, Faculty of Agriculture, Tottori University
1984. 4 ~ 1988.12	Associate Professor, Faculty of Agriculture, Tottori University
1988. 12 ~ 1997. 10	Professor, Faculty of Agriculture, Tottori University
1997. 10 ~ present	Professor, Graduate School of Veterinary Medicine, Hokkaido University

RESEARCH INTERESTS:

Rabies
Influenzal encephalopathy of childhood
Brain immunity

Intratheal vaccination as a possible therapeutic measure for rabies

Takashi Umemura

Laboratory of Comparative Pathology, Department of Veterinary Clinical Sciences,
Graduate School of Veterinary Medicine, Hokkaido University
E-mal: umemura@vetmed.hokudai.ac.jp

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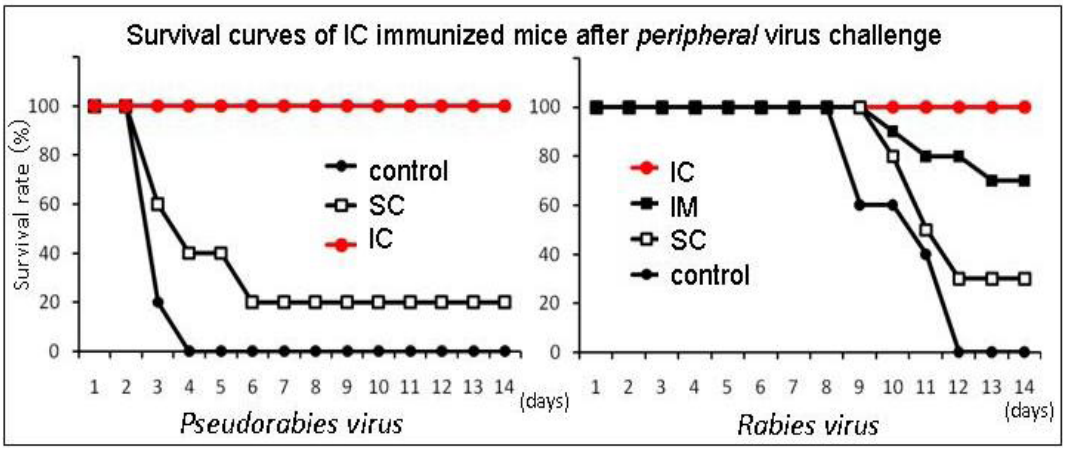
Rabies is one of the classical viral zoonoses and lethal in many mammals including humans. No effective treatment is available in rabid animals so far, since antibodies in the blood will neutralize the viruses only before they enter neurons or the nervous system and is ineffective for the viruses invaded the central nervous tissue (CNS) due to blood-brain barrier which interrupts antibody influx from blood to the CNS tissue.

In previous studies, we demonstrated that intrathecal immunization (direct inoculation of antigens into cerebrospinal fluid) induced specific antibody in cerebrospinal fluid (Shin *et al*, J Vet Med Sci 2007). The immunization was more effective at inducing a protective immune response against the transneural spread of rabies and pseudorabies viruses than ordinal subcutaneous and intramuscular immunizations (Shin *et al*, Microbiol Immunol 2006 and J Vet Med Sci 2009).



Furthermore, 80% of the mice given intrathecal immunization survived intracerebral inoculation of rabies virus without clinical signs, whereas only 25% of subcutaneously immunize and none of unimmunized mice survived the virus challenge (Sunden *et al*, Microb Infect 2010).

The final goal of our research is to clarify the mechanisms of brain immunity and treatment of rabid animals and humans using intrathecal immunization. In this joint symposium, I will introduce our recent research progress on those subjects.

Jae-Hong Kim

Professor
College of Veterinary Medicine
Graduate School of Seoul National University
Seoul National University

ACADEMIC DEGREES:

B.A.	1978	Seoul National University (Veterinary Medicine)
M.A.	1987	Seoul National University (Veterinary Medicine)
Ph.D.	1994	Seoul National University (Veterinary Medicine)

PROFESSIONAL APPOINTMENTS:

1981 - 2000	Scientific Researcher, National Veterinary Research Institute, MAF.
2001 - 2002	Director, Animal Diseases Control Division, National Veterinary Research & Quarantine Service (NVRQS), MAF.
2002 - 2005	Director, Avian Diseases Division, NVRQS. MAF.
2005 - 2007	Director general, Animal Diseases Research Department, NVRQS, MAF.
2007.3-present	Professor, College of Veterinary Medicine, Seoul National University

ACADEMIC ACTIVITIES

2009-Present	President, Korean Society of Poultry Science.
2005-2010	Vice-President, Korean Society of Veterinary Medicine.

