ISTC2014

International Symposium on Technologies against Cancer 2014

March 8th (Sat.)-9th (Sun.), 2014
Katsushika Campus, Tokyo University of Science, Tokyo, Japan
(***http://www.tus.ac.jp/en/campus/katsushika.html***)
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ABSTRACT BOOK

Center for Technologies against Cancer
Tokyo University of Science

RIST
Research Institute for Science and Technology

TOKYO UNIVERSITY OF SCIENCE since 1881

ISTC2014

International Symposium on Technologies against Cancer 2014

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ORGANIZER

Center for Technologies against Cancer (CTC)
Research Institute of Science and Technology (RIST)
Tokyo University of Science, Japan

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Ryo ABE , Shin AOKI, Masanori HAYASE, Hayato OHWADA

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SCOPE

In 2008, Tokyo University of Science (TUS) established a new research center, "Center for Technologies against Cancer (CTC)." The objective of the establishment is the projection of all of the potential sciences and technologies onto interdisciplinary and innovative technologies to solve problems relating to cancer medicine. The project is based on the interdisciplinary collaboration between TUS and the National Cancer Center Hospital East of Japan. The symposium "International Symposium on Technologies against Cancer 2014 (ISTC2014)" will be held to report the achievement of the center to date and promote the discussions for the "future technologies against cancer."

KEYNOTE SPEAKERS

Prof. Shuichi TAKAYAMA, *University of Michigan, Michigan, USA*
Prof. Tzu-Chen YEN, *Chang Gung Memorial Hospital, Taiwan, R. O. C.*

PROGRAM

March 8th, 2014 (Day1)

9:50-10:20	*Registration*		
10:20-10:55	*Opening*	***Prof. Ryo ABE, Director, CTC, TUS*** ***Prof. Akira FUJISHIMA, President, TUS*** ***Mr. Shigeru NAKANE, Chairman of the Board of Directors, TUS***	
10:55-11:10	*Introductory Remarks*	***Prof. Hiroyasu ESUMI, RIBS, TUS***	

Keynote Session

K1	11:10-11:50	Shunichi TAKAYAMA	*Microfluidics for Cancer Studies*	1
	11:50-13:20	*Lunch Break*		
K2	13:20-14:00	Tzu-Chen YEN	*Integrating Imaging Data with Information from Genomics in Oncology*	2
	14:00-14:20	*Break*		

Session A: Immunology and Informatics *Chair: Prof. Hayato OHWADA*

A1	14:20-14:40	Toshihiro Suzuki and Ryo Abe	*Initial IL7R signaling in lymphopenia regulates the expansion of effector precursors against tumor antigen*	3
A2	14:40-15:00	Kazutaka HORIE	*The effect of neutron irradiation on immune induction*	5
A3	15:00-15:20	Masato Okada, Shinya Ariyasu, Shotaro Togami, Shin Aoki and Hayato Ohwada	*Docking Score Calculation by Machine Learning and an enhanced inhibitors database*	6
A4	15:20-15:40	Hiroyuki Nishiyama and Hayato Ohwada	*Rule Generation to Predict Liver Cancer Recurrence using Inductive Logic Programming*	7
	15:40-16:00	*Break*		

Poster Session *16:00-17:00* 21-49

17:30-19:30 *Banquet*

March 9th, 2014 (Day2)

Session B: From Various Size Scale *Chair: Prof. Masanori HAYASE*

12:00-13:30 *Lunch Break*

Session C: Design, Synthesis, and Evaluation of Chemical Systems for Diagnosis and Treatment of Cancer *Chair: Prof. Shin AOKI*

15:00-15:20 *Break*

Session D: Visualization and Recognition for Cancer Diagnosis and Treat *Chair: Prof. Kohei SOGA*

16:50-17:00 *Closing*

POSTER SESSION PROGRAM March 8th, 2014 (Day1) 16:00-17:30

K1

Microfluidic Studies of Cancer

Shuichi Takayama[1]

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[1] *Department of Biomedical Engineering and Macromolecular Science & Engineering Program Biointerfaces Institute, University of Michigan, Ann Arbor, MI, U.S.A.*

This presentation will describe use of microfluidic technologies to manipulate living cells to understand cancer, find therapeutics against cancer, and to treat side effects of cancer such as infertility. While the ability to maintain human cells in culture dishes has been around for several decades, unfortunately, the same cells can behave very differently depending on how they are cultured. Sub-optimal cultures can lead to (i) unreliable drug efficacy/toxicity predictions, (ii) sub-optimal cell therapies, and (iii) misunderstanding of disease mechanisms. Until relatively recently, most of the focus on improving cell culture has been to improve the media, or the liquid solution and additives used to grow the cells in. But there is an increasing realization that this alone is insufficient. There has to be modifications made to the hardware aspects of cell culture as well. We need to recreate fluid flow, mechanical stretching, chemical interactions between cells from different organs and tissues to better mimic what cells experience in our bodies. Only then can cells cultured outside of the body not just be prevented from dying, but really bring out the functions they would perform the way they would perform it inside the body in their physiological tissues and organs.

This presentation will give an overview of efforts in our laboratory to develop microfluidic systems with living cells that mimic different parts of our body. The seminar will also present some basic concepts in microfluidic technology that enable recreation of physiological microenvironments as well as enable cell sorting and biochemical analysis. Microfluidic topics to be covered include valving, scaling, laminar flow, and aqueous two phase systems. Specific biomedical topics that will be discussed include microfluidic tumor spheroid formation and drug testing [1], micropatterned analysis of cancer invasion genes [2], microscale collagen contraction assay [3], microfluidic studies of the role of chemokines in cancer cell migration [4, 5], and microfluidic in vitro fertilization systems to treat infertility and preserve fertility for cancer patients [6, 7, 8].

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K2

Topic:Integrating imaging data with information from genomics in oncology

Speaker:Prof. Tzu-Chen Yen

Email:yentc1110@gmail.com

Institution:Molecular Imaging Center, Chang Gung Memorial Hospital and University, Taiwan

As a physician and researcher in medical imaging, we have been fascinated by the rapid and dramatic improvement of imaging capabilities over the past decade. Meanwhile, we have also witnessed an explosive development of the 'omics' technologies - genomics, proteomics, metabolomics, etc. over the years. Now that we are living in the “-omics” era, it has become an emergent need to integrate imaging data with the “-omics” data. More importantly, role of imaging should be defined in such integration by identifying its advantages. With such integration, we see a person truly as whole - macroscopically and microscopically. However, such integration is challenging. A few groups have been working on this emergent topic so far, but not too many yet. We initiated our work since 2007 with the start-up project of head and neck cancers for at least two reasons. First, head and neck cancers are important cancers in Taiwan because it is the top ranking cancer in male between 30-60 years old in Taiwan. Second, the treatment regimens for head and neck cancers in our hospital will follow a fixed protocol and most of them will have a radical surgery. Therefore, we may have a chance to study the “-omics” from their bio- and tissue specimens. We hope that through this start-up study, we can achieve a few goals: (1) Promoting the importance of imaging/omics data integration; (2) Letting people know this is feasible as well as what are the state of the arts; (3) Facilitating the interdisciplinary collaboration between different groups for pooling data together and (4) Stimulating the technological developments for such endeavors. Herein, I wish to share with you some of our preliminary data.

Initial IL7R signaling in lymphopenia regulates the expansion of effector precursors against tumor antigen

Toshihiro Suzuki[1] and Ryo Abe[1,2]

[1,] Division of Immunobiology, Research Institute for Biomedical Sciences,

[2,] Center for Technologies against Cancer, Tokyo University of Science

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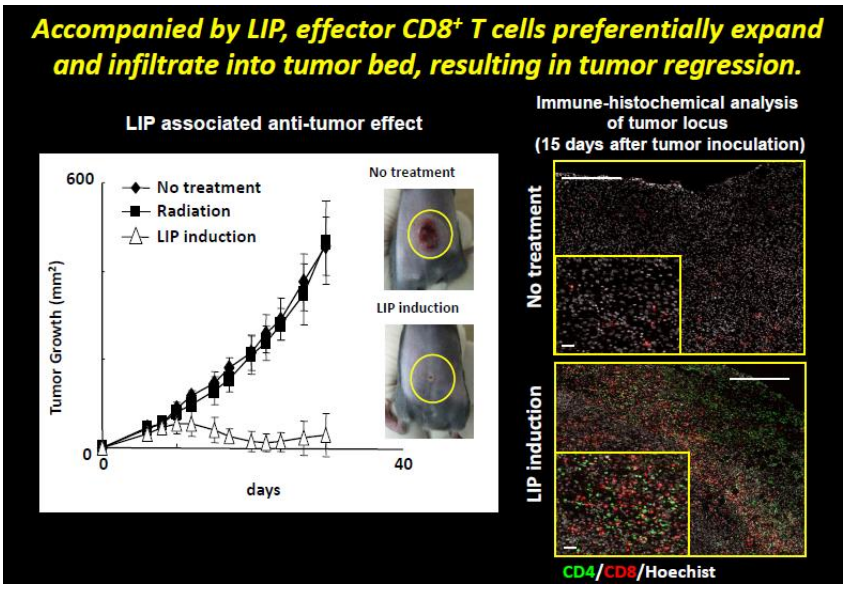
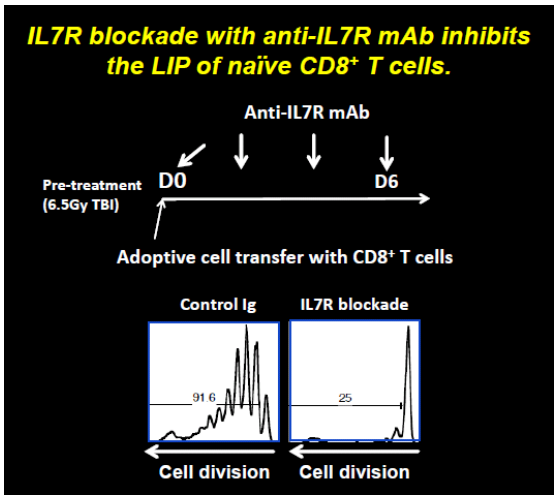
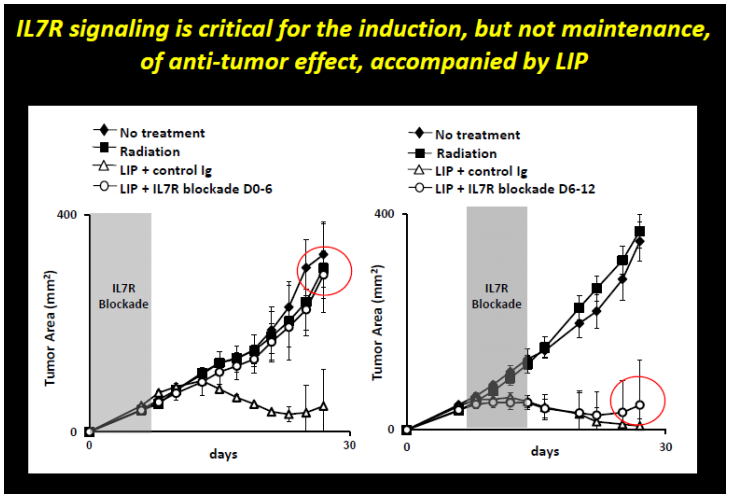
Transient lymhopenia and following lymphopenia induced proliferation (LIP) of T cells are usually assumed as potent initiator for the induction of anti-tumor immune response by adoptive immunotherapy. Previously, we demonstrated that CD28 signal is essential for the induction of anti-tumor immune response accompanied by LIP. Donor T cells lacking CD28 signal could not differentiate into CTLs in lymphopenic host, whereas LIP and following differentiation of donor CD8 T cell into $CD44^{high}$ $CD62L^{+}$ central memory T cell (T_{CM}), was also observed in the absence of CD28 signal. On the other hands, the distinct role of IL7 signaling and subsequent LIP of T cells for anti-tumor immune response has been still unclear, because multiple mechanisms are involved in the anti-tumor immune response under lymphopenic conditions.



