2014
HIROSAKI UNIVERSITY RESEARCH HIGHLIGHTS

Establishing a Global Identity
Creating with the Community

HIROSAKI UNIVERSITY

"The fluorescent L-Glucose (fLG), a Novel Probe for Cancer Detection"

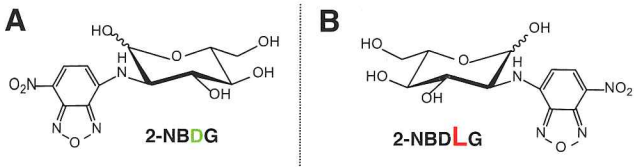
Purpose and Background of the Research

Our purpose is to develop a method to discriminate living cancerous/pre-cancerous cells from other cells by evaluating the uptake of non-natural L-form glucose analogues bearing a fluorescent substituent.

Background: Carbohydrates are absorbed from the intestine mainly in the form of glucose. Glucose, the most important energy source for living things, is taken up into mammalian cells in a stereoselective manner through glucose transporters such as GLUTs, whereby only D- and not L-, glucose is recognized.

Cellular uptake of D-glucose through GLUTs can be visualized by 2-NBDG, [1] a widely used D-glucose analogue emitting green fluorescence (Fig. 1A).[2-6] To precisely evaluate the stereoselectivity of the uptake, we developed 2-NBDLG, the first fluorescent L-glucose analogue (fLG), initially as a control substrate for 2-NBDG (Fig. 1B). [7-10]

Fig. 1 2-NBDG and 2-NBDLG, fluorescent D-, and L-glucose analogue, respectively.



Research Results

Beyond our expectations, however, 2-NBDLG was taken up into tumor cells showing malignant phenotypes.[11] 2-NBDLG is a promising probe for imaging cancer at the cellular level with lower background uptake by non-cancerous tissue compared to cases where D-glucose analogues are used. We are currently conducting clinical studies, hoping to realize a functional discrimination of cancerous/pre-cancerous lesions from surrounding tissues by using fLGs.

Future Prospects

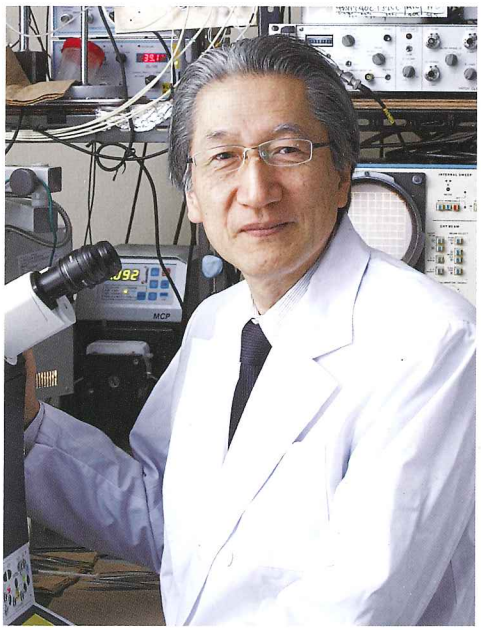
This method will facilitate accurate diagnosis and necessary and sufficient removal of lesions that have the potential of developing cancer, while sparing normal function as much as possible. Use of the method as a drug delivery system is another potential. We would like to proceed together with students and colleagues who share the belief that "freedom to pursue what 'you' think is important may open a new door to the future."

Funding

1. Hirosaki University Institutional Research Grant. FY2012-2014 12,750 Thousand Yen.
2. JST Collaborative Research Based on Industrial Demand. FY2012-2018 162,673 Thousand Yen.
3. JST A-STEP. FY2012-2018 300,000 Thousand Yen.
4. JST Intellectual Property Utilization Promotion Highway Support. FY2011 3,500 Thousand Yen.
5. Comprehensive Support Programs for Creation of Regional Innovation Science and Technology Incubation Program. FY2008-2010 78,000 Thousand Yen.
6. Research funds from Research Foundation for Opto-Science and Technology. FY2007-2008 4,000 Thousand Yen.

