2017

HIROSAKI UNIVERSITY RESEARCH HIGHLIGHTS

Establishing a Global Identity
Creating with the Community

HIROSAKI
UNIVERSITY

"The fluorescent L-Glucose (fLG), a Novel Molecule for Cancer Detection and Treatment"

Purpose and Background of the Research

Our purpose is to establish an early diagnosis and treatment method for cancer cells having malignant potential by visualizing their abnormal uptake of a fluorescently labeled L-glucose (**fLG**). Backgrounds: D-glucose, the most abundant sugar molecule in nature, is an energy source preferred by almost all cells. In contrast, L-glucose (the mirror image of D-glucose) is a sugar found rarely in our diet, and is thought to be a sugar that cells cannot take up. Our original aim was to visualize such stereoselective preference for glucose uptake using fluorophore-bearing D- and L-glucose molecules (Fig. 1).[1-3]

Research Results

Beyond our expectations, however, 2-NBDLG, the first fLG we developed, was abundantly taken up into three-dimensionally accumulated tumor cells that showed malignant phenotypes (Fig. 2).[6-7] The uptake of 2-NBDLG occurs specifically through a mechanism inhibited by phloretin, an aglycone of an apple tree polyphenol.[6] Living cancer cells in abdominal fluid of gynecological cancer patients emitted remarkable 2-NBDLG fluorescence.[7] Administration of 2-TRLG[8] (a membrane-impermeable fLG) with 2-NBDLG has demonstrated effective discrimination of malignant tumor from non-cancerous benign cells in *in vivo* confocal endoscopic detection of bile duct cancer[9] as well as *in vitro* confocal microscopic imaging of living biopsy specimens resected from patients with gastric cancer.[6,7,9,10] Safety tests according to GLP regulations have successfully been completed for these fLGs.

Future Prospects

The fLGs will be useful for early detection of malignant tumor cells and necessary and sufficient removal of lesions that have the potential of developing cancer. Use of the method as a drug delivery system is another potential.

Funding (Direct Cost)

1. Hirosaki University Institutional Research Grant. FY2016-2017 17,000,000 Yen; FY2012-2014 12,750 Thousand Yen.
2. JST Collaborative Research Based on Industrial Demand. FY2011-2016 136,942,000 Yen.
3. JST A-STEP. FY2011-2016 104,486,000 Yen.
4. Comprehensive Support Programs for Creation of Regional Innovation Science and Technology Incubation Program. FY2008-2010 58,000,000 Yen.

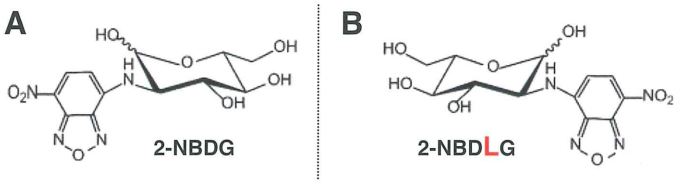


Fig. 1 2-NBDG[4] and 2-NBDLG[5], fluorescently labeled D-, and L-glucose analogue, respectively.

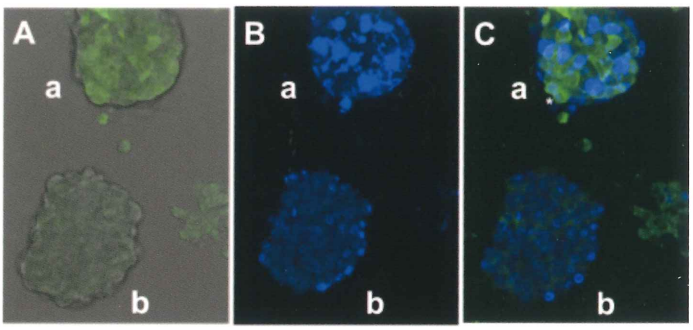
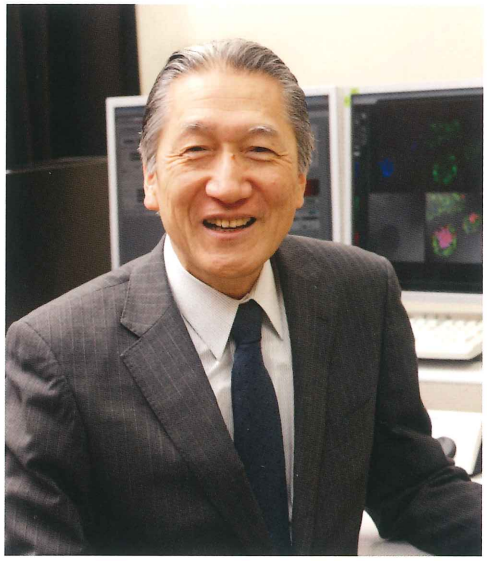