When LIP was occurred, the growth of transplanted lewis lung carcinoma cells, which stably expressed model antigen gp33 minigene, was markedly diminished. In lymphatic tissue and tumor focus, effecter $CD8^{+}$ T cells preferentially accumulated, and these populations maintained the IL7 receptor (IL7R) expression. From these results, it could be hypothesized that redundant IL7 in lymphopenic hosts directly improves the expansion and function of $IL7R^{+}$ effector $CD8^{+}$ T cells.

To inhibit LIP of naïve T cells at various time points, we used an IL7R blockade with anti-mouse IL7R mAb. This approach allowed us to reveal the role of LIP in the anti-tumor immune response, and whether IL7R signaling is required for the induction or maintenance of anti-tumor activity during LIP. As a result, IL7R blockade from the induction of LIP significantly diminished the expansion of effector $CD8^{+}$ T cells, and anti-tumor effect was disappeared. In contrast, blocking of IL7R signaling in the process of LIP did not arrest the expansion of CTLs, and anti-tumor effect was observed.

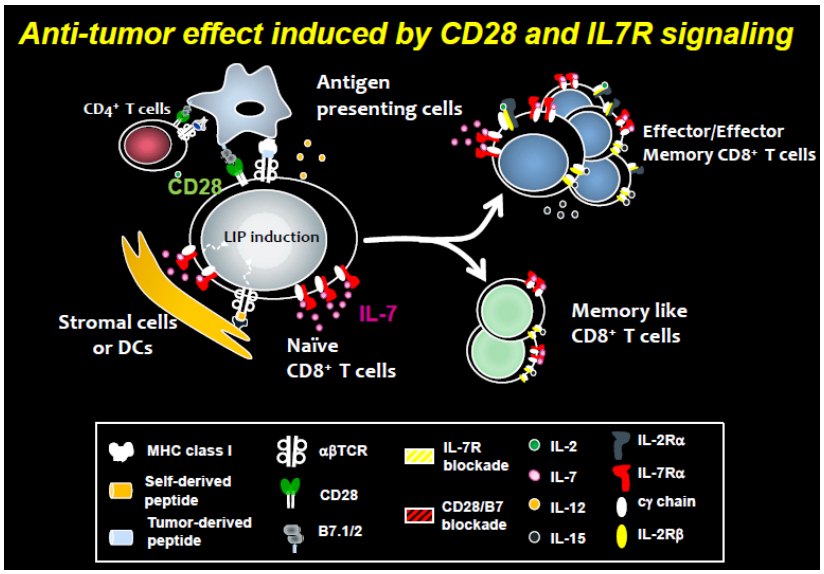




Therefore, the expression of IL7R on effector $CD8^+$ T cells was not a reason for the LIP-associated anti-tumor effect. Some reports focused on the role of IL7 for the improvement of effector T cell function by the down regulation and/or inactivation of suppressive molecules, PD-1, c-Cbl, p27, and Socs3. Assessing PD-1 expression on $CD8^+$ T cells in lymphopenic hosts in the presence or absence of IL7R blockade, we found that $PD\text{-}1^+$ $CD8^+$ T cells accumulated in LIP-induced hosts, and that PD-1 expression on $CD8^+$ T cells was not altered by IL7R blockade. We did not find any correlations between PD-1 expression on $CD8^+$ T cells and the LIP-associated anti-tumor effect in the presence or absence of IL7R signaling. Our findings demonstrated that IL7R signaling at initial phase of LIP is critical for the induction of anti-tumor immune response in lymphopenic host, as regard to the regulation of proliferative ability of effector precursors, rather than the improvement of CTL functions.

In conclusion, our results demonstrate that in lymphopenic hosts, initial IL7R signaling regulates the proliferative capacity of effector T cells, inducing an anti-tumor effect and LIP of T cell. IL7 was thought to enhance the survival and function of effector $CD8^+$ T cells directly, but our results demonstrate that these effects of IL7 do not cause the LIP-associated anti-tumor effect.

Now, we investigate the regulatory mechanisms of proliferative ability of effector precursors under IL7R signaling. Understanding the precise functions of IL7R signaling for acquired immunity, in normal and in lymphopenic environments, could lead to development of novel, specific cancer immune-therapies.



Induction of anti-tumor immunity by ionizing radiation therapy. - Aiming for application to BNCT-

Kazutaka Horie[1], Toshihiro Suzuki[1,2], Mitsutoshi Tsukimoto[1,3], Yoshinori Sakurai[4], Shin-ichiro Masunaga[5], Hiroki Tanaka[4], Koji Ono[5], Shin Aoki[1,6], and Ryo Abe[1,2]

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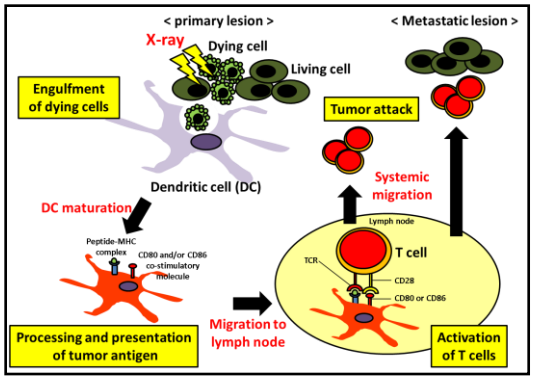
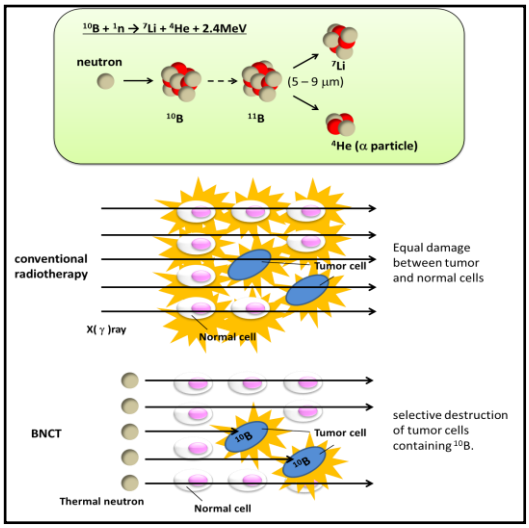
Radiotherapy (RT) with ionizing irradiation is commonly used to locally attack tumors. It induces a stop of cancer cell proliferation and finally leads to tumor cell death. Accumulated evidence supports the hypothesis that radiation therapy also has the potential to induce/enhance antitumor immunity. In this respect, we found that tumor-specific cytotoxic T lymphocytes (CTLs) were induced in not only tumor draining lymph nodes but also spleen of tumor-bearing mice by X-ray irradiation. The therapeutic effect of irradiation was attenuated in tumor-bearing mice by blocking CD28-B7 interaction, a critical role for full T cell activation, through human CTLA-4 Ig fusion protein administration. These indicates that local radiation against tumor can be systemic immune-adjuvant itself, and that RT followed by proper immunotherapy could synergistically overcome the tumor barriers and generate more tumor-reactive T cells that circulate systemically to eradicate micrometastasis. Therefore, we should revisit our current strategies and develop new approaches that can reduce tumor burden while boosting protective immunity.



RT induces adaptive immunity against tumor

In this conference, we introduce Boron neutron capture therapy (BNCT), a kind of RT. BNCT is based on the following nuclear reaction. Nonradioactive isotope ^{10}B atoms that absorb low-energy (<0.5 eV) neutrons (thermal neutrons) disintegrate into an alpha (^{4}He) particle and a recoiled lithium nucleus (7Li). The two particles generated in this reaction have a high linear energy transfer (LET) along their very short path (<10 μm), a range of roughly the diameter of one or two tumor cells. It is theoretically possible to kill only tumor cells without affecting adjacent healthy cells, if ^{10}B atoms can be selectively accumulated in the interstitial space of tumor tissue and/or intracellular space of tumor cells. BNCT is an effective tool for local tumor control in the treatment of brain tumor, melanoma, and so on. However, BNCT is not applicable to systemic malignancy to date. Therefore, local BNCT modality needs to be combined with an effective systemic treatment to significantly impact metastatic disease. In this respect, immunotherapy could offer an attractive candidate for combination treatments aiming at systemic antitumor effect. However, the effect of neutron irradiation on immune system has not been fully understood. Here, we show the effect of neutron irradiation on immune induction in a murine vaccination model.



Concept of BNCT

A3

Docking Score Calculation by Machine Learning and an enhanced inhibitors database

Hayato Ohwada[1,2] Masato Okada[1], Shinya Ariyasu[2], Shotaro Togami[2], and Shin Aoki[2,3]

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In this paper, we will show the docking score calculation with an enhanced inhibitors database and various machine learning tools. The docking score calculation is the prediction of the binding of the compounds with the protein by computer. In this calculation, the docking software which simulate the binding based on the force fields have been used widely [1], however, we clarify the highly performance of machine learning on the prediction of binding. Especially, the DUD-E (A Database of Useful Decoys: Enhanced) is one of inhibitors database and includes various target proteins, ligands, and decoys of each target protein. For this reason, this database is suit to the machine learning for calculation of the docking scores and comparison between docking software and machine learning. In this paper, we select the target protein which can be calculated the docking scores by docking software with high accuracy and calculate the docking scores of the compounds by classification methods including SVM (Support Vector Machine) and Random Forest. Using these data and tools, we will show the docking score performance with ROC curve which shows the classification performance in the various thresholds and superiority of machine learning.

References

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Rule Generation to Predict Liver Cancer Recurrence using Inductive Logic Programming

Hiroyuki Nishiyama and Hayato Ohwada

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In this study, we generate a set of rules that will focus on how to predict the recurrence of cancer from the patient database, perform the inductive learning for the diagnostic data of the ablation front and back in hepatocellular carcinoma is associated with a recurrence of liver cancer. Features of our study is that it is possible to convert the qualitative information, information obtained from the patient database, the relationship of the information ablation around, and was used as the differential information related diagnostic data between after resection. Then, by performing the inductive learning for the information, we were able to generate 19 pieces of rules for cancer recurs and 9 rules if the cancer does not recur. As a result of the accuracy evaluation by cross-validation based on rules obtained, the accuracy was 81.2%.

B1

Recent advances from bio device team

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Bio device team is one of five research teams in the Center for Technologies against Cancer, Tokyo University of Science. The team mainly consists of members whose major were not biological science and technology, and was established for exploring new approaches to cancer remedies and studies. During these five years, joint research themes were newly discussed and studied, and each member's interest was certainly expanded into medical fields. In this presentation, recent activity in the bio device team will be introduced.

Pneumatically-Driven Surgical Robot with Force Perception

Name- Kenji Kawashima
Affiliation- Tokyo Medical and Dental University, Japan

We have worked on the development of a master-slave robotic system for laparoscopic surgery. For the forceps manipulator on the slave side, pneumatic actuators are used to detect external forces based on pressure values without a force sensor. The forceps has two-degree of freedom (DOF) on the tip. A high performance spring component with wire actuation is employed for two-DOF bending joint, and two-DOF tendon drive system is implemented by four pneumatic cylinders. Some in-vivo experimental results are demonstrated. We confirmed that the suturing task in the abdomen is smoothly accomplished by the operator.