References

1) 2-[N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose
2) Yoshioka, et al., *Biochim. Biophys. Acta* 1289: 5 (1996).
3) Yamada, et al., *J. Biol. Chem.* 275: 22278 (2000).
4) Ohtsubo, et al., *Cell* 123: 1307 (2005).
5) Yamada, et al., *Nature Protocols* 2: 753 (2007).
6) Rouach, et al., *Science* 322: 1551 (2008).
7) 2-[N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-L-glucose
8) Yamamoto, et al., *Tetrahedron Lett.* 49: 6876 (2008).
9) Yamada, et al., WO2010/016587.
10) Yamamoto, et al., *Bioorg. Med. Chem. Lett.* 21: 4088 (2011).
11) Yamada, et al., WO2012/133688.



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Activation of Chemically Inert Small Organic Molecules on Semi-stabilized Transition-metal Reaction Sites

Purpose and Background of the Research

It is necessary to construct a sustainable social system that does not rely on fossil fuels in relation to resource problems and global warming issues. If carbon dioxide can be utilized as an energy resource without the consumption of further energy, the clue to solving energy problems and global warming at the same time can be provided. This project aims at the development of semi-stabilized transition-metal reaction sites, on which chemically inert carbon resources such as carbon monoxide, carbon dioxide, and nitriles would be converted into useful substances for human beings.

Research Results

1. Functionalization of carbon monoxide on the tetrairon core

Four carbon monoxide molecules were converted into two acetylene molecules on the tetrarion core through a reaction with $LiAlH_4$. Further treatment with *N*-bromosuccinimide (NBS) and primary amines led to the formation of isocyanides. The redox-responsive facile structural changes of the tetrairon core play a crucial role in these novel transformation reactions of carbon monoxide molecules (Figure 1).

2. Activation of nitriles through the cleavage of the carbon–nitrogen triple bonds

Thermal reaction of dinuclear and trinuclear ruthenium clusters with nitriles led to the unprecedented cleavage of the carbon–nitrogen triple bond of nitriles. The resulting carbyne–imido cluster has been unequivocally determined by X-ray diffraction analysis (Figure 2).

3. Activation of carbon dioxide by a three-membered metallacycle composed of titanium, silicon, and nitrogen atoms

Silanimines, based on the $R_2Si{=}NR$ structure, have attracted much attention since they represent heavier analogues of imines. Coordination of silanimines with the titanium center through σ-donation–π–back donation interaction resulted in the isolation of a three-membered metallacycle composed of titanium, silicon, and nitrogen atoms, the structure of which has been uniquely characterized by an X-ray diffraction study. At the metallacycle, carbon dioxide was smoothly converted to carbon monoxide at room temperature(Figure 3).

Future Prospects

In our country where natural resources are scarce, it is necessary to make molecular catalysts using base metals. However, iron and titanium are not efficient as catalysts. The methodology we have developed to handle the accumulation of iron and titanium will enable the construction of reaction sites with high catalytic activity, which can be applied to various reactions.

Funding

1. Hirosaki University Institution Research Grant FY2011–2013 11,400 Thousand Yen
2. JSPS Funding Program for Next Generation World Leading Researchers FY2010-2013 116 Million Yen
3. JSPS KAKENHI Grant Number 20350027 FY 2008-2010 13,200 Thousand Yen

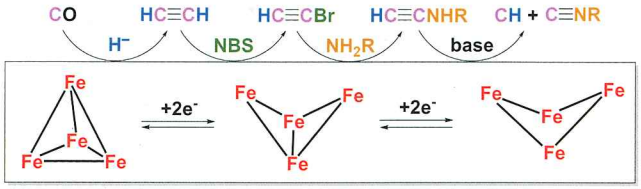

Figure 1. Transformation of CO at the redox-responsive tetrairon core

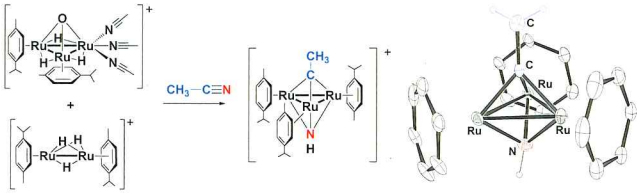

Figure 2. Activation of the C–N triple bond and X-ray structure analysis of the product

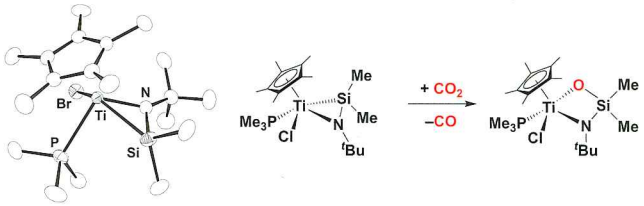

Figure 3. Crystal structure of the three-membered metallacycle and activation of carbon dioxide

