2015
HIROSAKI UNIVERSITY RESEARCH HIGHLIGHTS

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HIROSAKI
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RESEARCH HIGHLIGHTS 1

Development of a novel preventive strategy against neurodegenerative diseases through generation of innovative anti-aging methods

Purpose and Background of the Research

With the increasing number of elderly people in Japan, age-related diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) are also increasing, and we are facing a serious social problem of expanding health care costs. In age-related diseases, the balance between the generation of stress (such as oxidative stress and protein misfolding stress) and stress protective mechanisms has shifted toward stress generation, leading to cell degeneration and death. In this grant program, we will clarify the mechanisms of stress generation mechanisms by environmental or genetic causes through multidisciplinary collaboration. Furthermore, we aim to prevent age-related diseases especially by fortifying the stress-responsive mechanism by natural and/or synthetic chemicals to adjust this imbalance.

Research Results

- Oxidative stress plays a major role in age-related diseases. Nrf2 is a master transcription factor that regulates the oxidative stress response in mammals. We found that another stress-responsive transcription factor, ATF4, cooperates with Nrf2 to activate a set of antioxidant genes such as the cysteine transporter xCT and heme oxygenase 1 (HO-1). We also found that the HO-1 is regulated by Nrf2-depenent mechanisms involving Z-DNA formation and enhancer RNA generation that are specific to HO-1.
- Mitochondria play an important role in age-related diseases. We developed a peptide inhibitor against mitochondrial calpain and demonstrated that it prevented neurodegeneration in the rat model of retinitis pigmentosa. Furthermore, we demonstrated that the peptide inhibitor suppressed excitotoxicity-induced apoptosis in human neuroblastoma HT22 cells.
- Amyloid β (Aβ) generation is enhanced in Alzheimer's. We found that carnosic acid from the herb rosemary suppressed Aβ generation in neurons and astrocytes, and that it also prevented Aβ-induced apoptosis.
- Protein aggregation plays a key role in neurodegenerative disorders. We found that activation of autophagy by trehalose suppresses Lewy body formation in culture cells and that it activates autophagy in the mouse model of Lewy body disease.

Future Prospects

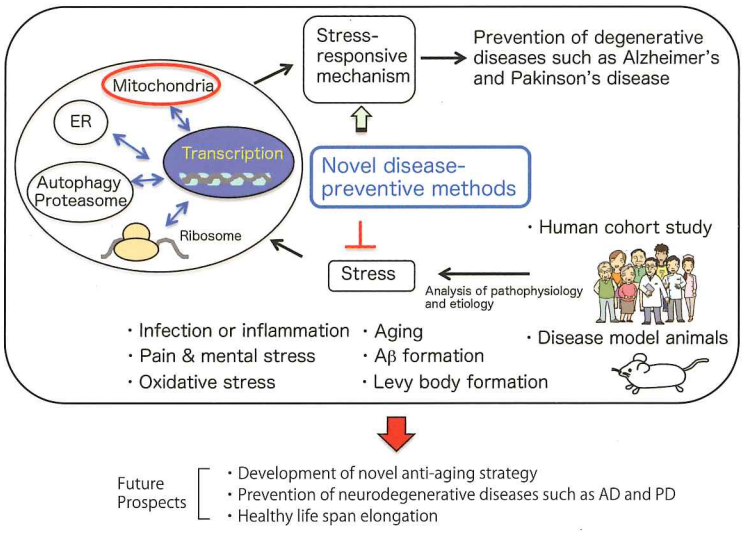
Under strong industry-government-academia partnership, Hirosaki COI (Center of Innovation) is establishing an innovative strategy for predicting neuronal diseases using the big health data collected from cohort research conducted in Aomori prefecture. In collaboration with Hirosaki COI, this project will develop preventive strategies based on the disease prediction.

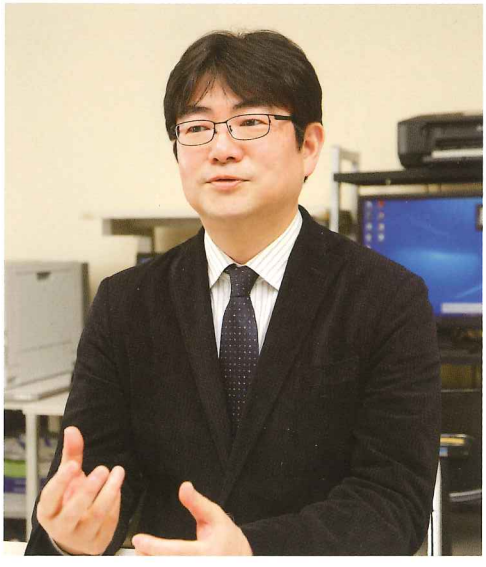
Funding

1. Hirosaki University Institutional Research Grant FY2013-2015 25,500,000 Yen
2. JSPS/MEXT COI program, FY2013-2015 600,000,000 Yen
3. JSPS/MEXT KAKENHI Grant Number 26111010, FY2014-2018 80,000,000 Yen