Cell Manipulation by Dielectrophoresis

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Most cancer deaths involve the metastasis caused by penetrated tumor cells shed to vasculature, so-called circulating tumor cells (CTCs)[1)]. Efficient detection, separation and capture of rare CTCs from the blood have been technically tough challenges for improved prognosis and treatment. A microfluidic approach could be one of methods with high capability for label-free characterization of CTCs in a tiny platform[2,3)]. So far, size-based separation using deterministic lateral displacement[4)] is successful for the separation of cells with different sizes in the blood. However, size would not be an exclusive criterion of CTCs from the other cells. The cell separation with similar or even almost the same size is still an issue to be addressed as an additional handling process to the size-based sorting.

In our study, we focus on dielectrophoresis in a microfluidic chip to separate cells with similar size. Fig. 1 shows a schematic of our prototype cell separator using multi-step dielectrophoresis. At the first step, a downforce area was set to make all the cells flowing in the channel to close to the bottom wall using negative-DEP so that efficient DEP force could act on the cells downstream. Then, the cells can be laterally migrated in slant electrode, namely separation area. Difference of response of SP2/O-HEL and lymphocyte both from BALB/c mice to electric field were measured (Fig. 2). The result implies the potential of the separation of both cells using dielectrophoresis. In the preliminary experiments, we have confirmed the good separation performance of SP2/O-HEL in our multi-step DEP chip.

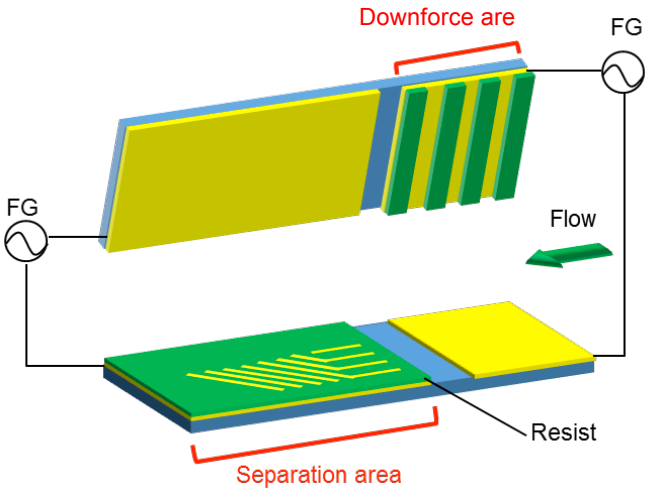

Fig. 1 Microfluidic cell separator using multi-step dielectrophoresis.

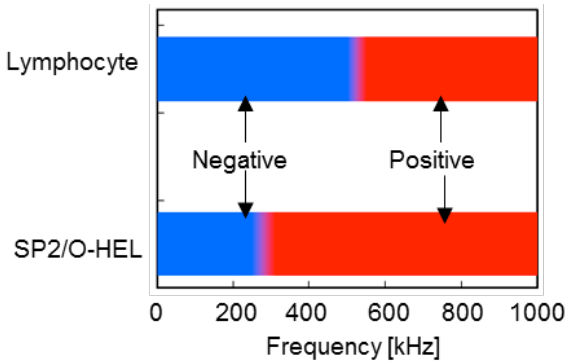

Fig. 2 Frequency-based response of SP2/O-HEL and lymphocyte.

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[4] L. R. Huang *et al.*, Continuous particle separation though deterministic lateral displacement, Nature, 304, pp. 987-990 (2004).

Suppression of cancer growth by purinergic antagonists

Mitsutoshi Tsukimoto*, Shizuka Seki, Wakako Kaji, Fumie Hattori, Satomi Tanaka, Akina Suzuki, Yasuhiro Ohshima, and Shuji Kojima

(tsukim@rs.noda.tus.ac.jp)

Department of Radiation Biosciences, Faculty of Pharmaceutical Sciences,

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1. Anti-angiogenic effect of P2X7 receptor antagonist oxidized ATP as a mechanism of anti-tumor growth

Extracellular ATP accumulated in tumor microenvironment activates P2X7 receptor on cellular membrane of cancer cells. Recently, an importance of P2X7 receptor in cancer growth or malignancy has been suggested. We have reported an inhibitory effect of oxidized ATP (oxATP), which is an irreversible antagonist of P2X7 receptor, on melanoma growth. However, the mechanism has not yet been established. In this study, we investigated the effect of oxATP on angiogenesis *in vitro* and *in vivo* to reveal the mechanism of anti-tumor growth by oxATP. We found that oxATP strongly suppressed cell migration and wound healing in mouse endothelium b.End3 cells, indicating the suppressive effect of oxATP on angiogenesis *in vitro*. We further investigate the effect of oxATP on angiogenesis *in vivo*. We performed ligation of the femoral artery and vein of BALB/c mouse, and a laser doppler perfusion image analyzer recorded blood flow postoperatively. The blood flow of hind limb was significantly decreased by the operation and recovered within 1-2 weeks, indicating angiogenesis. However, administration of oxATP to mice significantly suppressed the recovery of blood flow. Increase of serum matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) levels contribute to angiogenesis. The serum MMP-2, MMP-9, and VEGF levels were lower in oxATP-treated mice than in control mice. Moreover, production of VEGF in RBL-2H3 mast cells was suppressed by treatment with oxATP. These results suggest that oxATP inhibited angiogenesis *in vivo* via suppression of MMP-2, MMP-9 and VEGF production. We conclude that oxATP has an anti-angiogenic effect, which would contribute to suppression of cancer growth.

2. Adenosine A_{2B} receptor antagonist PSB603 suppresses tumor growth by inhibiting induction of regulatory T cells

Regulatory T cells (Treg) play a role in suppression of immune response, including anti-tumor immunity. We have recently reported that treatment of naïve CD4 T cells with adenosine A_{2B} receptor antagonist PSB603 under Treg-skewing conditions inhibits expression of Foxp3, a marker of differentiation to Treg, without blocking IL-2 production or CD25 expression, which are activation markers, in CD4 T cells. We hypothesized that PSB603 suppresses cancer growth by inhibiting induction of Treg, thereby facilitating anti-tumor immunity. In this study, we examined the effect of PSB603 on tumor growth in B16 melanoma-bearing C57BL/6 mice. Administration of PSB603 significantly suppressed the increase of tumor volume as well as the increase of Treg population in these mice. The populations of CD4 and CD8 T cells were higher and splenic lymphocyte-mediated cytotoxicity towards B16 melanoma was significantly increased in PSB603-treated mice. We confirmed that PSB603 did not reduce the viability of B16 melanoma cells *in vitro*. Overall, our results suggest that A_{2B} receptor antagonist PSB603 enhances anti-tumor immunity by inhibiting differentiation to Treg, resulting in a delay of tumor growth.

Summary Report of Research Projects of Drug Discovery Team in Pharmaceutical DDS Group (PDG)

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Drug discovery team of pharmaceutical DDS group is constituted by organic, inorganic, and biological chemists and our effort has been devoted to the following projects, since the establishment of CTC. In this presentation, the summary of these results will be reported.

1) Detection of acetaldehyde in blood for cancer diagnosis.
2) Design and synthesis of receptors and sensors of intracellular metals and signal messengers.[1]
3) Luminescent pH sensors for diagnosis of cancer and photochemical cell death induction.[2]
4) Development of new devices for isolation & collection of specific ligand-receptor complexes and live cells.[3]
5) Design and synthesis of anti-cancer agents targeting *a*-tubulin.[4]
6) Development of new synthetic methologies for the efficient synthesis of ant-cancer drugs.[5]
7) Isolation and total synthesis of naturally occurring anti-cancer compounds.[6]
8) Nano drug delivery system (nDDS) for diagnosis and treatment of cancer.[7]
9) Design and synthesis of radioprotectors to reduce side effects of cancer radiotherapy.[8]
10) *In Silico* design and synthesis of zinc(II) hydrolase inhibitors.[9]
11) Drug (+ *in silico*) screening of compound library in TUS.
12) Treatment of anastomotic leak after resection operation of colorectal cancer.

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Design and Synthesis of C_3-Symmetric Cyclometalated Iridium Complexes Having DR5 Binding Peptides for Selective Staining and Cell Death Induction of Cancer Cells

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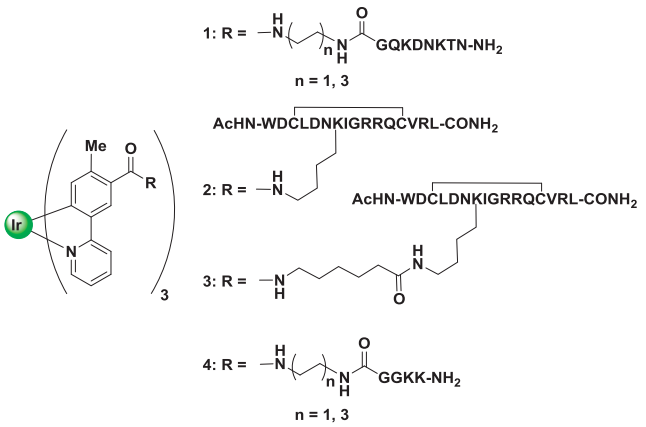
TNF-related apoptosis-inducing ligand (TRAIL) is categorized into TNF superfamily and induces the apoptosis in various tumor cells and tissues through the cell-extrinsic pathway, independently of p53.[1] TRAIL forms a C_3-symmetric homotrimeric structure by Zn^{2+}-centered self-assembly of monomeric unit and is capable of interacting with five cell surface receptors such as death receptors (DRs). Among known death receptors, death receptor 4 (DR4) and DR5 containing death domains send an apoptotic signals that activate caspase-8 (-10) to accelerate effector caspases (e.g. caspase-3, -6, and -7) and finally induce apoptosis.[1] Since TRAIL has very small influence on normal cells and tissues, antibodies and artificial compounds having TRAIL-like activity have been proposed as promising agents for cancer therapy.

In this presentation, we report on design and synthesis of peptide conjugated C_3-symmetric tris-cyclometalated iridium (Ir) complexes **1-3** and **4** (as a reference compound). The complex **1** has heptapeptide (QKDNKTN) that is important amino acid sequence of TRAIL (residues: 199-205) to interact with DR5.[2] The complexes **2** and **3** have cyclic peptide that specifically recognize DR5.[3] These complexes are expected to have agonistic activity for apoptosis induction of cancer cells and function for luminescent imaging of extra- and intracellular events.

For the purposes mentioned above, the Ir complexes **1-4** were synthesized utilizing regioselective substitution reactions reported by us[4] and coupled with the corresponding peptides. Affinity of these Ir complexes toward DR5 was evaluated by 27-MHz quartz-crystal microbalance (QCM) competitive assay, and self-assembly of DR5 with **3** was also observed on fast-scanning atomic force microscopy (FS-AFM). The green luminesce spots of **2** and **3** were observed on the cell membrane of Jurkat cells, and these spots showed overlap with staining area of anti-DR5 monoclonal antibody. The results of these experiments and cell viability assay of **1-4** will also be presented.