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Theory on a method of searching novel matter, energy and space-time in our universe

Purpose and Background of the Research

Normal matter, including human bodies, consists of chemical elements in the known periodic table. Over the last few decades, however, astronomers and physicists have made the surprising discovery that the mass and energy in our universe are dominated by two unknown components. They are called "dark matter" and "dark energy," respectively. Remarkably, the atoms that we know well are a minor component of the universe (a small percentage in mass). In 2011, three scientists received the Nobel Prize in Physics for "the discovery of the accelerating expansion of the universe through observations of distant supernovae." Their findings strongly suggest the existence of a dark energy that is totally beyond the standard theory of physics.

At present, therefore, it is very urgent and important to understand the nature of dark matter and dark energy. Furthermore, it is quite fascinating to search for a third (and even fourth) unknown component in the universe. Moreover, recent progress in physics, especially string theories, suggests that the theory of general relativity that is the most established gravity theory, by Albert Einstein, might require some modification. Ideas for such modification are of major concern in modern physics.

Research Results

(1) It is usual to consider cosmological observations in testing gravity modification. However, our group has made a new proposal for a ground-based experimental test. The first proposal for "testing Chern-Simons modified gravity using a quantum interferometer" was published in the journal "Physical Review Letters" (Okawara, Yamada and Asada, 2012).

(2) According to the theory of general relativity, gravity is described as a curved space-time. In this theory, non-trivial topology is possible. For instance, a "wormhole" is a kind of a space-time tunnel that has been intensively studied by theoretical physicists and mathematicians. Our group has shown theoretically that wormholes are distinguishable from normal celestial objects such as stars using an astronomical method called astrometry (Toki et al. 2011). In addition, we have corrected a formula (described in an earlier work) for the deflection angle of light in a wormhole space-time (Nakajima and Asada, 2012).

(3) By using big data called the Sloan Digital Sky Survey (SDSS), we have put the first cosmological limit on such exotic celestial objects (Takahashi and Asada, 2013).

Future Prospects

We are now performing further studies testing parity violation in gravity including a Chern-Simons modified gravity model. Furthermore, we are investigating gravitational lensing as a tool to search for a novel space-time structure. I sincerely hope our group maintains an attitude of constantly taking on new challenges in research.

Funding

1. JSPS Grant-in-Aids for Scientific Research, FY2014-FY2017, Grant Number 26400262 (2,800 Thousand Yen)

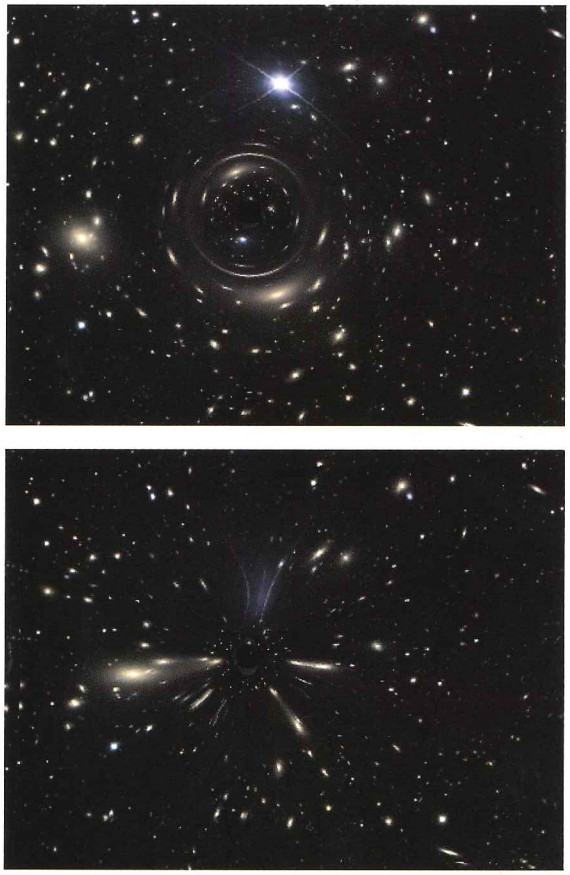
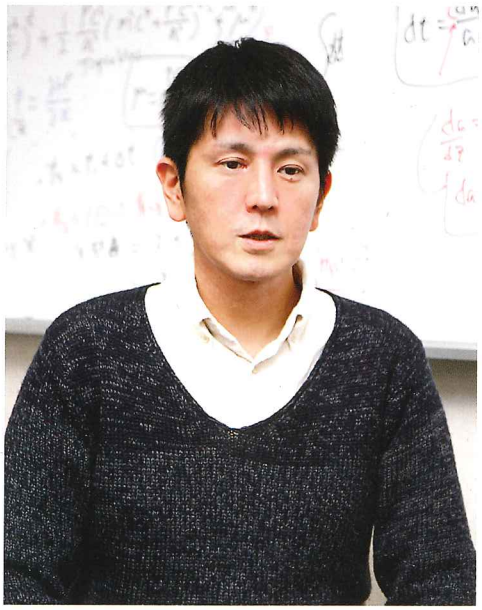