Fig. 2 Three-dimensional aggregates of mouse insulinoma cells taking up 2-NBDLG.
A, Fluorescence image of 2-NBDLG-positive (a) and -negative (b) MIN6 mouse insulinoma cell aggregates. Differential interference contrast image is overlaid. B, Live nuclear staining with DAPI. The upper one (a) shows heterogeneous nuclei (malignant phenotype), whereas the lower one (b) consisted of cells having normal small nuclei stained only weakly. C, Fluorescence and nuclear images were overlaid. Aggregates showing abnormal nuclei (a) took up abundant 2-NBDLG (Reproduced with permission by Ref. 6).

References
1)Yamada, et al., ***J. Biol. Chem.*** 275: 22278 (2000); Yamada, et al., ***Nature Protocols*** 2: 753 (2007).
2)Yamamoto, et al., ***Tetrahedron Lett.*** 49: 6876 (2008).
3)Yamada, et al., EP2325327; JP5682881; US8986656.
4)2-[N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose.
5)2-[N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-L-glucose.
6)Sasaki, et al., ***Human Cell*** 29: 37-45 (2016).
7)Yamada, et al., WO2012/133688. JP6019500; ZL201280015126.5; Granted on Nov. 13, 2017 by EPO. Yamada et al., WO2016/047676.
8)Yamamoto, et al., ***Bioorg. Med. Chem. Lett.*** 21: 4088 (2011).
9)Yokoyama, et al., ***Human Cell*** 29: 111-121 (2016).
10)Yamada, et al., ***J. Physiol. Sci.*** 67: 539-548 (2017).



PROFILE

Katsuya Yamada
Associate Professor,
Department of Physiology,
Hirosaki University Graduate School of Medicine

Website
http://www.med.hirosaki-u.ac.jp/~physio1/index.html

Research for new health function of blackcurrants: Toward "General production of Aomori cassis"

Purpose and Background of the Research

In Japan, Aomori Prefecture has the largest production of blackcurrant (cassis). In December 2015, "Aomori Cassis" was selected as the first issue of the "Geographical Indication Protection System" by the Ministry of Agriculture, Forestry and Fisheries to protect agricultural products and food as regional brands by the country. Blackcurrants contain high concentrations of four anthocyanins: delphinidin-3-glucoside, delphinidin-3-rutinoside (D3R), cyanidin-3-glucoside, and cyanidin-3-rutinoside (C3R). D3R and C3R are specific blackcurrant anthocyanins. These components are known to have an effect of improving blood flow, but there are many other unknown functions. Therefore, we clarified the new health function of blackcurrant and found added value of blackcurrant.

Research Results

This study has been mainly performed by young scientists of Hirosaki University. Some of the major research accomplishments are listed below:

1. We investigated the possibility of blackcurrant anthocyanins binding to estrogen receptor β, and show that blackcurrant anthocyanins induced estrogen receptor β transcriptional activity.
   Molecules 2018, **23**, 74; doi:10.3390/molecules23010074.
2. We revealed that anthocyanin-rich blackcurrant extract induces G0/G1 cell cycle arrest and apoptosis in normal breast epithelial cell line MCF10A. Blackcurrant extract dysregulated Polo-like kinase signaling and reduced breast cancer cell–proliferation-related genes such as Aurora kinase A and lysine demethylase 5B. These findings suggest that blackcurrant anthocyanins could be useful as a component of breast cancer prevention foods.
   Mol Med Rep. 2017, **16**, 6134-6141.
3. We analyzed the potential phytoestrogenic effects via estrogen receptor α of blackcurrant extract (BCE) in breast cancer (MCF-7) and human endometrial cancer (Ishikawa) cell lines that over-express estrogen receptor α, as well as in immature female rats. Our results suggest that blackcurrant anthocyanins act as phytoestrogens *in vitro* and *in vivo*.
   Mol Nutr Food Res. 2015, **59**, 2419-31.