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Evaluation of Radioprotective Ability of Zinc-Chelator Derivatives

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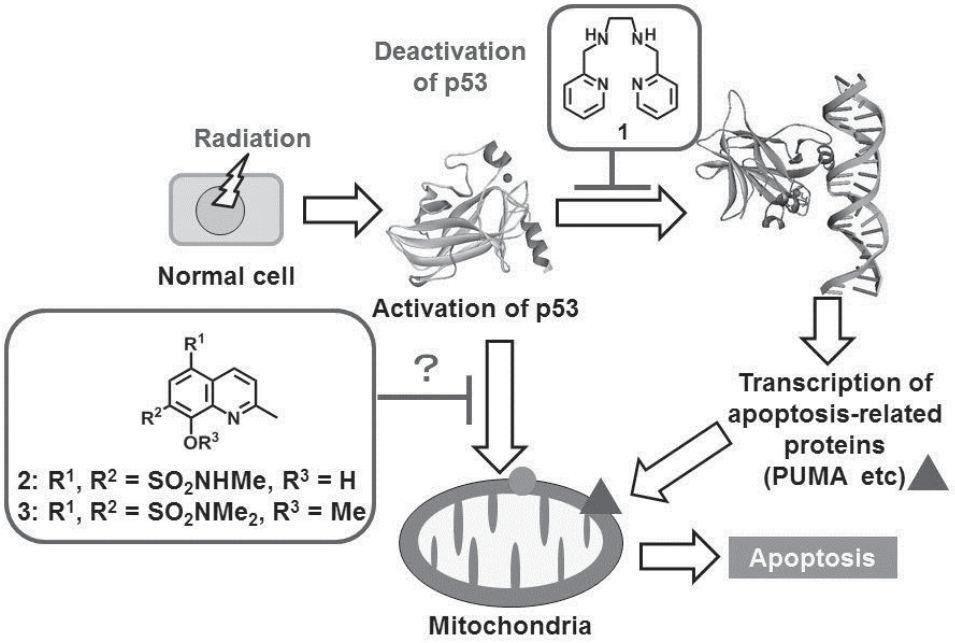
Radiation therapy and chemotherapeutic agents mainly target the DNA of growing cancer cells. Such therapies frequently have adverse side effects on normal tissues and cells, including p53-induced apoptosis. p53 protein, which is one of the important transcriptional factors, can activate its downstream targets in sequence specific manner to induce apoptosis. In contrast, many types of cancers tend to have a lower incidence of p53-mediated apoptosis, because the function of their p53s is often suppressed or lost during cancer development. Chemical agents that suppress p53-mediated apoptosis would be expected to prevent the damage of normal tissues during treatments of p53-deficient tumors.[1)] p53 protein contains zinc ions as structural factor in itself, and the zinc binding site in the p53 is essential for DNA transcription, and thus chelation can cause structural alterations, resulting in the inactivation of the p53 protein. Therefore, we expected that removing the zinc ion from p53 protein would be an effective means of inhibiting p53-mediated apoptosis induced by radiation.

In this presentation, we evaluated zinc-chelator derivatives such as Bispicen (N,N'-bis(2-pyridylmethyl)-1,2-ethanediamine) and 8-quinolinol as novel radioprotectors. The effect of some zinc chelators on intracellular p53 activity was examined p53-dependent apoptosis in irradiated MOLT-4 cells that are p53-active leukemia cells. The results of the dye-exclusion test suggest that Bispicen **1** and several 8-quinolinol derivatives such as **2** potently suppressed apoptosis induced by γ ray radiation. In addition, 8-methoxyquinoline derivatives such as **3**, whose affinity to zinc ion is very low, exhibit high radioprotective effect with low cytotoxity. The mechanistic study based on circular dichroism (CD) spectroscopy and electrophoretic mobility shift assay (EMSA) reveled that conformational changes of p53 are induced by **1**.[2)] On the other hand, it is very interesting that the mechanism of radioprotective effect **2** and **3** are different from that of **1**.



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Design and Synthesis of Sulfoquinovosylacylglycerol Hybrid Molecules as Tumor Theranostic Agents

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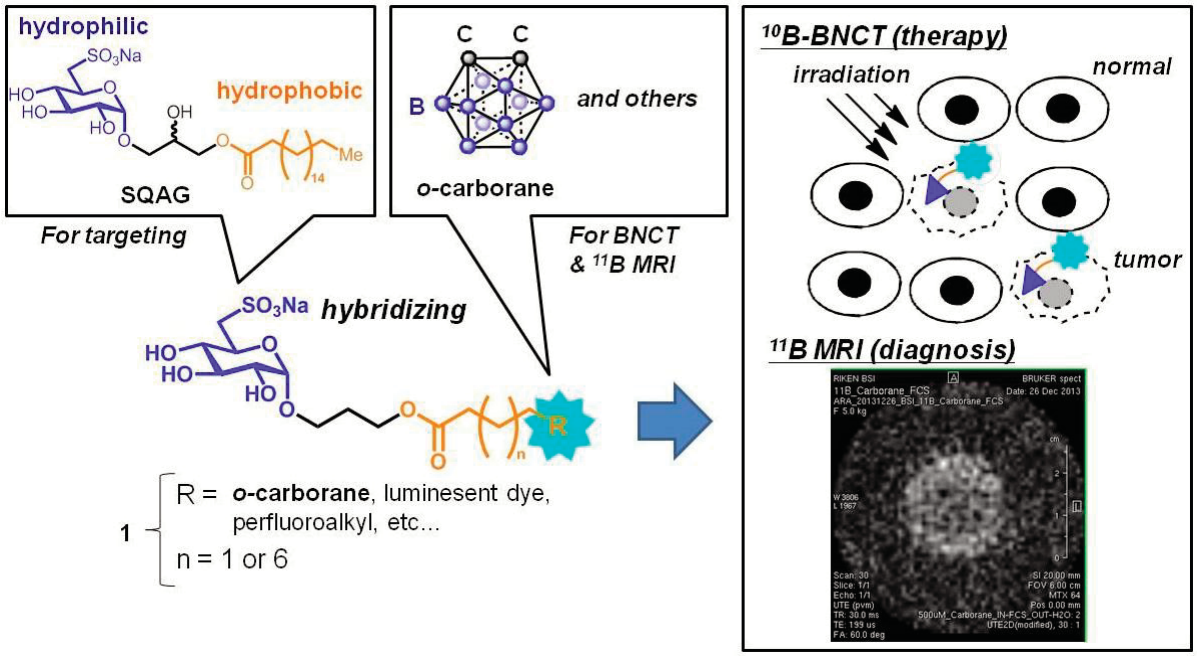
Sulfoquinovosylacylglycerol (SQAG) is a natural products that was isolated from See algae and identified by Prof. Sakaguchi and Prof. Sugawara.[1] SQAG is an amphiphilic glycolipid bearing 6-sulfo-quinovose and long-chain fatty acid linked via a glycerol linker. Recently, it has been found that SQAG has anti-tumor and radio-sensitizing activities.[2] SQAG and its analogues are accumulated at tumor cells, but with low cytotoxicity against normal cells. Based on this knowledge, we designed and synthesized SQAG analogues connected with biofunctional units for diagnosis and therapy of cancer. For instance, boron(^{10}B/^{11}B)-cluster "*o*-carborane" was introduced into the fatty acid part of SQAP, because *o*-carborane derivatives are recognized as a potent compound for boron neutron capture therapy (BNCT) and magnetic resonance imaging (MRI), respectively.

Synthesis of SQAG analogues **1** having different alkyl chains have been achieved from commercially available penta-*O*-acetyl D-glucoside. Physical behaviors of **1** and **2** such as critical micelle concentration (CMC), vesicle size and ζ-potential were measured. Intracellular uptake and cytotoxicity of these molecules were also evaluated by ICP-AES and MTT assay, respectively. In this presentation, these experimental data and the results of ^{11}B MRI experiments will be presented.



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Nano-drug Delivery System Containing Metalloporphyrin (Part 5): Investigation with Various Active Targeting Liposome Systems

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We have found that nano-drug delivery systems containing metalloporphyrins (MP/n-DDS) are non-cytotoxic and toxic hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) from a superoxide anion radical ($O_2^{\bullet -}$) are efficiently generated through the metal ions such as iron and manganese catalyzed dismutation and the Fenton-like reaction [1]-[3]. In this paper, we report for the investigation with iron and manganese porphyrins-loaded liposomes modified with active targeting sites such as glucose, folate and transferrin groups as a novel design of anticancer MP/n-DDS and their comparison of anticancer properties.

The MP/n-DDSs used were 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), Pluronic F68^{TM} (block-copolymer of polyetylene glycol-polypropylene glycol-polyetylene glycol, PEG) and glucose-, folate- and transferrin-modified polyetylene glycol-1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine (Glc-, FA- and Trf-PEG-DSPE) containing ion complexes composed of iron and manganese porphyrin and sodium stearate. The MP/n-DDSs were prepared by ultrasonic irradiation of aqueous solution containing phospholipids, surfactants and ion complexes. The particles of MP/n-DDSs modified with Glc-, FA- and Trf-groups were spherical, highly dispersed ($\phi < 200$ nm) and stable in water and phosphate-buffered saline for several days at 37°C from the results of DLS particle size distributions and FF-TEM images. This would provide at least an opportunity of physiology-based targeting of MP/n-DDSs modified with Glc-, FA- and Trf-groups to these pathological areas via the active and passive targeting effects.

An alamar blue exclusion assay of the modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method was used to detect the cytotoxicity induced by the MP/n-DDSs. Colon 26, HeLa and L1210 as the tumor cell was treated with the MP/n-DDSs modified with and without Glc-, FA- and Trf-groups. An effective concentration of drug required to produce 50% lethal dose against cell (IC_{50}) was determined as shown in Table 1. Damage to such impregnated tumor cells was observed in active targeting systems. We demonstrated that the MP/n-DDSs modified with Glc-, FA- and Trf-groups as active targeting sites present a potential model for the design and development of novel anticancer drug.

Table 1 *in vitro* antitumor capacities of various MP/n-DDSs modified with Glc-, FA- and Trf-groups.

MP/n-DDSs and tumor drugs	MP	IC_{50} (μ M)	Cancer cells
Glc-modified MP/n-DDS	FeHpD	47.3	Colon 26
MP/n-DDS		77.0	
FA-modified MP/n-DDS	FeT4MePyP	9.5	HeLa
		40.1	L1210
MP/n-DDS		9.7	HeLa
		41.2	L1210
Trf-modified MP/n-DDS	$MnT4Me_2SuP$		Colon 26
sulfide-binding site / PEG 2000		28.7	
/ PEG 5000		28.5	
amido-binding site / PEG 2000		32.7	
/ PEG 5000		30.7	

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D1

Next Generation Technologies for Visualizing Cancer

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Since 2008, as a group of the Center for the Technologies against Cancer (CTC) of the Tokyo University of Science, the Visualization and Recognition Group (VRG) of the CTC played an active role to produce innovative technologies for the visualization and recognition of cancer. The major three themes of the project were:

1) Application of over-1000-nm (OTN) near infrared (NIR) bioimaging for cancer imaging;
2) Interdisciplinary approach by medical science, engineering and artistic design; and
3) Medical device development for endoscopic diagnosis for Cancer.

The achievements of the third topic will be reported in the following presentations by T. Zako and K. Kaneko.

The OTN-NIR bioimaging is the original technique for the deep tissue imaging, which has been originally developed by the authors in the Tokyo University of Science. The optical loss of in a live body is basically decided by optical scattering and absorption. In the OTN-NIR wavelength range between 1000 and 2000 nm, the decrease of the both of the scattering and absorption meet to form a valley in the optical loss spectrum. The OTN-NIR wavelength range has long been known as a “biological window” since 1980s. However, most of the currently used cameras based on Si semiconductor can only detect the light with the wavelength up to 1000 nm. A lot of efforts have been made to elongate the wavelength in to 900-nm region by using dyes such as ICG or Q-dots. The imaging depth for fluorescence bioimaging for small animals has been limited up to several mm. The authors has predicted and proved that the depth can be extended to several cm. The first achievement of the project is developing the probes and small animal imaging. A revolutionary small animal fluorescence imaging system for the OTN-NIR imaging was developed by the collaboration between our group and a company. The system is planned to be launched into market in this year, 2014. The deep tissue imaging by the OTN-NIR fluorescence imaging has advantages such as dynamic, multicolor and sensitive characters. However, the imaging is currently only limited for two-dimensional use. Nuclear medicine imaging (NMI) including single photon counting CT (SPECT) is a method good at three-dimensional and quasi quantitative small animal imaging. By developing hybrid probes for the OTN-NIR fluorescence imaging and the NMI can propose a new cancer diagnosis method which can utilize both of the merits of the two imaging scheme. The precise report will be given in the following presentation by H. Fujii.