Figure Our numerical simulations for distorted images by a "non-standard object" (Izumi and Asada).



PROFILE

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Creation and Functional Development of Fluorinated Polymeric Nanocomposites and Their Applications

Purpose and Background of the Research

Fluoroalkyl end-capped oligomers [R_F-(M)n-R_F; R_F = fluoroalkyl groups; M = radical polymerizable monomers] are attractive functional materials, because they exhibit various unique properties such as high solubility, surface active properties, biological activities and nanometer size-controlled molecular aggregates which cannot be achieved by the corresponding non-fluorinated, randomly or block-type fluoroalkylated polymers, and low-molecular weight fluorinated surfactants. Thus, from the developmental viewpoint of new fluorinated polymeric materials, it is of particular interest to develop fluoroalkyl end-capped oligomeric nanocomposites–encapsulated with a variety of guest molecules such as inorganic particles, organic dyes and fullerene. Here we demonstrate the creation and functional development of fluoroalkyl end-capped oligomeric (polymeric) nanocomposites–encapsulated with a variety of guest molecules, including their applications.

Research Results

A variety of fluoroalkyl end-capped oligomer/guest molecule nanocomposites are developed from the interaction of fluoroalkyl end-capped oligomeric aggregates with these guest molecules. In fact, fluoroalkyl end-capped oligomer/silica nanocomposites and /metal particle nanocomposites have been prepared under mild conditions. Fluorinated polymer/silica composites in general exhibit a clear weight loss characteristic corresponding to the contents of fluorinated polymers in the composites through the calcination process at around 800℃. This point is quite different from our present fluoroalkyl end-capped oligomer/silica nanocomposites, and some fluoroalkyl end-capped oligomers in the silica nanocomposite matrices can exhibit no weight loss behavior even after calcination at 800℃. Fluoroalkyl end-capped oligomers/gold, /titanium oxide, and /hydroxyapatite nanocomposites were found to exhibit not only a surface active characteristic imparted by the fluoroalkyl groups but also unique properties related to these inorganic nanoparticles (see ***Figure*** for the fluorinated gold nanocomposites).

Future Prospects

Our fluoroalkyl end-capped oligomeric nanocomposites–encapsulated with numerous guest molecules can exhibit not only the surface active characteristic imparted by the fluoroalkyl groups but also unique properties related to these guest molecules. Therefore, these fluorinated nanocomposites have a high potential as novel fluorinated functional materials including applications in a wide variety of fields.

Funding

1. JSPS KAKENHI (2009-2011), Grant Number 21550179 (4,940 Thousand Yen)
2. JST A-STEP (2010-2011), Grant Number AS2211149D (7,720 Thousand Yen)
3. JSPS KAKENHI (2012-2014), Grant Number 24550220 (5,590 Thousand Yen)

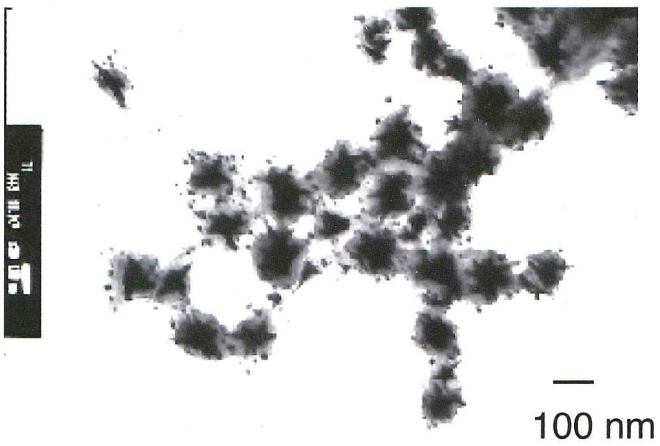

Figure TEM(transmission electron microscopy)images of fluoroalkyl end-capped oligomer/Au nanocomposites in methanol

PROFILE

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Comprehensive study for prevention and clarification of etiology for modern diseases at the Iwaki Health Promotion Project

Purpose and Background of the Research

The background of this research is based on the following two points.