Future Prospects

Blackcurrant anthocyanins may be effective for improvement of various disorders associated with estrogen such as menopausal disorder and breast cancer. We would like to clarify these new health functions of blackcurrant and lead to the development of blackcurrant functional food.

Funding (Direct Cost)

Hirosaki University Institutional Research Grant for Young investigators FY2016-2017 6,000,000 yen
JSPS KAKENHI Grant Number 16K00844 FY2016-2018 3,500,000 yen
JSPS KAKENHI Grant Number 25640058 FY2013 2,200,000 yen
JSPS KAKENHI Grant Number 22770119 FY2010-2011 3,200,000 yen

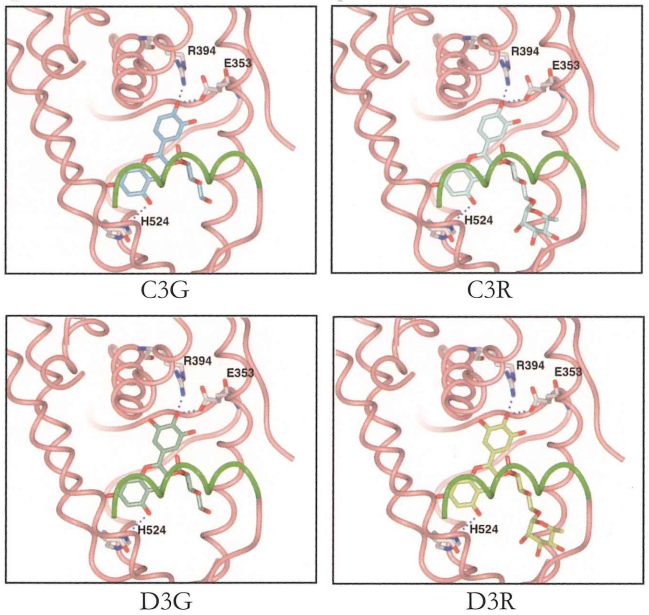
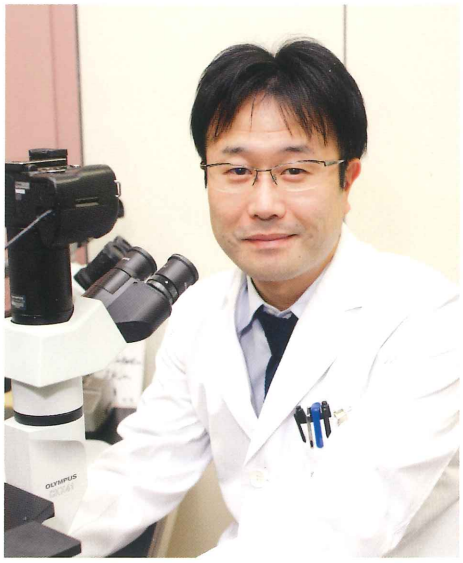


Figure 1. Ligand-binding pocket of the active estrogen receptor α conformation showing interactions with cyanidin-3-glucoside (C3G), cyanidin-3-rutinoside (C3R), delphinidin-3-glucoside (D3G), delphinidin-3-rutinoside (D3R).



PROFILE

Naoki Nanashima
Lecturer,
Department of Bioscience and Laboratory Medicine,
Hirosaki University Graduate School of Health Sciences

E-mail
nnaoki@hirosaki-u.ac.jp

Investigation of novel photocatalysis reactions by utilizing organic p-n bilayers as photocatalyst materials