On the other hand, the group efforts for the second topic started up an activity for producing and maintaining a unique community for interdisciplinary discussion among the medical doctors, scientists, engineers, as well as design artists and psychologists. The group has hosted a workshop named “Biomedical Interface (BMI)” workshop every year. The workshop has a unique structure consists of the complaints by the medical doctors for daily diagnosis and treatments and the answers and discussions by the researchers of the above wide range fields of academy. Already, various interdisciplinary projects have been born from this workshop for solving the clinical problems of the cancer disease.

Some of the projects of the group were publicly called. The achievement of the publicly-called projects will also be reported in the poster sessions.

The group activity finally has achieved new materials, devises, systems and community which will contribute in future for the innovative technologies solving the clinical problems of the cancer disease.

Development of Devices for OTN-NIR Laparoscopic Cancer Surgery

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Laparoscopic surgery for colorectal cancer has been established as a minimally invasive surgery. Tattooing into the submucosal layer of the colon is generally performed in for localization of tumors during laparoscopic surgery. However, it sometimes leads to difficulty in recognition of cancer site due to faint tattoo and diffused tattoo, which causes spread resection of the colon.

Previously we have proposed a new method for tumor localization during laparoscopic colorectal cancer surgery using near infrared (NIR) light (Fig. 1) [1]. NIR light in the wavelength region between 800 and 2000 nm, which is called "biological window", has received particular attention since water and biological tissues have minimal optical loss due to scattering and absorption in this region. We have developed rare earth ion-doped ceramic nanoparticles (RED-CNP) for OTN (over-1000 nm) NIR bioimaging, which show strong NIR emission under NIR excitation. For example, ytterbium (Yb) and erbium ion (Er)-doped yttrium oxide nanoparticles (Y2O3:YbEr-NP) showed NIR fluorescence (1550 nm) with NIR excitation (980 nm), which is strong enough to penetrate swine colon walls [1]. In our proposed method, RED-CNP-coated materials such as medical clips (NIR clip) are used to mark cancer site with endoscopy. Cancer site can be recognized during cancer surgery through the intestinal wall by NIR fluorescence from the NIR clips fixed inside the colon using NIR-NIR imaging system.

Here we report a noble NIR-laparoscopy system which consists of NIR laparoscopy, NIR excitation laser diode and NIR camera (Fig. 1). We also carried out laparoscopic surgery using this NIR laparoscopy system on a swine model, and demonstrated that determination of the proper resection margins for curative resection during surgery is possible, which we believe is much more advantageous compared with the current procedure using tattoo.

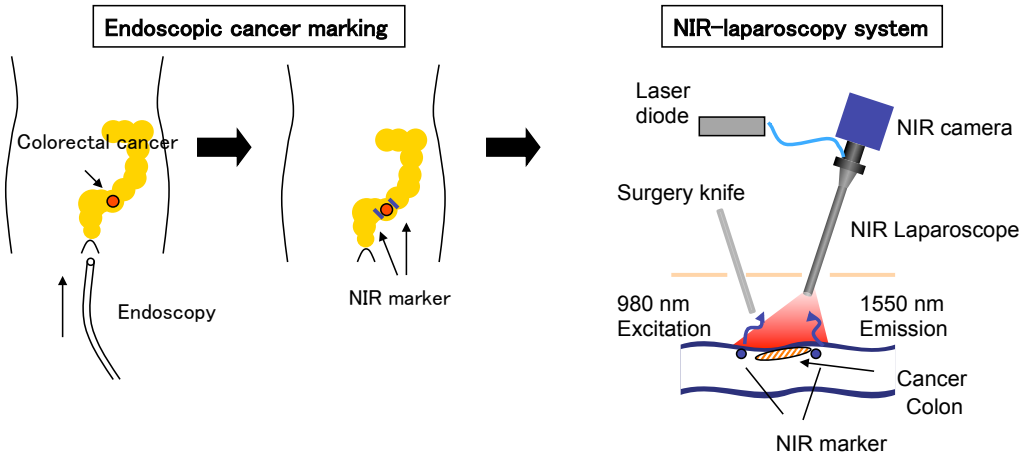

Fig.1 OTN-NIR laparoscopic cancer surgery system

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Frontiers of Endoscopic Diagnosis for Cancer

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Abstract: In recent years, image-enhanced endoscopy (IEE) such as narrow-band imaging (NBI) or blue laser imaging (BLI) has been used to better visualize the vascular patterns in the surface of the mucosa for enhanced diagnosis, such as histological diagnosis, distinguishing between neoplasia and non-neoplasia, and expectation of the depth of infiltration. However, further innovation regarding endoscopy will be required in the near future. Now, we perform development of new endoscopic systems. A novel imaging system using near-infrared light (NIR), which appears the microstructure of intramucosa and muscraris mucosa in horizontal direction, can lead the new strategies for diagnosis and treatment. Micrometer-Volumetric Optical Imaging System (μ-VOIS) is based on Optical Coherence Tomography (OCT). The μ-VOIS can visualize a three-dimensional (3D) cross sectional image at any directions. The 3D imaging after segmentation of tissue microstructure is useful for greater understanding of tissue structure. Furthermore, molecular imaging endoscopy using small molecule, peptide or nanoparticles is innovated. The clinical use of this system will be very innovative, and molecular imaging endoscopy is mentioned to a next generation endoscopy. In contrast, we focused a function or metabolism regarding cancer progression, and a novel imaging system equipped with a laser source is innovated in our hospital. We introduce these systems according to endoscopic classifications.

D4

Hybrid imaging probes for dual modality imaging of near-infrared light and radionuclides

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In vivo visualization of biological process by imaging tests is now important in research fields of biomedical science. There are many kinds of imaging tests such as optical imaging, X-ray test, magnetic resonance imaging (MRI) and radionuclide imaging. Each test has both advantage and disadvantage and multi-modality imaging can cancel disadvantages of each imaging test. Therefore, multi-modality imaging is attracting attention and hybrid imaging probes for multi-modality imaging are currently actively investigated.

Optical imaging is an imaging method to use fluorescence or luminescence of various kinds of materials and this imaging modality is simple and suitable for dynamic observation due to excellent time and spatial resolutions. But, optical method can observe only superficial areas because light can easily attenuate in materials. On the contrary, radionuclide imaging, scintigraphy, is a useful test to observe deep areas of objects and 3D imaging is possible. Moreover, radionuclide imaging has excellent quantitative features although its spatial and time resolutions of are suboptimal. If hybrid imaging probes for both optical imaging and radionuclide imaging are available, they would be quite useful.

We are investigating new type of hybrid imaging probes made of rare-earth doped ceramic nanoparticles (RED-CNPs), which are known to efficiently emit over 1000-nm near-infrared (OTN-NIR) fluorescence under NIR excitation. OTN-NIR fluorescence bioimaging (FBI) can provide higher transparency than conventional NIR imaging *in vivo*. Our previous study revealed that RED-CNPs could be labeled by ^{111}In, which is a radionuclide commonly used in radionuclide imaging. Our first hybrid probes, ^{111}In-labeled YPO_4 nanoparticles (^{111}In-YPO_4 NPs), whose average size was 110 nm, were applicable for both OTN-NIR FBI and NMI. However, these probes were rapidly trapped in the reticuloendothelial system (RES) after the injection into mice and their retention in blood was poor. It was hard to visualize target lesions or tissues by using these hybrid probes.

To improve the biodistribution of these hybrid probes, we modified these probes by following two strategies: 1) modification of probe surfaces by functional polymer such as polyethylene glycol (PEG) and 2) downsizing of probe particles.

First, we modified surfaces of ^{111}In-YPO_4 NPs by PEG-*b*-poly(2-(N,N-dimethylamino)ethyl methacrylate) (PEG-*b*-PAMA). Obtained probes (^{111}In-YPO_4 NPs modified with PEG-*b*-PAMA) were injected into mice and their biodistribution was evaluated. At 5 min after the injection of these probes, ^{111}In accumulation ratios in the liver and spleen were 19.8 and 13.1 %AD/g, respectively. These results indicated that simple PEG modification of ^{111}In-YPO_4 NPs failed to avoid RES-trapping of probes.

Then, we used oleic acid coated $NaYF_4$ NPs (OA-$NaYF_4$ NPs) instead of YPO_4 NPs. Average particle size of $NaYF_4$ NPs (OA-$NaYF_4$ NPs) was 20 nm. OA-$NaYF_4$ NPs were modified in chloroform suspension of 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [amino (polyethylene glycol)]-1,4,7,10-tetraazacyclo dodecane-1,4,7,10-tetraaceticacid (DSPE-PEG-DOTA) and labeled by ^{111}In. The smallest particle size of obtained probes was 60nm and dispersion stability of these obtained probes was highest under physiological conditions. Biodistribution of these hybrid probes can be improved, compared with our old ones, ^{111}In-YPO_4 NPs.

Wide Wavelength Range Tranasparent Image Detection
by Head-Scanning Mechanism

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Recent years, Biomedical Imaging by the light is attracting attention. As a wavelength gets longer, the light comes to have little damage to the body[1]. NIR whose wavelength range is between 700 and 2500nm is called "Biological Window". NIR are few absorption and dispersion. It is able to observe depth near a tissue surface of the body by using NIR[2]. If an endoscope using NIR, it is able to observe depth near a tissue surface of the body and early detection of cancer, but Si-CCD camera cannot capture NIR. InGaAs-CCD camera can capture NIR, but InGaAs-CCD camera is so large to use for endoscope.

In this paper, we propose the head-scanning mechanism for a NIR endoscope which is able to capture NIR. The head-scanning mechanism mainly consists of a photo detector and a 2-axis Micro electro mechanical system (MEMS) mirror (Fig.1).NIR laser is irradiated to beam splitter. Beam splitter split laser into two. One side laser is irradiated to 2-axis MEMS mirror. MEMS mirror scanned two dimensional surfaces. The reflection light is captured by photod etector. Image scanning experiments use 975nm wavelength laser and shading. This experiment inspects influence of covered with meet or not. Imaging experiments were conducted by using the proposed head-scanning mechanism. The experimental results suggest that the proposed method is able to capture NIR image and is useful for observe of depth near a tissue surface of the body.