Selected Papers

1. Ye P. et al. Nrf2- and ATF4-dependent upregulation of xCT modulates the sensitivity of T24 bladder carcinoma cells to proteasome inhibition. Mol Cell Biol 2014.
2. Ozaki T. et al. Delivery of Topically Applied Calpain Inhibitory Peptide to the Posterior Segment of the Rat Eye. PLoS ONE 2015.





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Multistep leukemogenesis in Down Syndrome

Purpose and Background of the Research

Trisomy 21, the genetic hallmark of Down Syndrome (DS), is the most frequent human chromosomal abnormality. In children with DS, there is an approximately 20-fold higher incidence of leukemia than in unaffected children. The majority of leukemia cases associated with DS in early infancy are acute megakaryocytic leukemia (AMKL), and its incidence is approximately 500-fold higher than in non-DS children. Interestingly, about 5% to 10% of DS neonates develop transient abnormal myelopoiesis (TAM), which is characterized by unrestricted proliferation of megakaryocytic progenitors. AMKL develops in approximately 20% to 30% of the cases with TAM within the first 4 years of life. Almost all cases of these disorders harbor mutations in the gene encoding the GATA1 transcription factor, which is essential for erythropoiesis and megakaryocytic differentiation. Human tumors have been shown to progress through the accumulation of genetic abnormalities. The malignant transition from TAM to AMKL makes AMKL associated with DS is an excellent model of cancer pathogenesis. This project aims to understand the multistep mechanism of leukemogenesis in DS.

Research Results

Whole exome sequencing of 15 TAM and 14 DS-AMKL, whole genome sequencing of 4 paired TAM/DS-AMKL samples and follow-up targeted sequencing 41 TAM, 49 DS-AMKL and 19 non-DS-AMKL cases revealed new aspects of the pathogenesis of DS-related myeloid proliferation. First, the initial TAM phase was characterized by a paucity of somatic mutations. The mean number of non-silent mutations per sample was surprisingly small compared with that reported in other human cancers. Excluding common *GATA1* mutations, no other recurrent mutations were identified with only 0.7 non-silent mutations per case, indicating that TAM could be caused by a single acquired *GATA1* mutation in addition to constitutive trisomy 21. Second, subsequent AMKL evolves from a TAM clone acquiring additional mutations, where mutations of cohesin (53%), such as *RAD21* and *STAG2*, *CTCF* (20%), epigenetic regulators including *EZH2* and signaling pathways play a major role (Figure 1). Strikingly, all mutations/deletions in different cohesin components were completely mutually exclusive, suggesting that the cohesin function was the common target of these mutations (Yoshida et al, **Nature Genetics** 2013).

Future Prospects

Cohesin, CTCF, and other epigenetic regulators, such as EZH2 regulate various epigenetic phenomena including DNA methylation. We would like to understand the impact of these epigenetic phenomena on DS-AMKL development.

Funding

1. JSPS KAKENHI (2011-2013), GRANT NUMBER 23118501, (19,370,000 Yen)
2. JSPS KAKENHI (2011-2012), GRANT NUMBER 23118501, (13,650,000 Yen)
3. AMED-CREST (2012-2016), GRANT NUMBER 122734, (17,500,000 Yen)
4. JSPS KAKENHI (2015-2016) GRANT NUMBER 20168339, (3,640,000 Yen)
5. JSPS KAKENHI (2014-2017) GRANT NUMBER 26253061, (41,080,000 Yen)