RESEARCH INTERESTS:

- Molecular Epidemiology of Avian Influenza Viruses and Development of Advanced Diagnostic Techniques.
- Productivity of Reassorted Avian Influenza viruses
- Molecular Epidemiology and Protection of Avian Infectious Bronchitis Coronaviruses.
- Poultry Viral Diseases and Diagnositics.
- Poultry Salmonellosis and Related Food-hygiene.

Surveillance strategy for early detection of highly pathogenic avian influenza viruses and recent results of virus isolation in Korea

Kim, J-H.[1)], Kwon H-J.[1)], Kim I-H.[1)], Park C-K[2)]., Kim H-R[2)]
1) College of Veterinary Medicine, Seoul National University, Korea
2) National Veterinary Research and Quarantine Service, Korea
E-mal: kimhong@snu.ac.kr

Three different epizootics of highly pathogenic avian influenza (HPAI) caused by H5N1 avian influenza virus (AIV) have occurred in Korea since 2003. Among them, the 3rd epizootics of HPAI introduced on April, 2008 caused the most severe economic impact on the poultry industry in terms of the speed of disease spread, range of host species affected (6 species), number of outbreaks (33 cases) and total cost for eradication (about $ 550 million). Since then, an intensified active surveillance strategy of HPAI viruses focusing in winter season has shifted to the surveillance system throughout the year for early detection.

According to the Ministry for Food, Agriculture, Forestry and Fisheries (MIFAFF) and National Veterinary Research and Quarantine Service (NVRQS), 186,243 field samples were tested for AIV antigen and/or antibodies in 2009, in collaboration with avian diseases laboratories in 9 Colleges of Veterinary Medicine, Korea. Among them, 364 AIVs were isolated and subtyped, and finally identified as a low pathogenic AI (LPAI) virus, consisting of 20 H1, 2 H2, 25 H3, 15 H4, 9 H5, 82 H6, 4 H7, 190 H9, 4 H10, 12 H11, 1 (H3 + H6) viruses. Commercial chicken and swine farms were tested all negative for H5 or H7 subtypes of AIV, with the exception of H9N2 which showed nationwide outbreaks, whereas several AIV subtypes, H1, H3, H4, H6 and H9, were isolated from meat duck and breeder farms and one H5 (LPAI) from a duck breeder farm. And H11, H1, H4, H5, H6, H10, H2, H7 and H10 were frequently detected, in order, from feces of migratory birds around wetland. From live bird markets, H9, H6, H3 and H7 were often isolated. No isolate of subtype H8 and H12 to H16 was detected in 2009.

Although our active surveillance system of AIV covers commercial poultry farms, pet birds, migratory birds and captured wild birds, live bird markets and swine farms, as well as clinical observation data of poultry farmers, much more data should be collected and analyzed for long time in order to establish the early detection and early warning system.

Kiichi Kajino

Associate Professor
Department of Collaboration and Education
Hokkaido University
Research Center for Zoonosis Control

ACADEMIC DEGREES:

1992	M.D.,B.M.	Hokkaido University School of Medicine
1996	Ph.D. (Immunology)	Hokkaido University Graduate School of Medicine

PROFESSIONAL APPOINTMENTS:

1996 - 1999	Post Doctoral Researcher, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine
1999 - 1999	Clinical Fellow, Shiga University of Medical Science
1999 - 2005	Research Associate, Shiga University of Medical Science

CTL Peptide Vaccine against Influenza A Virus Infection

Kiichi Kajio
Department of Collaboration and Education
Hokkaido University Research Center for Zoonosis Control
E-mal: kiichi@czc.hokudai.ac.jp

Current influenza vaccines induce antibody production providing protection against virus infection. One of the major failings of these vaccines is the ineffectiveness for different viral subtypes or for escape mutants of viral surface proteins, hemagglutinin (HA) and neuraminidase (NA). Therefore, we focused on cytotoxic T lymphocyte (CTL) to develop a cross-protective Influenza vaccine, because CTLs are able to recognize the epitopes derived from conserved internal viral proteins. To this end, we designed immunogenic CTL epitope selection system and developed a CTL inducing influenza vaccine for human use.