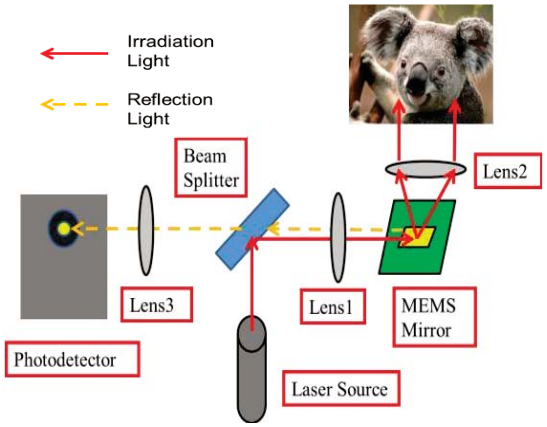

Fig.1 Hed-scanning system

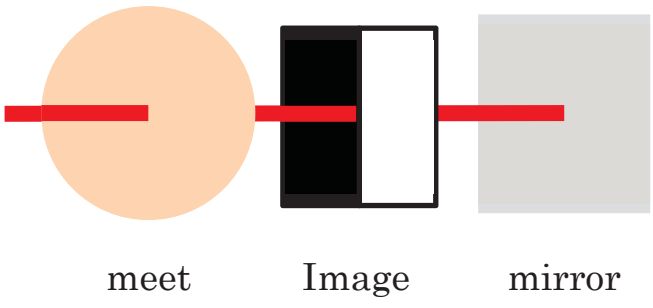

Fig.2 Transmission scanning experimental

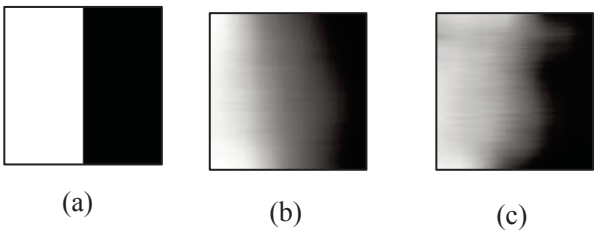

Fig.3 Detection image
(a) sample(b)NIR(c)transmission

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Support System Making Supervised Data Base for Development of Auto Pathological Diagnosis

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In Japan, cancer is the most cause of death, and the number of patients who are suffering with cancer is still increasing [1]. However pathologists are almost constant [2]. This makes them lot busy, and tends to cause the fault of the diagnosis. To prevent the fault of the diagnosis, auto pathological diagnosis is needed. A lot of methods for auto pathological diagnosis have been proposed [3][4], for example, how to calculate features, how to make computer learn and so on. However, pathological images are usually unique format, and researchers need to have special knowledge to work on these images. Therefore we propose the Support System for Development of Auto Pathological Diagnosis (SSDAPD). SSDAPD enables us to handle unique format with OpenCV. In addition, SSDAPD have the other functions to make developing auto pathological diagnosis easily. SSDAPD consists of the following three processes (Fig.1);

(1) Making the supervised database
(2) Machine learning from the database
(3)Detecting cancer using the learning data

In this paper, we developed the system which makes the supervised database. The format is “ndpi” developed by Hamamatsu Photonics K.K. when images are added into the database, we obtain supervised data to surround cancer areas (Fig.2).

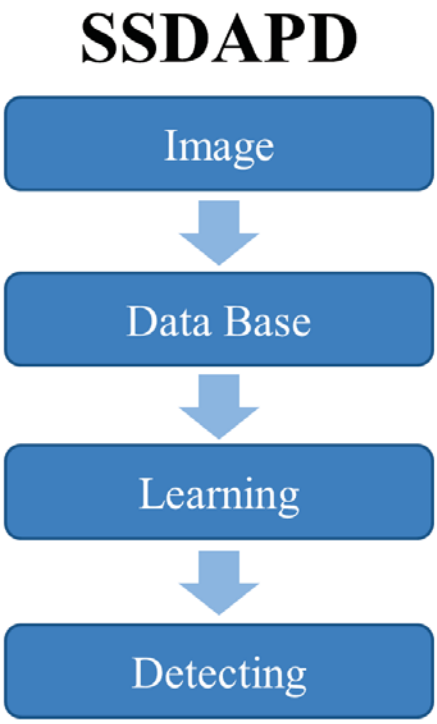

Fig. 1 SSDAPD

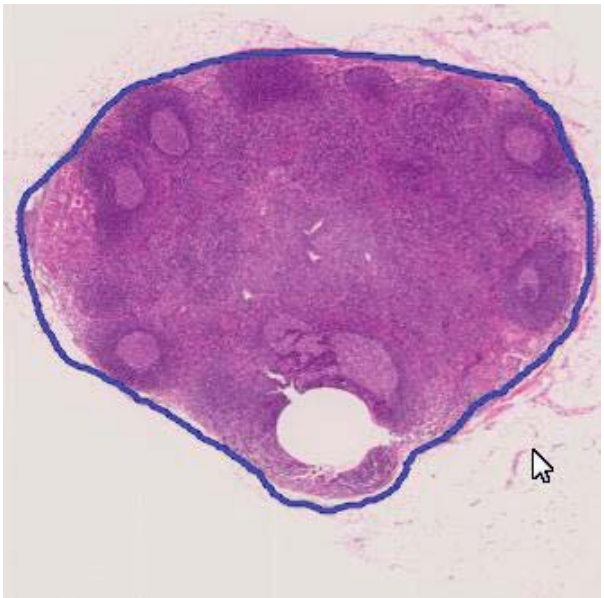

Fig. 2 Supervised image

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[4] Oliver Faust, U. Rajendra Acharya, and Toshiyo Tamura, “Formal Design Methods for Reliable Computer-Aided Diagnosis,” IEEE REVIEWS IN BIOMEDICAL ENGINEERING, Vol.5, (2012), pp.15-28.

Development of real-time image composite system for novel laparoscope with NIR camera

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Recently, a near-infrared (NIR) Fluorescence Biomedical Imaging (FBI) attracts increasing attention from many researchers. As shown in Fig.1, the light wavelength region from 1000 nm to 1700 nm has been known to be a "biological window" where the both tails of scattering and infrared absorption decrease to form a valley in optical loss spectra of biological objects. The inside of biological objects which cannot be usually seen in a normal laparoscope can be seen by using NIR, because of NIR light can penetrate in biological objects, and the further development of medical equipment is expected by using NIR. We are developing towards the realization of a novel laparoscope with NIR camera which composes of a NIR camera and a visible light camera. The visible light camera can observe the surface in biological objects, and the NIR camera can observe the inside of biological objects. The novel laparoscope with NIR has a potential of the helpful in the early detection of tumor. In our proposed system [2], the images captured by a NIR laparoscope and a normal laparoscope are shown on the separate screens simultaneously. The operator need to more experience to use the system. It was difficult to figure out the clinical usefulness of the NIR laparoscope with a visible light camera. We proposed the image composite method using affine transformation and a distance correction. As a result, we can composite two the grayscale NIR images and the colorscale visible images (Fig.2). The proposed method, compared to conventional methods, is simple and fast. The proposed composite method of the two images captured by a NIR camera and a visible light camera can facilitate the diagnosis and the surgery. The proposed system can reduce not only operator's burden but also patient one. We are applying the proposed method to a laparoscope and conducting the evaluation experiment of the proposed method.

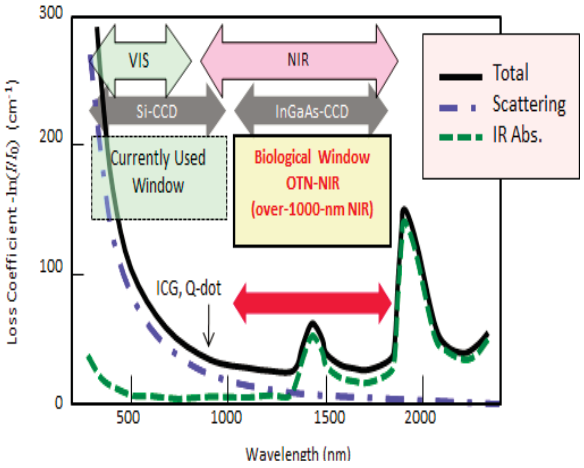

Fig.1 Optical loss of human skin[1]

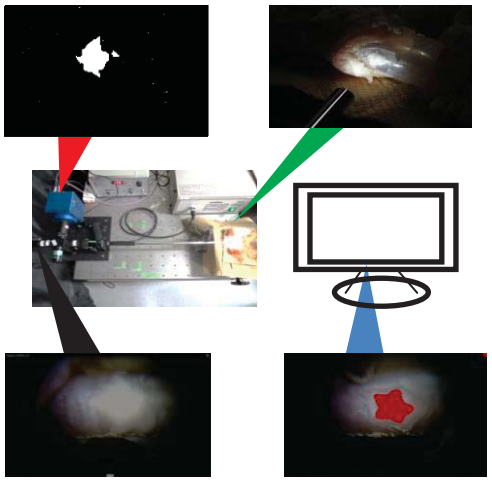

Fig.2 Situation of the composite experiment and results using the colon of the swine

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P4

Design, Synthesis and Evaluation of ^{11}B NMR Probes of d-Block Metal Ions

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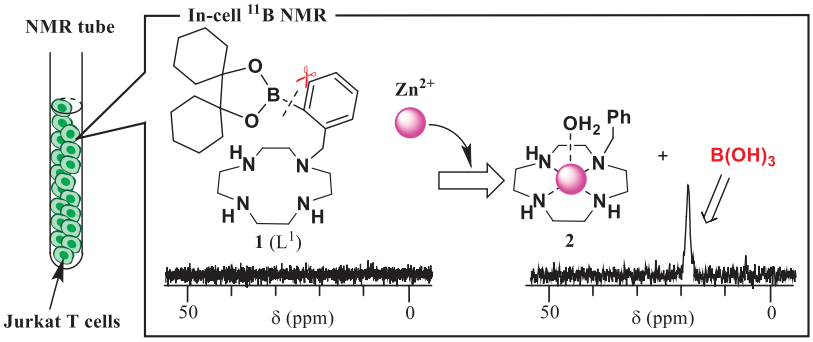
It is well established that Zn^{2+} levels is markedly decreased in prostate cancer and other cancer cells.[1] Therefore, the Zn^{2+} and other metal ions are potent candidates as biomarkers for its diagnosis. Accordingly, considerable efforts have been devoted to the development of fluorescent molecular sensors for the detection of metal cations in living systems. Unfortunately, this sensing technique sometimes suffers from the quench of the fluorescence and invisibility especially in tissue more than a few millimeters in depth due to light scattering and absorption. In addition, fluorescent sensors for cellular paramagnetic metal ions such as copper, iron, and nickel have remained underdeveloped, because these metals typically act as fluorescence quenchers.

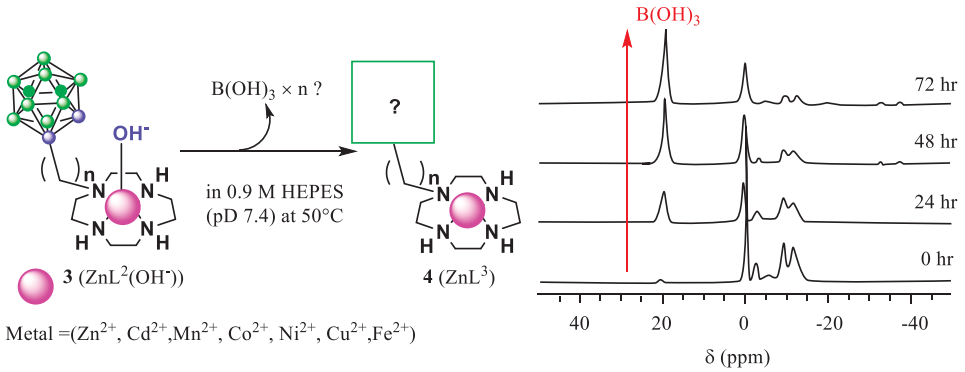
In this regard, noninvasive magnetic resonance imaging (MRI) based on ^{1}H NMR signals is a powerful method for the *in vivo* imaging of intracellular molecules and for monitoring various biological events. However, it has a drawback of low resolution due to background signals from intrinsic water protons. In this context, it is assumed that the ^{11}B NMR signals which can be applied to a "chemical shift imaging (CSI)" technique, have certain advantages, since boron is an ultratrace element in animal cells and tissues.