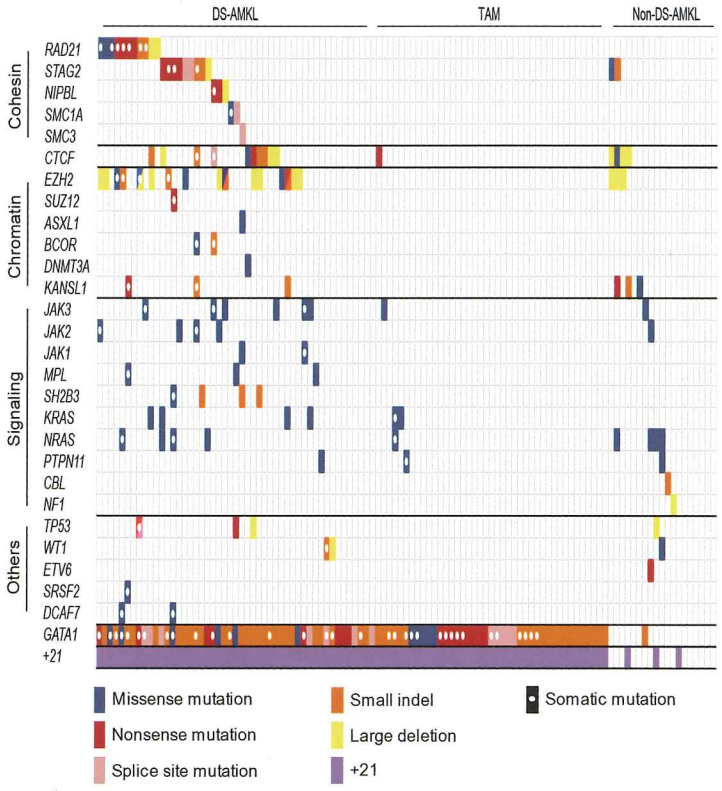

Figure 1. The landscape of somatic mutations in Down Syndrome-related myeloid disorders.

PROFILE

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Study on functions of staphylococcal superantigenic toxins and development of vaccines against *Staphylococcus aureus* infections

Purpose and Background of the Research

Staphylococcus aureus, especially MRSA (methicillin-resistant *S. aureus*), is a leading cause of human diseases in the hospital setting as well as in the community. The diseases by this bacterium range from superficial skin infections to more life-threating diseases. *S. aureus* produces a variety of exotoxins, including staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1). These toxins are superantigens, which have the ability to stimulate a large repertoire of the Vβ elements of T cell receptors. SEs are also emetic toxins causing staphylococcal foodborne poisoning.

We have explored the molecular functions and pathogenicity of staphylococcal superantigens as well as developed a vaccine against *S. aureus* infection (Figure 1)

Research Results

1. **Development of superantigen-based vaccines against MRSA infectious diseases**

   As most of clinical isolates from hospital-acquired MRSA infections harbor the TSST-1 encoding gene, we developed a vaccine from superantigenic-deficient mutant of the toxin (mTSST-1). We demonstrated that mTSST-1 vaccination provides interleukin 17A-dependent host defense against *S. aureus* and prevents mice from systemic MRSA infection (Pathog Dis 73(4):ffv023, 2015).

2. **Regulation of autophagy by TSST-1**

   We demonstrated that TSST-1 suppresses autophagosomal accumulation in human epithelial cells, independent of superantigenic activity. Although *S. aureus* is an extracellular bacterium, it can grow intracellularly in epithelial cells. Therefore, it is possible that TSST-1 regulates *S. aureus* infection in epithelial cells through autophagic suppression (PLoS ONE 9(11):e113018, 2014).

3. **Mechanism of staphylococcal enterotoxin-induced emesis**

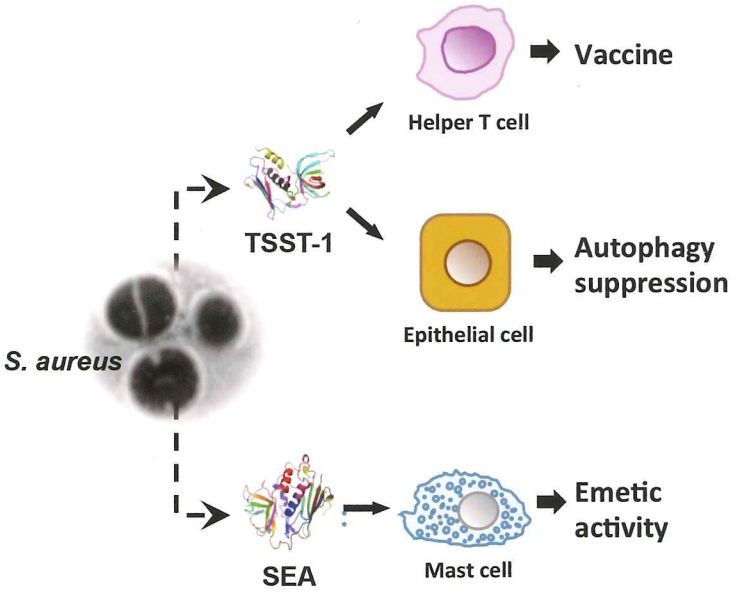
   We established the house musk shrew *Suncus murinus* as a suitable animal model for emetic assay of SEs. In this model, we demonstrated that orally-ingested SEA stimulates serotonin secretion from mast cells in the gastrointestinal tract, resulting in depolarization of enteric nerves. Thus, mast cells and abdominal vagal afferent nerves are the major pathways for SEA-induced emesis (Eur J Pharmacol 722(1):95-107, 2014, Appl Environ Microbiol 81 (20):7034-40, 2015).