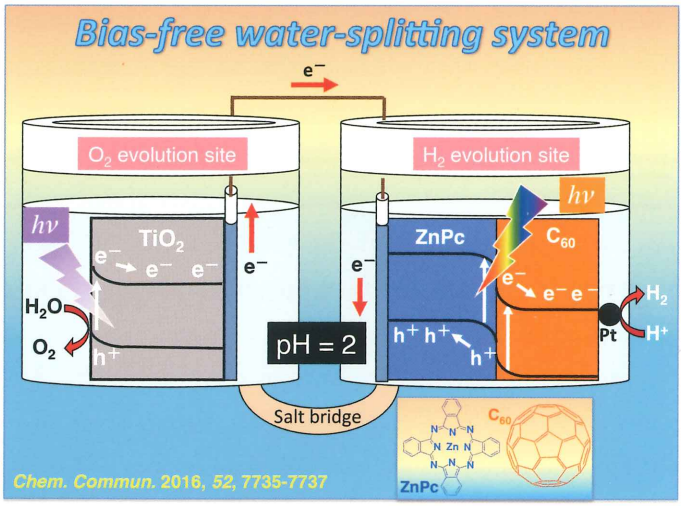
Purpose and Background of the Research

Photocatalysts can simultaneously lead to oxidation and reduction, which are induced by oxidizing and reducing powers generated at a photocatalyst, respectively. Photocatalysts are applied to two types of reactions, i.e., spontaneous but kinetically inconvenient reaction (i.e. down-hill reaction of $\Delta G^\circ<0$) and artificial photosynthetic reaction (i.e. up-hill reaction of $\Delta G^\circ>0$). Generally, inorganic semiconductors have been utilized as photocatalysts, which usually show more efficient photocatalysis at shorter wavelengths. This is due to the trade-off relation between photocatalytic activity and the magnitude of photon energy available for photocatalytic reaction. Towards decomposition or elimination with a great deal of substance, an active photocatalyst, capable of making efficient use of solar spectrum, must be developed. However, such photocatalysts have so far been absent.

Research Results

In order to suggest a novel approach for an efficient photocatalytic reaction under solar irradiation, we have studied organic p-n bilayers in terms of photoelectrodes and photocatalysts. The organo-bilayer can generate oxidizing and reducing powers at p-type and n-type surfaces, respectively, through the series of photophysical events (i.e. light absorption, exciton formation, migration of its excitation energy, charge separation into electron and hole carriers and carrier conduction). The organo-photocatalyst also meets the desirable requirements with regard to the utilization of solar energy for reactions.

Our recent instances are introduced as follows: I) bias-free water splitting was found to occur in the system of TiO_2 photoanode featuring an efficient organo-photocathode (*ChemCommun* 2016), although the reference system of TiO_2 (O_2 evolution site) and Pt wire (H_2 evolution site) was needed to apply some biases to the system. Separate generation of a reducing power at the photocathode was the key event distinct from the reference system (*JPC C* 2011, *Int J. Hydrogen Energy* 2015). II) We demonstrated dual-functional catalysis for the same reaction as pollutant degradation by an organic p–n bilayer with and without irradiation (*JMC A* 2017). There has been no evidence of such catalysis in practical TiO_2. III) When p-type NIR-responsive lead phthalocyanine was combined with an n-type organic semiconductor, the organic p-n bilayer was recognized as demonstrating a photocatalytic reaction (*Appl. Catal. B* 2017), which was superior to the control system of a p-type material responding to only the visible-light region.



Future Prospects

The application of organic p-n bilayers for matter in chemical reactions is considered to be effective, so our efforts will continue to discover new photocatalysis systems for redox reactions that have never been induced under solar irradiation.

Funding (Direct Cost)

JSPS KAKENHI Grant number 25107505 FY2013-2014
3,000,000 Yen
JSPS KAKENHI Grant number 15K05595 FY2015-2017
3,800,000 Yen

PROFILE

Toshiyuki Abe

Professor,
Department of Frontier Materials Chemistry,
Graduate School of Science and Technology

E-mail
tabe@hirosaki-u.ac.jp

Transplantation of Multilineage-differentiating stress-enduring cells after spinal cord injury.