In our laboratory, the sensing of biologically indispensable d-block metal cations such as zinc, copper, iron, cobalt, manganese, and nickel based on ^{11}B NMR signals of phenylboronic acid-pendant cyclen **1**(L^1) (cyclen = 1,4,7,10-tetraazacyclododecane), in aqueous solution at physiological pH has already been reported.[2] The results indicate that the carbon–boron bond of $\mathbf{L^1}$ is cleaved upon the addition of Zn^{2+} and the broad ^{11}B NMR signal of **1** at 31 ppm is shifted downfield to 19 ppm, which corresponds to the signal of $B(OH)_3$. ^{11}B NMR signals of **1** is hardly affected by cell-derived noise. However it doesn't show high sensitivity because of small amount of $B(OH)_3$ released from $\mathbf{L^1}$ and small cellular uptake.

In this presentation, we will report on the sensing of d-block metal cations based on ^{11}B NMR signals of cyclen containing *o*-carborane **(L^2)** which is a B_{10} cluster. We expected that hydrophobic carborane analogs would be efficiently introduced into living cells and release of ten molecules of $B(OH)_3$ by the full decomposition of one *o*-carborane unit would result in much better sensitivity than that of **1**.

In this paper, the results ofdegradation reactions of **3**(L3, n=1~4) and *o*-carborane upon addition of metal ions such as Zn^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+} and other transition metals will be discussed. The transport of these ligands into living cells will also be reported.





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Design and Synthesis of Red-Color Emitting Cyclometalated Iridium(III) Complexes as pH Probes and Singlet Oxygen Sensitizers

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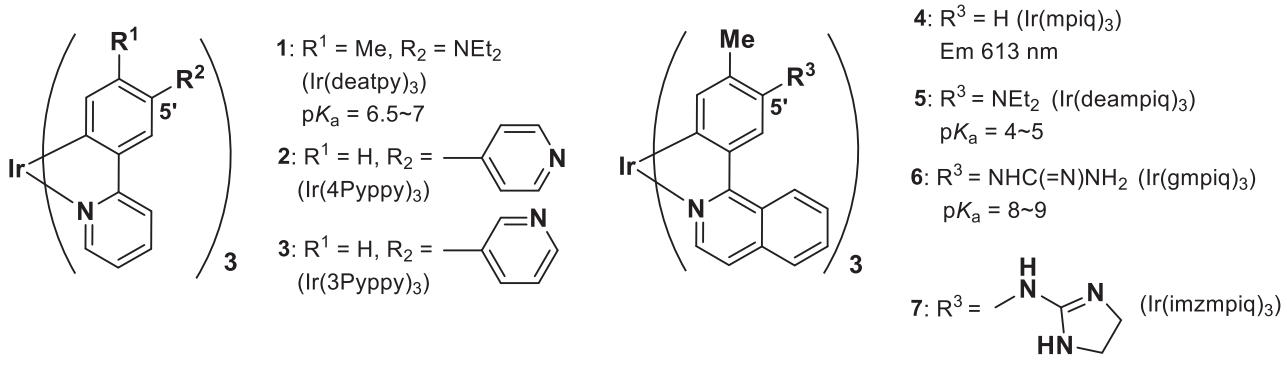
Cyclometalated iridium (III) complexes are receiving considerable attention because of their potential photophysical properties and are important candidates for use as luminescent probes for cellular imaging. We previously reported regioselective aromatic substitution reaction of $Ir(tpy)_3$ (tpy:2-(4'-tolyl)pyridine) at 5'-position of tolyl group,[1)] and prepared pH-sensitive iridium complex that contains three *N*,*N* –diethylamino groups, **1** ($Ir(deatpy)_3$), (deatpy:2-(5'-*N*,*N*-diethylamino-4'-tolyl)pyridine) [2)] and three pyridyl groups **2** ($Ir(4Pyppy)_3$) and **3** ($Ir(3Pyppy)_3$), (ppy:2-phenylpyridine) .[3)] It was found that **1**~**3** exhibit considerable pH-dependent emission change due to the protonation of their basic groups. [2)] The co-staining of HeLa-S3 cells with **1** and LysoTacker suggested that **1** is capable of staining lysosome, an acidic organelle in cells. In addition, **1~3** function as pH-dependent singlet oxygen ($^1O_2$) generators and induce necrosis-like cell death.[3)] However, observation of green emission of **1** is sometimes hampered by autofluorescence from the living tissues.

In this work, we designed and synthesized some new pH-responsive Ir(III) complexes; **5** ($Ir(deampiq)_3$), **6** ($Ir(gmpiq)_3$) and **7** ($Ir(imzmpiq)_3$) that basically emit red light and contain dialkyl, guanidyl and imidazolyl groups at the 5′-position of the mpiq ligand (mpiq:1-(4'-methylphenyl)isoquinoline). The pK_a values of these derivatives were predicted based on the pK_a values of the corresponding aniline derivatives by SciFinder database. It was found that the emission of **5** was almost silent at > pH 5 and a red luminescence emission was observed at < pH 5 and that necrosis-like cell death of HeLa-S3 cells is induced by photoirradiation of **5**, **6** and **7** at 465 nm. The synthesis and photoirradiated properties of **4**~**7** will be presented.



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Design and Synthesis of Artificial Death Ligands Based on Self-Assembling Homotrimeric Metal Ion Complex

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Apoptosis is the programmed cell death that is essential for the control of number of cells in organisms by the removal of cells at the appropriate time. Tumor necrosis factor related apoptosis inducing ligands (TRAIL) are reported to be effective apoptosis inducers without significant cytotoxicity.

TRAIL forms a homotrimeric structure by Zn^{2+}-centered self-assembly of monomeric unit and is capable of interacting with death receptors (DR)[1]. As shown in Figure 1, TRAIL has two important sites for complexation with DR5, which are called Patch A and B.[2] Our purpose in this work is to develop artificial death ligand whose DR-binding activity is switched "ON" upon the formation of trimeric structure around intracellular metal ions like TRAIL trimers. In this context, we decided to design and synthesize the hybrid compounds (L) of DR-binding peptides with metal chelators that coordinate to Zn^{2+} and Fe^{2+} to form trimeric complexes (ML_3), responding to the concentrations of these metals.

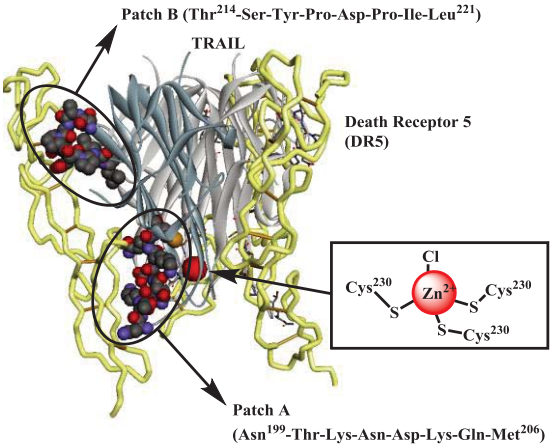
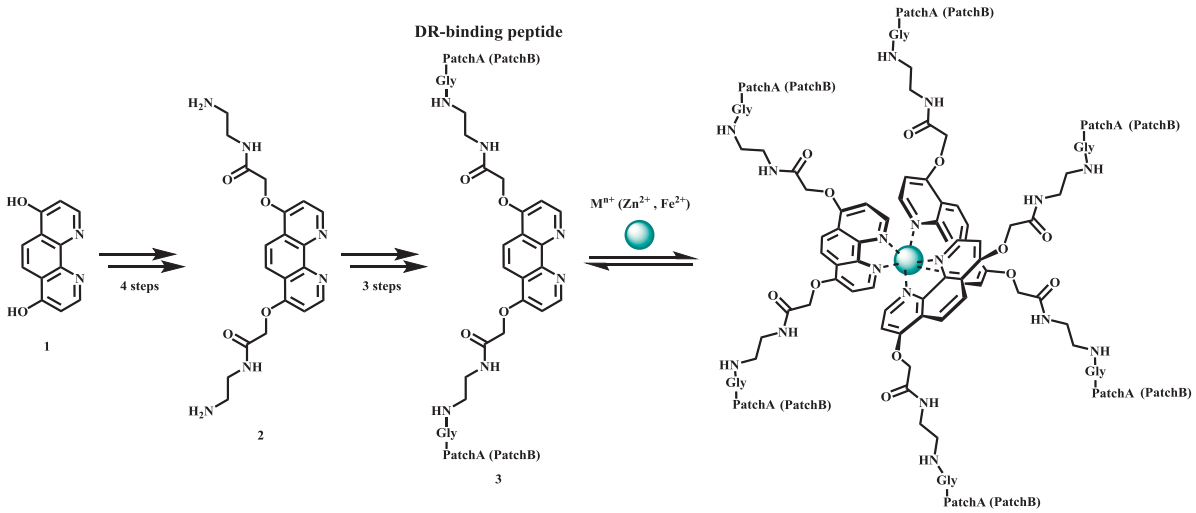


Figure 1. Co-crystal structure of TRAIL-DR5

Previously, we synthesized 2,2-bipyridine-based ligands having Patch A peptide. However, the affinity of these ligands with Zn^{2+} was very weak in aqueous solution. In this work, we tested several Zn^{2+} chelators and found that 1,10-phenanthroline **1** (1,10-Phen) is the best chelator for the formation of 3:1 complex with Zn^{2+} at neutral pH. We thus synthesized DR binding peptides by solid-phase synthesis and introduced them onto the 1,10-Phen scaffold **2** to obtain the 1,10-Phen-peptide hybrid **3** (Scheme 1). These results, their complexation with metal ions and their apoptosis activity will be reported.



Scheme 1. Synthesis of artificial death ligand

References

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[2] Sarah, G.-H.; Hans, W.-C.; Germaine, F. et al. *Mol. Cell* **1999**, *4*, 563-571.

Design and Synthesis of Artificial Death Ligands Based on C_3 Symmetric Iridium Complexes

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TNF-related apoptosis-inducing ligand (TRAIL) is a C_3 symmetric protein and induces apoptosis in various tumor cells by complexation with cell surface receptors such as death receptor 4 or 5 (DR4 or DR5).[1] As shown in Figure 1, TRAIL has two important sites for complexation with DR5, which are called Patch A and B.[2] Since it is described that TRAIL does not influence normal cells, the compounds having TRAIL-like activity have been proposed as promising agents for cancer therapy with lower side effects than those of the currently used anti-cancer agents. However, the reported instance of the artificial molecules having TRAIL-like activity have been limited.

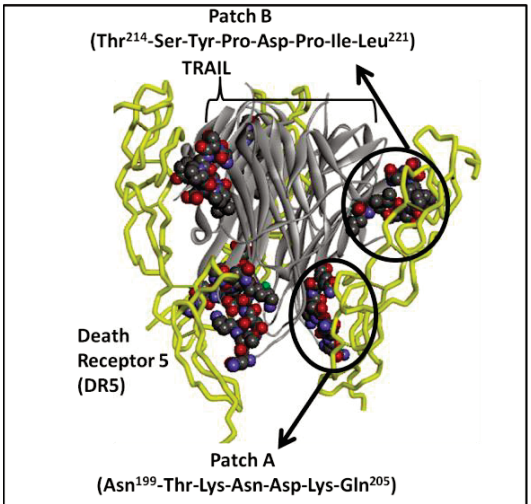

Figure 1. Co-crystal structure of TRAIL-DR5

Meanwhile, cyclometalated iridium (III) complexes such as *fac*-Ir(tpy)$_3$ **1** (tpy = 2-(4-tolyl)pyridine) have excellent luminescent properties and possess C_3-symmetric structure like TRAIL and DR's. C_3-symmetric Ir complexes having Patch A and/or B were designed and synthesized for mimicking TRAIL, luminescent imaging of cancer cells, and for mechanistic study of TRAIL-mediated apoptosis.