Several epitope peptides from H5N1 Influenza A virus internal proteins were selected by CTL epitope prediction programs. These peptides were further narrowed down in terms of the immunogenicity using in vivo cytotoxicity assay. To evaluate protective efficacy of each CTL peptide, HLA-A*2402 transgenic (A24Tg) mice, in which human CTL immune system have been reconstituted, were immunized with CTL peptides, then challenged with several Influenza A virus subtypes. After virus infection, the survival rate and the body weight were daily monitored. In addition, lung virus titers of immunized mice were also compared.

A24Tg mice immunized with the mixture of CTL peptides survived after H5N1 Influenza A virus infection. The body weight loss of intra-nasally immunized mice was not observed after virus challenge. Lung virus titers at infection day 5 of immunized groups were significantly lower than those of unimmunized groups. , Furthermore, this peptide vaccine was also effective for another two influenza A virus subtypes, H1N1 and H3N2. We have demonstrated that the CTL peptide vaccine elicit a cross-protective immunity against Influenza A virus infection. These results provide the basis of CTL-inducing Influenza vaccine development for human use.

Joon-seok Chae

Associate Professor
Veterinary Internal Medicine
College of Veterinary Medicine
Seoul National University
599 Gwanangno, Kwanak-gu, Seoul 151-742
Republic of Korea
Phone/Fax: +82-2-880-1279/1213

ACADEMIC DEGREES:

DVM	1987	Chonbuk National University (College of Veterinary Medicine), Korea
MS	1990	Chonbuk National University (Department of Veterinary Medicine, Graduate School), Korea
PhD	1994	Chonbuk National University (Department of Veterinary Medicine, Graduate School), Korea

PROFESSIONAL APPOINTMENTS:

1992. 10. ~ 1992. 11.	Visiting Research Scientist, Hokkaido University, Japan
1995. 6. ~ 1997. 12.	Post-Doc, College of Veterinary Medicine, Texas A&M University
1998. 1. ~ 2000. 2.	Post-Doc, School of Veterinary Medicine, University of California-Davis
2000. 3. ~ 2006. 12.	Associate Professor, College of Veterinary Medicine, Chonbuk National University
2000. 6. ~ 2003. 2.	Director/Co-president, Mol-BioNet Research Institute/Scientec Lab Center Co., Ltd
2002.7. ~ 2004. 6.	Director, VMTH/CVM, Chonbuk National University
2006. 1. ~ 2006. 12.	Visiting Professor, School of Veterinary Medicine, University of California-Davis
2006. 12. ~ Present	Associate Professor, College of Veterinary Medicine, Seoul National University

RESEARCH INTERESTS:

- Climate change and arthropod-borne diseases
- Equine stem cell research and therapy
- Equine respiratory diseases

New genetic variants of *Anaplasma phagocytophilum* and *Anaplasma bovis* from Korean water deer (*Hydropotes inermis argyropus*)

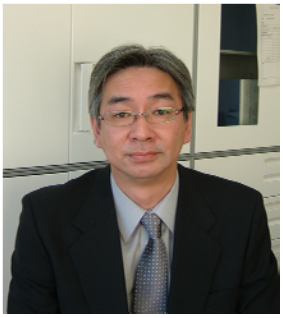
Jun-gu Kang[1], Sungjin Ko[1], Young-Jun Kim[2], Hyo-Jin Yang[2], Hang Lee[2], Nam-shik shin[3], Kyoung–seong Choi[4], Joon-seok Chae[1*]

[1]Laboratory of Veterinary Internal Medicine, [2]Conservation Genome Resource Bank for Korean Wildlife, [3]Laboratory of Zoo and Wildlife Medicine, Research Institute and BK21 Program for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea. [4]Department of Animal Science, College of Ecology and Environmental Science, Kyungpook National University, Sangju 742-711, Korea.