Purpose and Background of the Research

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into adipocytes, chondrocytes, osteoblasts, and myoblasts *in vitro* and undergo differentiation *in vivo*. MSCs are currently being applied in a number of clinical studies targeting numerous diseases because of their accessibility, nontumorigenicity, and powerful trophic effects. We have shown MSCs transduced with a multineurotrophin were effective in promoting cell growth and improving sensory function after spinal cord injury (SCI). These novel data also provide insight into the neurotrophin-receptor dependent mechanisms through which cellular transplantation leads to functional improvement after experimental SCI. However, other studies indicate no significant functional improvements after transplantation of MSCs into injured spinal cord. Multilineage-differentiating stress-enduring (Muse) cells in adult human MSCs have been reported as a novel population of stem cells with pluripotent characteristics. Muse cells comprise a small percentage of MSCs, are able to generate cells representative of all three germ layers from a single cell, and are nontumorigenic and self-renewable. The goal of this study is to repair spinal cord after SCI using Muse cells from mouse tissue. At first, this study investigated whether SSEA-3 positive cells could be sorted from mouse adipose-MSCs. Second, it was demonstrated that the SSEA-3 positive cells had the characteristics of Muse cells. Third, their neuronal/glial differentiation potentials were assessed. At last, we are going to transplant Muse cells to mice spinal cord injury model.

Research Results

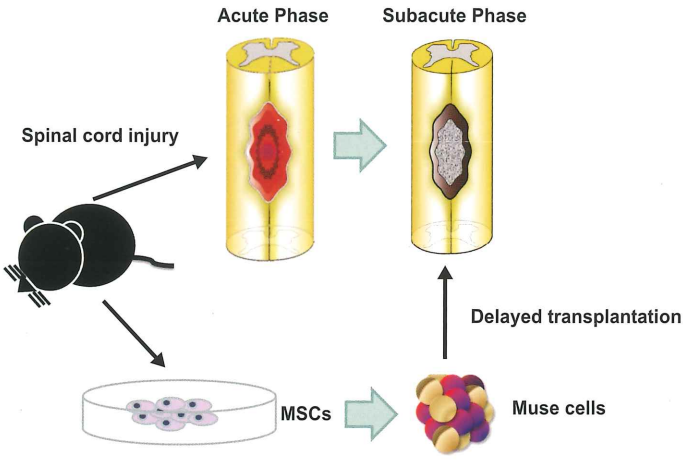
Muse cells were isolated from adult mouse adipose tissue successfully. SSEA-3 positive Muse cells could be sorted from mouse adipose-MSCs. Mouse SSEA-3 positive Muse cells had capacity to self-renew and expressed pluripotency markers (Nanog, Oct4, and Sox2). And, they could differentiate into ectodermal, mesodermal, and ectodermal cells *in vitro*. Mouse SSEA-3 positive cells differentiated into NeuN positive neuron cells, O4 positive oligodendrocyte cells, and GFAP positive astrocyte cells. We also established spinal cord injury model in mice for assess anxiety, motor, and sensory function (Fujita T, Journal of Neurotrauma. 2017).

Future Prospects

We are going to transplant mice Muse cells to mice spinal cord injury model. We will assess anxiety behavior, motor, and sensory functional recovery after transplantation.

Funding (Direct Cost)

Hirosaki University Grant for Distinguished Researchers FY2017
600,000 Yen



Transplantation of Muse cells into a mouse SCI model.

PROFILE

Yasuyuki Ishibashi
Professor,
Department of Orthopaedic Surgery, Hirosaki University Graduate School of Medicine

Website
http://www.hirosaki-u-ortho.jp/web/index.html

A study on the pseudogap state of unconventional superconductors by preparing new single crystals

Purpose and Background of the Research

Superconductivity is a phenomenon whereby the electrical resistivity drops to zero below critical temperature (Tc), so it is expected as an environmentally friendly technology for saving energy. In recent years, the so-called unconventional superconductors with high-Tc such as cuprates or ion-based compounds have been discovered and, thus, attracted much attention. However, the mechanism has not been solved. The most puzzling phenomenon observed is the pseudogap that depletes the electronic density of states (DOS) around the Fermi level from characteristic temperatures (T^*) far above Tc. In order to find the origin, we prepare unique single crystals.

Research Results

① It is not known whether the pseudogap is a necessary ingredient (precursor) for high-Tc superconductivity or if it is a different ordered state. The difference should appear at the heavily overdoped state. To investigate the difference, we have succeeded in preparing a heavily overdoped $Bi_2Sr_2CaCu_2O_{8+\delta}$. Usui et al., J. Phys. Soc. Jpn. 83, 064713 (2014).