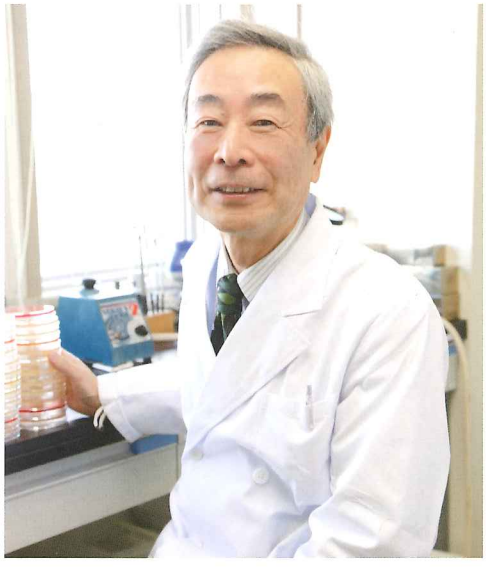
Future Prospects

S. aureus causes life-threating diseases in immunocompromised hosts, while the bacterium colonizes the nasopharyngeal cavity and the skin as a commensal microorganism in healthy persons. Elucidation of a mechanism for persistent infection of *S. aureus* in hosts is a critical point for the eradication of MRSA infectious diseases. Alternatively, staphylococcal superantigens exert various effects on host cells. Therefore, the goal of our study is the prevention of MRSA infectious diseases by clarifying the mechanism of persistent infection through staphylococcal superantigens.

Funding

1. JSPS KAKENHI Grant number 25670207, FY2013-2015, 2,800,000 Yen.
2. JSPS KAKENHI Grant number 23390100, FY2011-2014, 15,000,000 Yen.
3. JSPS KAKENHI Grant number 23659217, FY2011-2012, 2,800,000 Yen.





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Research on the potential of living organisms to adapt to a new environment

Purpose and Background of the Research

What are the differences between artificial products of a network consisting of many elements, *i.e.*, a computer, and living organisms that have a genetic network consisting of many elements, *e.g.*, genes, enzymes, and metabolites? One of the differences is that living organisms have the potential to adapt to a new environment. Determining the mechanism underlying adaptation to a new environment contributes not only to obtaining crucial insights into evolutionary biology but also to providing an approach for establishing a continuously developing society.

To elucidate this mechanism, we have used experimental evolution systems with bacteria and bacteriophages (*i.e.*, viruses that infect bacteria). Because of the short generation time and small genome size of these organisms, we can trace and analyze the adaptation processes over many generations in short periods and can understand the relationships between genetic and phenotypic changes more easily than would be possible for eukaryotes. In addition, replicates of evolution, which is an adaptation process involving genetic changes, are available simultaneously.

Research Results

1. Coevolution with *Escherichia coli* and RNA bacteriophage Qβ
   Copropagation experiments with *E. coli* and the lytic RNA bacteriophage Qβ in a spatially unstructured environment were conducted. Whole genome analysis of *E.coli* and Qβ revealed accelerated molecular evolution in comparison with their sole propagation. Fitness analysis showed that they were in an arms race (Figure 1). *PLoS Genetics* vol.7, e1002188, (2011), *Frontiers in Microbiology*, vol.6, 124, (2015).
2. Thermal adaptation of RNA bacteriophage Qβ
   We performed a thermal adaptation experiment of Qβ where the culture temperature was increased from 37.2°C to an inhibitory temperature of 43.6°C in a stepwise manner (Figure 2). The phage became able to grow at the inhibitory temperature after 2 months. Whole-genome and fitness analyses clearly showed that silent mutations play important roles in the adaptation of Qβ to inhibitory environments. *Journal of Virology*., vol.88,11459, (2014).