- The life expectancy of Aomori prefecture has remained the shortest among all 47 prefectures for a long time.
- Modern diseases such as lifestyle-related diseases, dementia etc. are multifactorial, and closely related, in a complex manner, to our modern life.

To address these issues, we started the Iwaki Health Promotion Project in 2005, as a comprehensive study in the community. This is a field-based study that has multiple purposes, with the main aim being the promotion of health in the local community to prolong the lifespan of residents. This project, however, also acts as a platform where people from various backgrounds, such as students, local residents, medical researchers, local companies and health professionals take part in whatever they can do. The "big data" we gain from this project will be used to develop effective methods to prevent modern diseases. The biggest event of this project is a 10-day drop-in health check-up in Hirosaki City in Aomori Prefecture. Approximately 1,000 adults attend the health check-up each year (Figure 1).

Research Results

There were a number of academic achievements, through the support of the Hirosaki University Institutional Research Grant, between 2012 and 2014. Some of the major research accomplishments are listed below.

- Original articles – 35 articles in 2012, 40 articles in 2013 and 42 articles in 2014
- Number of international conference presentations – 6 in 2012, 6 in 2013 and 8 in 2014
- Number of domestic conference presentations – 55 in 2012, 49 in 2013 and 66 in 2014
- Number of academic meetings held – 1 in 2012, 1 in 2013 and 2 in 2014

One finding, based on big data, is shown in Figure 2

Future Prospects

The Iwaki Health Promotion Project is set to continue for 10 or more years. Therefore, findings will be broadened based on further analysis of big data in the future. The COI (Center Of Innovation) STREAM is a national project, launched by the Ministry of Education, Culture, Sports, Science and Technology in Japan. The Iwaki Health Promotion Project is a core activity of the Hirosaki COI Center, which is one of twelve main centers in Japan. Achievements based on this research should spread worldwide and contribute to human wellness.

Funding

1. Hirosaki University Institutional Research, FY 2013-2015. 35,000 Thousand Yen
2. JSPS, Grant-in-Aids for Scientific Research, Grant Number 20380045、FY2013-2014、3,900 Thousand Yen, Grant Number 24659311、FY 2013、1,170 Thousand Yen, Grant Number 23659342、FY 2012、1,170 Thousand Yen, Grant Number 22500610、FY 2012、910 Thousand Yen, Grant Number 22591873、FY2012、1,170 Thousand Yen, Grant Number 22500559、FY 2012-2014、1,950 Thousand Yen, and Grant Number 22249019、FY 2012-2014、12,090 Thousand Yen, Grant Number 21500676、FY2012-2013、2,470 Thousand Yen
3. COI (Center Of Innovation) STREAM, FY2013-2014、330,000 Thousand Yen

Figure 1. One of the drop-in health check-up days—part of the Iwaki Health Promotion Project.

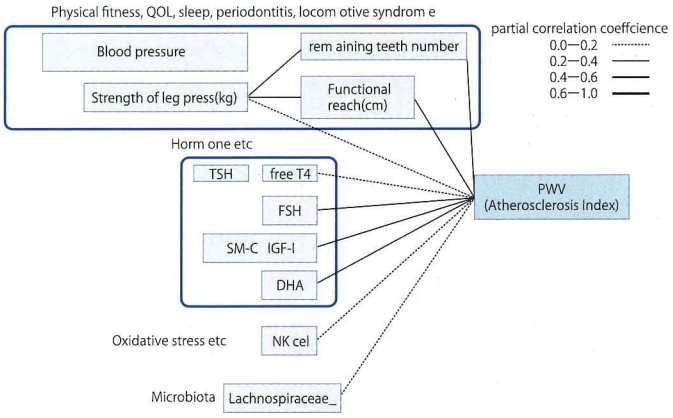

Figure 2. Correlation between atherosclerosis indices and other parameters from the big data obtained in the Iwaki Health Promotion Project

PROFILE

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Exosome research: Novel diagnostic and therapeutic tools for diseases

Purpose and Background of the Research

The aim of this study is to search disease- and stimulus-specific biomarkers in body fluids and analyze the biological function of exosomes. Recently, a number of microRNAs were found in extracellular spaces, and some of these were embedded in exosomes. Exosomes are extracellular vesicles, 40-200 nm in diameter, which are secreted from cells such as epithelial, endothelial, cancer, dendritic, and mesenchymal stem cells, which play various roles in cell-to-cell communications. Exosomes are present in biological fluids and are potential candidate biomarkers for disease. This study will help in the search for novel diagnostic markers and could clarify the biological roles of exosomes.