E-mal: jschae@snu.ac.kr

Wild deer are one of the important natural reservoir hosts of *Anaplasma* and *Ehrlichia* species which cause granulocytic anaplasmosis in equines, canines, and humans. The objective of the present study was to determine whether and what species of *Anaplasma* and *Ehrlichia* naturally infect Korean water deer (KWD) in the Republic of Korea (ROK). A total of 66 spleens from KWD carcasses were collected by the Conservation Genome Resource Bank for Korean Wildlife (CGRB) in Korea between March 2008 and May 2009. Polymerase chain reaction (PCR) was performed using 16S ribosomal RNA, with *ank*A, *gro*EL, and *msp*2 gene primers to amplify the genes of *Anaplasma* and *Ehrlichia*. Using 16S rRNA-based nested PCR, *Anaplasma phagocytophilum* and *Anaplasma bovis* were detected in 42 (63.6%) and 23 (34.8%) of 66 KWD spleens, respectively. The 42 *A. phagocytophilum* were classified into five genotypes and the 23 *A. bovis* were classified into two genotypes by sequence analysis. By *ank*A, *gro*EL and *msp*2-based nested PCR, *A. phagocytophilum* was detected in 1 (1.5%), 7 (10.6%), and 3 (4.6%) of 66 samples, respectively. These gene sequences had only one genotype. Five out of seven obtained 16S rRNA gene sequences have never been identified. The *ank*A, *gro*EL, and *msp*2 obtained gene sequences represented new genotypes. This is the first report of *A. phagocytophilum* and *A. bovis* in KWD, suggesting that they may act as reservoirs for anaplasmosis zoonotic pathogens.

Kazuhiko Ohashi



Professor
Laboratory of Infectious Diseases
Department of Disease Control
Graduate School of Veterinary Medicine
Hokkaido University

ACADEMIC DEGREES:

D.V.M. 1983 Hokkaido University (Veterinary Medicine)
M.S. 1985 Hokkaido University (Veterinary Medicine)
Ph.D. 1993 Cornell University (Veterinary Medicine)

PROFESSIONAL APPOINTMENTS:

1985 - 1989 Research fellow, Central, Research Labs., Sanraku Incorporated, Japan
1993 - 2000 Assistant professor, Graduate School of Veterinary Medicine, Hokkaido University.
2001 - 2008 Associate professor, Graduate School of Veterinary Medicine, Hokkaido University.
2008 - Professor, Graduate School of Veterinary Medicine, Hokkaido University.

RESEARCH INTERESTS:

- molecular mechanism of viral pathogenesis
- molecular mechanism of viral oncogenesis
- immune responses to infections in animals (chickens, cattle)

Studies on the molecular mechanisms for Marek's disease virus to increase its virulence in the field

Ohashi, K., Murata, S., Konnai, S.
Laboratory of Infectious Diseases, Department of Disease Control,
Graduate School of Veterinary Medicine, Hokkaido University
E-mal: okazu@vetmed.hokudai.ac.jp

Marek's disease virus (MDV), the etiological agent of Marek's disease (MD), causes malignant lymphoma in chickens. Currently, MD is effectively controlled by the vaccination with nonpathogenic strains of MDV. However, neither molecular mechanism of the pathogenesis of MDV nor the protection mechanism of MD vaccines has been clarified. In addition, recent field MDV isolates tend to increase their virulence, and the risk of future outbreaks is pointed out. Besides, survey of MDV in the wild goose populations showed that highly virulent MDV is widespread in white-fronted geese in Japan and Far East region of Russia. Thus, the characterization of recent highly virulent MDV isolates in chickens and wild waterfowls will be necessary to understand the molecular basis of the increase in virulence of MDV, and to develop the new methods to control MD in the field.

The Meq protein, present only in pathogenic MDV strains, plays an important role as a transcription factor in the MDV oncogenesis, and the diversity or insertion in Meq has been reported among a variety of MDV strains. Sequence analysis showed that distinct diversity and point mutations are present in Meq of highly virulent strains from chickens and wild birds, whereas L-Meq, containing a 60-amino-acid insertion in Meq, is identified in low virulent strains. Further analyses showed that both transactivation and transformation activities of Meq were altered when mutations were introduced into the transactivation domain, whereas those of L-Meq was significantly lower. Since L-Meq is found only in "old" low virulent MDV strains, such as CVI988, and Meq is usually found in "recent" highly virulent MDV strains, the transition from L-Meq to Meq might be one of the mechanisms for MDV to increase its virulence.

Han Sang Yoo

Professor
Department of Infectious Diseases
College of Veterinary Medicine
Seoul National University
Seoul 151-742, Korea
Tel: +82-2-880-1263
Fax:+82-2-874-2738

ACADEMIC DEGREES:

DVM	1982	Seoul National University (Veterinary Medicine)
MS	1984	Seoul National University (Veterinary Microbiology)
Ph.D.	1995	University of Minnesota, USA (Veterinary Microbiology)

PROFESSIONAL APPOINTMENTS:

1997 - 1999	Lecturer, College of Veterinary Medicine, Seoul National University
1999 - 2003	Assistant Professor, College of Veterinary Medicine, Seoul National University
2003 -2008	Associate Professor, College of Veterinary Medicine, Seoul National University
2008-Present	Professor, College of Veterinary Medicine, Seoul National University
1999 - 2001	Secretary for Academic Affairs and Editor-in-Chief The Korean Society for Veterinary Sciences and Korean J. of Veterinary Research
1996 - 2007	Secretary for Academic Affairs and Editor-in-Chief The Korean Association for Buiatrics and Korean J. for Buiatrics
2003 -Present	Associate Editor, Journal of Veterinary Science
2005 -2006	Visiting Associate Professor, School of Veterinary Medicine University of Wisconsin-Madison, Madison, WI, USA
2009 -2012	Adjunct Professor, Department of Pathobiological Sciences School of Veterinary Medicine, University of Wisconsin-Madison, WI, USA
2009-Present	Adjunct Assistant Professor, Department of Veterinary Population Medicine College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA Vice President of The Korean Society for Veterinary Sciences.

RESEARCH INTERESTS:

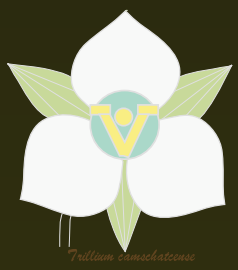
- Development of needle-free vaccines against porcine pleuropneumoniae and atrophic rhinitis in swine
- Studiens on Zoonotic Diseases ; Bovine brucellosis, prion diseases, swine Hepatitis E
- Studiens on the Animal Health ; seroprevalence, antimicrobial resistance, etc.

Investigation of Prion Disease Neuropathogenesis using Stable Knockdown Neuroblastoma Cells Established by Chained MicroRNA-mediated RNA Interference

Roh, YM., Kang SG, Yoo HS.

Department of Infectious Diseases, College of Veterinary Medicine, BK21 for Veterinary Science, Seoul National University, Seoul 151-742, Korea
E-mal: yoohs@snu.ac.kr

Prion diseases are fatal transmissible neurodegenerative disorders. In the pathogenesis of the disease, the cellular prion protein (PrP^{c}) is required for replication of abnormal prion (PrP^{Sc}), which results in accumulation of PrP^{Sc} and expense of PrP^{c} in the brain. Although there have been extensive studies using prion protein gene (*Prnp*) knockout system, the normal function of PrP^{c} and neuropathogenesis still remain ambiguous. Compared with conventional germline knockout technologies or transient naked siRNA-dependent knockdown system, newly constructed durable chained primary miRNA could provide the prion disease cell culture model. The selective silencing of a target gene by RNA interference (RNAi) is a powerful approach to investigate the unknown function of genes *in vitro* and *in vivo*. In the dual targeting primary microRNA encoding cassette (pri-miRdual), the pri-miRdual, which targets N- and C- termini of *Prnp* simultaneously, more effectively suppressed prion expression compared with conventional single site targeting method. Furthermore, gene expression analysis of PrP^{c} interacting or associating genes was done with newly established PrP^{c} ablated cells. Among selected genes, *670460F02Rik* and *Plk3*, *Ppp2r2b* and *Csnk2a1* were up-regulated in the present study. These genes have been reported to be involved in cell proliferation and mitochondria mediated apoptosis. *Ppp2r2b* enconding the regulatory subunit of proapoptotic phosphatase PP2A has been reported to predispose neuronal cells to apoptosis by driving Drp1-dependent mitochondrial fragmentation. In regard to PrP^{c} depletion, a possible pathogenic mechanism of prion disease was speculated from remarkably up-regulated *Ppp2r2b*. Based on this hypothesis, the major protein in mitochondrial fission, Drp1, was increased at 24h after apoptosis induction in N2amiRdual followed by up-regulated cytosomal cytochrome c which indicates mitochondria dysfunction. Caspase 3 activity also 11.8 fold increased in N2amiRdual compared to control cells. The proportion of apoptotic cells bound to Annexin V was increased by 211% at 24h after apoptotic stimulation in N2amiRdual whereas most of cells showed the resistance against apoptosis stimulation in pri-miRNA non-treated cells. Altogether, prion knockdown neuronal cell line achieved by dual targeting approach with pri-miRdual against *Prnp* will be useful for dissecting the PrP^{c} physiological function as well as neuropathogenesis. It could be inferred by analysis of altered gene expression and selected gene involved signaling pathway following *Prnp* knockdown. In addition, this newly established *Prnp* specific pri-miRdual provides a potential therapeutic strategies for prion disease in the future.



Graduate School of Veterinary Medicine, Hokkaido University
http://www.vetmed.hokudai.ac.jp