Future Prospects

In nature, some living organisms coexist with other organisms in a symbiotic relationship, which might originally have been a host-parasite interaction. We would like to elucidate the mechanism underlying the process by which host-parasite interactions can change to symbiotic interactions by using an experimental ecosystem consisting of *E. coli* and Qβ.

Funding

1. Hirosaki University Institutional Research Grant for Young Scientists FY2014-2015, 6,000 Thousand Yen
2. JSPS KAKENHI Grant Number 26440194, (2014-2016), 5,070,000 Yen
3. JSPS KAKENHI Grant Number 23570268, (2011-2013), 5,460,000 Yen
4. JSPS KAKENHI Grant Number 21770255, (2009-2010), 4,290,000 Yen

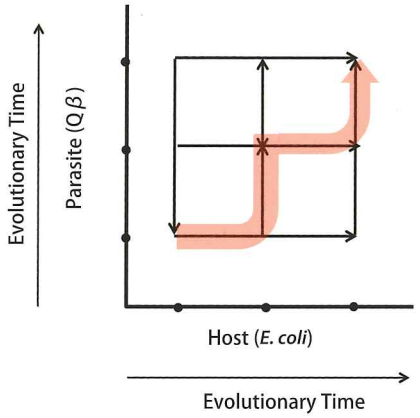

Figure 1. **Estimation of evolutionary path in our coevolution experiment.**
The direction of the arrowhead on each arrow indicates higher fitness. The red line indicates the plausible route for fixing.

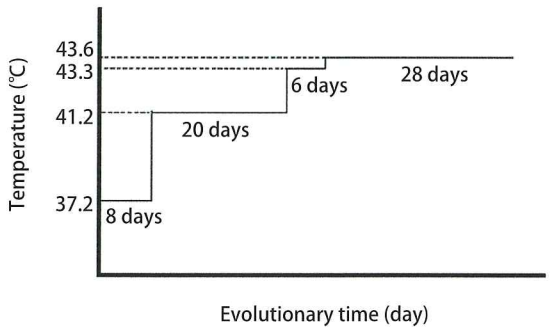

Figure 2. **Culture conditions in the evolution experiment**
Thermal adaptation evolution experiments with Qβ were carried out at 37.2, 41.2, 43.3, and 43.6°C.

PROFILE

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Interdisciplinary study on environmental transfer of radionuclides from the Fukushima Daiichi Nuclear Power Plant accident: Fate of radionuclides in the ocean.

Purpose and Background of the Research

A magnitude 9.0 earthquake and subsequent tsunami caused severe damage to the Fukushima Daiichi Nuclear Power Plant on 11 March 2011. This accident has resulted in a substantial release of radioactive materials into the atmosphere and ocean. The project of Interdisciplinary Study on Environmental Transfer of Radionuclides from the Fukushima Daiichi Nuclear Power Plant Accident (ISET-R) has been adopted as a Grant-in-Aid for Scientific Research on Innovative Areas, the Ministry of Education, Culture, Sports, Science, and Technology, Japan 2012. I am one of the Principal Investigators of eight multidisciplinary research groups of ISET-R. The objectives of my group are (1) to measure the spatial and vertical distributions of radionuclides concentrations (^{134}Cs, ^{137}Cs, ^{90}Sr, ^{129}I, Pu isotopes, ^{3}H) in seawaters from the Fukushima coast and North Pacific Ocean and trace temporal changes of radionuclides inventories in the water columns; (2) to measure the spatial and vertical distributions of ^{134}Cs, ^{137}Cs and Pu isotopes in marine sediments from the Fukushima coast; (3) to collect settling particles by using the time-series sediment traps and discuss the form and particle-scavenging process of the radiocesium ; and (4) to discuss the mechanisms controlling the environmental dynamics of radionuclides in the ocean.