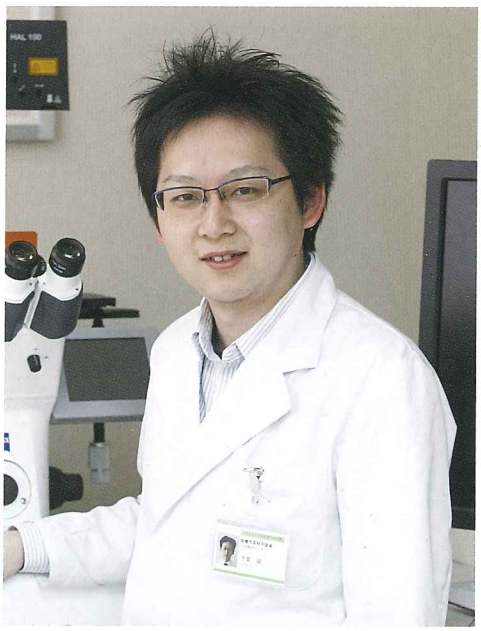
Research Results

This study has been mainly performed by young scientists of Hirosaki University including M. Chiba, N. Nanashima, A. Maruyama, R. Kanezaki, and S. Monzen. Some of the major research accomplishments are listed below:

1. The release mechanisms of microRNAs from culture cells. We proved that sphingomyelin phosphodiesterase 3 was involved in the secretion of microRNAs. *Oncol Rep.* 33 (1):67-73, (2015).
2. Identification of potential candidate biomarkers for colorectal cancer and breast cancer. We have been performing searches for diseases-specific microRNAs using human and animal sera.
3. Identification of up-regulated microRNAs in sera by exposure of ionizing radiation (IR). We searched biomarkers in sera and urine immediately after IR exposure. *Mol Clin Oncol.* in press.
4. The roles of exosomes derived from pancreatic cancer in tumor microenvironments. Exosomes derived from pancreatic cancer cells enhance angiogenesis and may be involved in cancer metastasis.
5. Stability of extracellular small RNAs against various conditions. Extracellular small RNAs in purified culture supernatants were stable for 4 weeks at room temperature, after 20 freeze-thaw cycles and exposure to pH 2.0, and were also resistant to ribonuclease A degradation. *Oncol Rep.* 33 (1):67-73, (2015).
6. Searching of exosomal unknown transcripts using a deep-sequencer. We identified a number of exosomal transcripts other than microRNAs.

Future Prospects

We hope that exosomes will be used as novel biomarkers and useful therapeutic tools for intractable diseases. Future studies are required to establish rapid detecting methods of exosomal biomarkers, to understand biological roles of exosomes in body, and to clarify the release mechanisms of exosomal RNAs into extracellular spaces.



Funding

1. Hirosaki University Institutional Research Grant for Young Scientists, FY2013-2014: 6,000 Thousand Yen.
2. JSPS KAKENHI Grant Number 25670264 FY2013-2014 3,770 Thousand Yen (M. Chiba)
3. JSPS KAKENHI Grant Number 25640058 FY2013 2,860 Thousand Yen (N. Nanashima)
4. JSPS KAKENHI Grant Number 25860202 FY2013-2014 4,160 Thousand Yen (A. Maruyama)
5. JSPS KAKENHI Grant Number 26461559 FY2013-2016 4,810 Thousand Yen (R. Kanezaki)
6. JSPS KAKENHI Grant Number 25861054 FY2013-2015 4,160 Thousand Yen (S. Monzen)

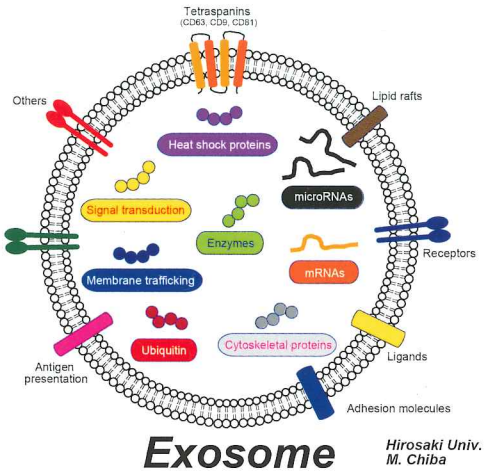


Figure 1. Schematic presentation of exosomes. Exosomes are composed of a lipid bilayer and contain some proteins and nucleic acids (mRNAs and microRNAs).