② There was a problem that T^* and the onset temperature for the superconducting fluctuation, $Tscf$, were difficult to distinguish at the overdoped states. Here, we have found that we can distinguish them by measuring magnetic field dependence of out-of-plane resistivity. Watanabe et al., Phys. Rev. B 94, 174517 (2016). Usui et al., Physics Procedia 65, 49 (2015).

③ It is empirically known, in high-Tc cuprates, that Tc increases on increasing the number of CuO_2 planes in a unit cell, n, from n = 1 to n = 3. However, the microscopic mechanism underlying this behavior is not known. To investigate the origin, we have succeeded in growing high quality single crystals of $Bi_2Sr_2Ca_2Cu_3O_{10+\delta}$ (Figure 1). Adachi et al., Physics Procedia 65, 53 (2015). Adachi et al., J. Phys. Soc. Jpn. 84, 024706 (2015).

④ Among the many Fe-based superconductors, $Fe_{1+y}Te_xSe_{1-x}$ system is suitable for basic research, since the crystal structure is the simplest. However, an incorporation of excess Fe in the crystals has been a serious problem. Here, we have succeeded in growing high quality $Fe_{1+y}Te_xSe_{1-x}$ single crystals (Figure 2) and proposed an effective annealing method for removing excess Fe from the crystals. Koshika et al., J. Phys. Soc. Jpn. 82, 023703 (2013).

Future Prospects

By measuring the physical properties for these crystals in collaboration with some other research groups, we reveal the relationship between the pseudogap and superconductivity. Based on the knowledge obtained, we search for new superconducting materials with higher-Tc of above room temperature.

Funding (Direct Cost)

1. Hirosaki University Grant for Distinguished Researchers FY2017-2018, 1,160,000 Yen
2. JSPS KAKENHI Grant Number 25400349, FY2013-2016, 3,800,000 Yen

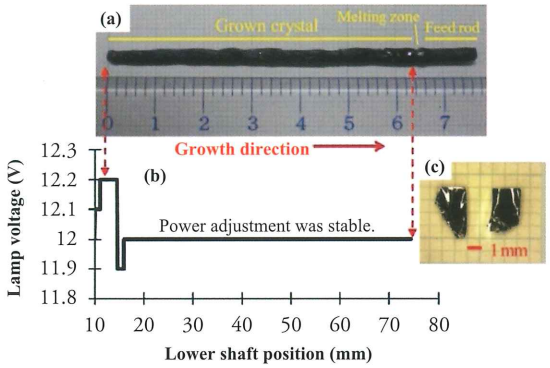
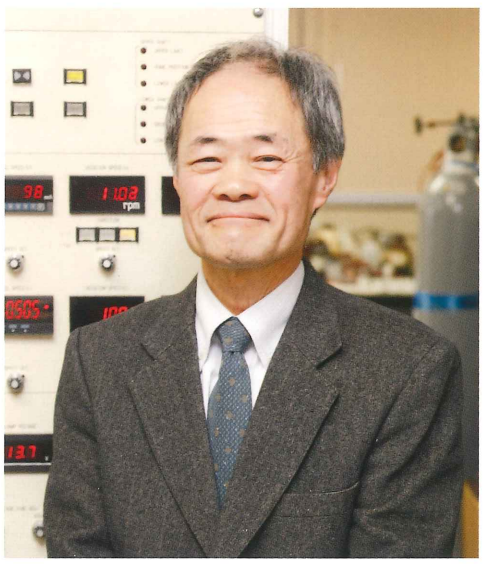


Figure 1. (a) Photograph of the grown $Bi_2Sr_2Ca_2Cu_3O_{10+\delta}$ boule. (b) Operation history: halogen lamp power vs. lower shaft position. (c) Grown crystals cleaved from the boule.

Figure 2. Photograph of as-grown $Fe_{1.03}Te_{0.8}Se_{0.2}$ single crystals.



PROFILE

Takao Watanabe
Professor, Department of Mathematics and Physics, Graduate School of Science and Technology

E-mail
twatana@hirosaki-u.ac.jp

Edited by Institute for the Promotion of Research and Innovation
University Corporation Hirosaki University
URL http://www.hirosaki-u.ac.jp/ MAIL jm3909@hirosaki-u.ac.jp
Edited in March 2018