Research Results

There have been a number of academic achievements: 30 original articles in 2012, 30 original articles in 2013, 15 original articles in 2014. Some of the major research results are cited below:

(1)High-density observations of ^{134}Cs and ^{137}Cs in surface seawater in the North Pacific were carried out by research vessels and cargo ships from March 2011 to March 2012. The main body of the radioactively-contaminated plume traveled along the 40° N meridian and reached the International Date Line on March 2012.
Aoyama et al., Biogeosciences, 10, 3067-3078 (2013)

(2)Vertical distributions of ^{134}Cs and ^{137}Cs were measured at stations along the 149° E meridian in the western North Pacific. The atmospheric-deposited radiocesium had been transported not only eastward along with surface currents but also southward due to formation/subduction of subtropical mode waters.
Kumamoto et al., Scientific Reports, 4:4276 (2014)

(3)The concentrations of $^{239+240}$Pu and ^{241}Pu in seawater and marine sediment were low compared with the background level before the accident. The release of Pu isotopes from the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident to the marine environment was negligible.
Bu et al., Environmental Science and Technology, 48, 9070-9078 (2014); Journal of Chromatography A, 1337, 171-178 (2014)

(4)The time-series sediment traps were collecting settling particles when the FDNPP accident occurred on March 2011. Sediment trap data revealed that FDNPP-derived radiocesium was quickly transported to the deep sea in the western North Pacific. The estimated sinking velocity of most particulate ^{137}Cs was about 50 m day^{-1} and most of the ^{137}Cs was likely incorporated into aluminosilicates.
Honda et al., Biogeosciences, 10, 3525-3534 (2013); Geophysical Research Letters, 41, 3959-3965 (2014)

(5)The FDNPP accident resulted in the release of radioactive materials to the ocean by two major pathways: direct release from the accident site and atmospheric deposition. The total amounts of directly released ^{134}Cs, ^{137}Cs and ^{131}I were estimated for 1 year after the accident to be 3.5 ± 0.7 PBq, 3.6 ± 0.7 PBq, and 11,1 ± 2.2 PBq, respectively.
Tsumune et al., Biogeosciences, 10, 5601-5617 (2013)

Future Prospects

Contributions from my group provided a solid scientific basis for understanding radioactive contamination and the processes controlling radionuclide transport in the ocean. Further studies are needed to predict long-term radionuclide migration in the ocean and the convergence of the Fukushima impacts through field monitoring and model simulation.

Funding

1. MEXT/JSPS, Grant-in-Aid for Scientific Research on Innovative Areas, Grant Number 24110004, FY2012-2016 (140,270 Thousand Yen)
2. JSPS, Grant-in-Aid for Scientific Research, Grant Number 24310002, FY2012-2014 (17,680 Thousand Yen)

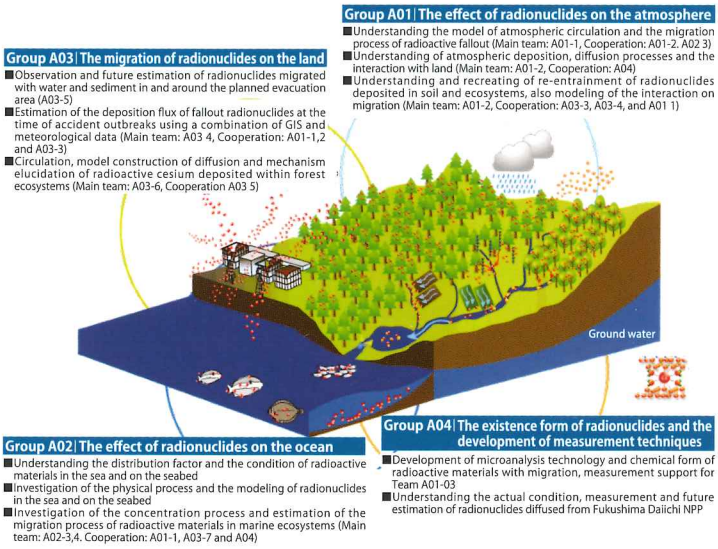
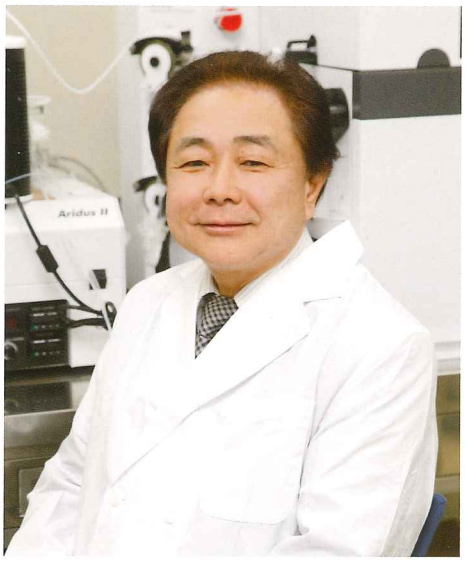


Fig.1 Research outline of ISET-R
(http://ied.tsukuba.ac.jp/hydrogeo/isetr/ISETRen/researchEN.html)



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