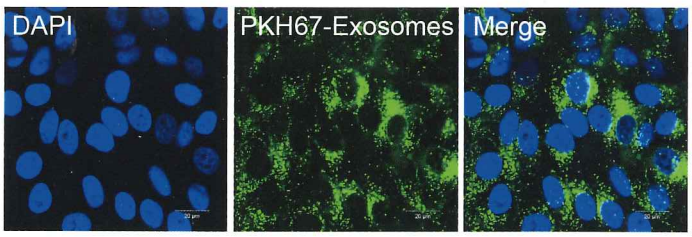


Figure 2. Uptake of exosomes labeled with fluorescence dye (PKH67) into cells. Green indicates exosomes uptaken into cells; blue indicates nucleus stained with DAPI.

PRO FILE

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About Hirosaki University

Principle

In the spirit of the Fundamental Law of Education, the aim of Hirosaki University is to acquire a wide store of knowledge for in-depth teaching of and research in the professional arts and sciences; to develop the intellectual, moral, practical, and applicable capacities of staff and students alike; and to produce future leaders supplied with insights sufficiently broad and well informed that they can help shape the future of human culture.

Goals

Hirosaki University is a medium-size university with Faculties of Humanities, Education, Medicine, Science & Technology, and Agriculture & Life Science. These five Faculties cover a broad and comprehensive range of undergraduate academic disciplines.
The university is also home to seven graduate programs, including the independent and interdisciplinary doctoral course in Regional Studies.
Hirosaki University has taken full advantage of its location in Aomori Prefecture to advance its educational and research goals. First of all, within Aomori Prefecture there is great potential for energy production and development, including the nuclear energy and nuclear fusion-related facilities located within its borders. The Shirakami Wilderness World Heritage Site, located along the border of Aomori and Akita prefectures, offers.

Educational Goals

Hirosaki University strives to provide a high level of education to future global and local leaders in a wide range of specialized fields. Theuniversity's general education program instills a strong sense of humanity and social responsibility, while the core curricula in the various specialized academic programs help students develop the know-how and the confidence to grapple with the rapid changes taking place in modern society. All of these academic programs are geared to conform to the university's commitment to the study of energy, the environment and food production. This commitment extends not only to the hard sciences, but to the human and social sciences as well.

Research Goals

Hirosaki University promotes research that examines modern global issues linked to the local themes of energy, global warming and the environment, as well as food and food production. It promotes internationally recognized research, cutting edge basic research, as well as research that contributes to local growth and development.

Community Service

Hirosaki University's community service program covers a wide range of activities that address many of the challenges that face the prefectures of northern Japan, including the loss of population in rural communities, low birthrates and an aging population. The University offers community medical care services, including care for those who have been exposed to radiation. Educational support programs to counteract diminished interest in the sciences amongst local elementary, middle, and high school students have been introduced as well. Projects to protect and build interest in local and regional culture and traditions have also been put into practice. Collabo HiroDai (Hirosaki University Collaboration Center) serves as the base for the cooperation between local industry, academia, and government that has made possible the implementation of these projects.

Outside Linkage

Hirosaki University actively promotes collaboration with local government and industry. It also seeks to create linkages with other universities and institutions of higher learning in the region through the Committee for the Promotion of Ties between the Three National Universities in Northern Tohoku and the cooperative industry-university-government linkage program. It also promotes academic and personnel exchanges with affiliated institutions of higher learning abroad.

University Administration

In order to successfully achieve the educational, research and community service goals it has set, Hirosaki University is working to strengthen its management system by soliciting the opinions of administrative staff and students and by giving full play to the leadership of the president. It also encourages its faculty and students to improve the quality of their work and to raise their levels of motivation. The University continues to promote the reforms necessary to reach these goals through a constant process of verification and assessment.

HIROSAKI UNIVERSITY

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