In this paper, design and synthesis of artificial death ligands **2-5**, whose Ir(tpy)$_3$ core is connected with N or C terminus of Patch A (N^{199}TKNDKQ^{205}) via linkers having different lengths, and artificial death ligand **6**, which has Patch A and Patch B (T^{214}SYPDPIL^{221}) through the L-lysine linker (Figure 2) will be reported. In addition, photochemical properties, and biological activity of these Ir complexes will also be presented.

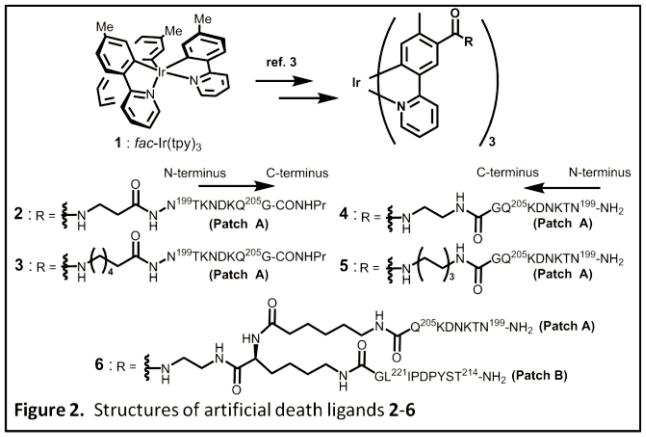

Figure 2. Structures of artificial death ligands **2-6**

References

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[3] Aoki, S.; Matsuo, Y.; Ogura, S.; Ohwada, H.; Hisamatsu, Y.; Moromizato, S.; Shiro, M.; Kitamura, M. *Inorg. Chem.* **2011**, *50*, 806-818

Design and Synthesis of Novel Zinc Inhibitors Based on the *in Silico* Screening by Machine Learning

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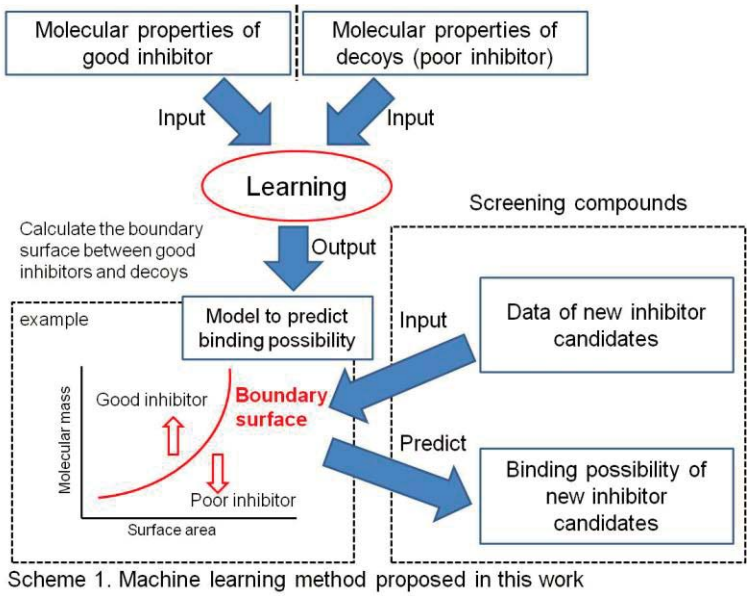
It is established that *in silico* screening is one of powerful ways of drug discovery. However, the application of the traditional structure-based and mechanism-based drug design is often hampered by the limited availability of the three dimensional structure of the target enzymes or proteins. We previously reported the selective inhibition of an aminopeptidase from *Aeromonas proteolytica* (AAP), dinuclear Zn^{2+} hydrolase, by 8-quinolinol (8-hydroxyquinoline, 8-HQ) and its derivatives.[1] The *in silico* docking study of 8-HQ with AAP using "CDOCKER" software program suggested that the "Pyr-in" mode is more favorable, while X-ray crystal structure analysis of the AAP-8-HQ complex disclosed that 8-HQ binds to AAP in the "Pyr-out" mode. There results suggest that the result of CDOCKER and related docking softwares have to be evaluated very carefully, and hence the alternative virtual methodology need to be developed.

This background prompted us to test new virtual screening method based on machine learning to predict the good inhibitors of specific enzymes, whose three dimensional structures are unknown.[2] In this method, the data of good ligands and decoys (poor inhibitors) are collected from the inhibitor's database "DUD-E". The molecular properties (physical and chemical parameters) of good ligands and decoys (ca. 60~70 parameters for each compound) were calculated by "Discovery Studio" and then input to the machine-learning software "LIBSVM" (SVM: Support Vector Machine), which calculates "boundary surface (hyperplane)" between good ligands and decoys and output the results as "model" files. Having these data, the molecular properties of new inhibitor candidates are input to "LIBSVM" to predict "binding possibility".

In this work, this technique was applied to the screening of inhibitors of carbonic anhydrase (CA), whose reaction mechanism and inhibitors have been extensively studied. The performance was visualized by the receiver operate characteristic (ROC) curve as shown in Figure 1, which proves the judgment accuracy of this method.



Scheme 1. Machine learning method proposed in this work

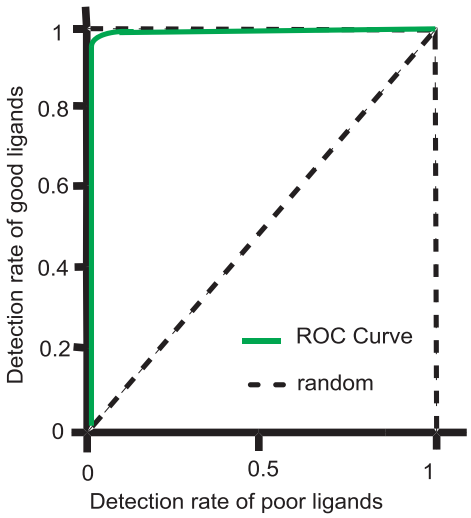


Figure 1. ROC curve for CA

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P9

Identification of human UGT isoforms responsible for glucuronidation of Arctigenin

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Arctigenin (AG) was identified from Burdock Fruit (Arctium lappa L), a traditional medicine as an antitumor agent having ability to eliminate cancer cells' tolerance to nutrient starvation. Burdock Fruit extract containing about 10% AG and its glycoside was elaborated and named GBS-01 at GMP grade is under clinical evaluation to treat patients with pancreatic cancer refractory to gemcitabine. During pharmacokinetic study in the phase I clinical trial, arctigenin was found to be conjugated with glucuronic acid quite rapidly. After oral administration, arctigenin was detected in the serum within 30 minutes at nM level and almost simultaneously arctigenin glucuronide (AGG) was detected about thousand time higher level although Cmax of AGG seemed to appear about 30 minutes later than that of AG. Among patients enrolled in the phase I trial, individual variation of pharmacokinetics was small but distinct. The 19 functional human UDP-glucuronosyl-transferases (UGTs) are classified into three subfamilies, UGT1A, UGT2A, and UGT2B and tissue specific expression of each isoform are known. In this study, we tried to determine site and isoform of UGT responsible for glucuronidation of arctigenin. In vitro assay, we detected strong activity both in human liver and intestinal microsomes. Among 13 recombinant human UGTs tested in vitro, UGT1A1, UGT1A3, UGT1A7, UGT1A9, UGT2B7, UGT2B15, and UGT2B17 were active. Glucuronidation activity of UGT1A9 is highest at unit protein and is more than 6 times higher than the other UGTs. IA1, IA3, 2B7, and 2B17 are expressed in the intestine and all the isoforms tested are expressed in the liver except 1A7. Arctigenin is secreted quite efficiently into the urine as AGG. UGT1A9 is known to be expressed in the kidney and might be related to the above fact.

P10

Mechanism of action of antiausterity agents

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Both tolerance to nutrients starvation and angiogenesis are essential for cancer progression because of the insufficient supply of nutrients to tumor tissue. Since chronic nutrient starvation seldom occurs in normal tissue, cancer's tolerance to nutrient starvation should provide a novel target for cancer therapy. We previously reported that arctigenin, pyrvinium pamoate, and kigamicin D, i.e., antiausterity agents, exhibited selective cytotoxicity against cancer cells under glucose-deprived conditions through inactivation of AKT in vitro and anti-tumor activity in vivo. We were conducted a phase Ⅰ clinical study to evaluate the activity of arctigenin in pancreas cancer patients refractory to gemcitabine and found to have a favorable clinical responses and safety profiles. However, precise molecular mechanism of action of these agents remained to be elucidated. In this work, we demonstrated that antiausterity agents selectively induced large amount of intracellular reactive oxygen species (ROS), especially hydrogen peroxide (H_2O_2) under glucose deprivation. When N-acetylcysteine (NAC), an antioxidant reagent, was added in the medium, antiausterity agents induced cell death in the absence of glucose was partially but clearly inhibited. The origin of ROS production was examined. We found that pyrvinium pamoate and arctigenin but not kigamicin D, inhibited oxygen consumption of PANC-1 cells. Taken together, ROS might be a common denominator of antiausterity agents regardless of different effect on mitochondrial respiration.

In-situ Transmission Infrared Spectroscopy of ATP in Aqueous Solution

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Organism maintains its life by spending an enormous amount of energy. In the human body, the energy is produced from adenosine triphosphate (ATP) through metabolism continuously. Mechanism of metabolism in the cells has been reported by many researchers, but details are yet under discussion. Decomposition reaction mechanism of ATP is one of them. Nucleotides such as ATP, are essential for many enzyme reactions in living matter. For example, it is well known that a hydrolysis of ATP, which produces an energy for muscle contraction, is promoted by Mg(II) ions[1] [2]. However, the reaction between Mg(II) and ATP in water is unclear, as it is difficult to analyze the reaction using conventional methods.

Transmission infrared (IR) spectroscopic measurement is known as an useful method for analyzing reactions, however, it does not suit for water or aqueous solution as an absorption of water is high in IR region. Thus, it is impossible to observe the chemical reaction in water using transmission IR spectroscopy. To solve this problem, we have developed a special cell, which optical path length is 1 μm, for water and aqueous solution. Accordingly, transmission IR spectra of sample in aqueous solution can be measured. In this study, transmission IR spectra of ATP solutions mixed with Mg(II) or Ca(II) ions are measured and compared to the nuclear magnetic resonance (NMR) results.

ATP (40mM) aqueous solutions with $MgCl_2$ or $CaCl_2$ have been prepared. And the pH value was adjusted to 7.0 by adding NaOH. The prepared solutions were introduced to the cell, of which body was hastelloy alloy and windows were CVD diamond, and then IR spectra were collected.

According to NMR spectra, ^{31}P peak position shifted to downfield by adding Mg(II) and Maximum downfield shift is observed at around $[Mg^{2+}]/[ATP]=1.0$.

In the transmission IR spectra, the two peaks at 1175 - 1300 cm^{-1} and 1050 - 1175 cm^{-1}, which are P=O and P-O stretching vibrational peaks, are detected. In the case of additive Mg(II), the peak of P=O vibration shifts to large wavenumber side. In the case of additive Ca(II), the similar shift was observed, however, integrated peak intensity of ATP-Mg is smaller than ATP-Ca. This result indicates that the number of oscillator in ATP-Mg is less than in ATP-Ca.

As conclusion, we successfully observed additional effects of Mg(II) (or Ca(II)) ions on ATP solution by using NMR and transmission IR spectroscopy. It was revealed that three phosphate groups of ATP molecule exhibit different behavior with Mg(II) (or Ca(II)) ions.

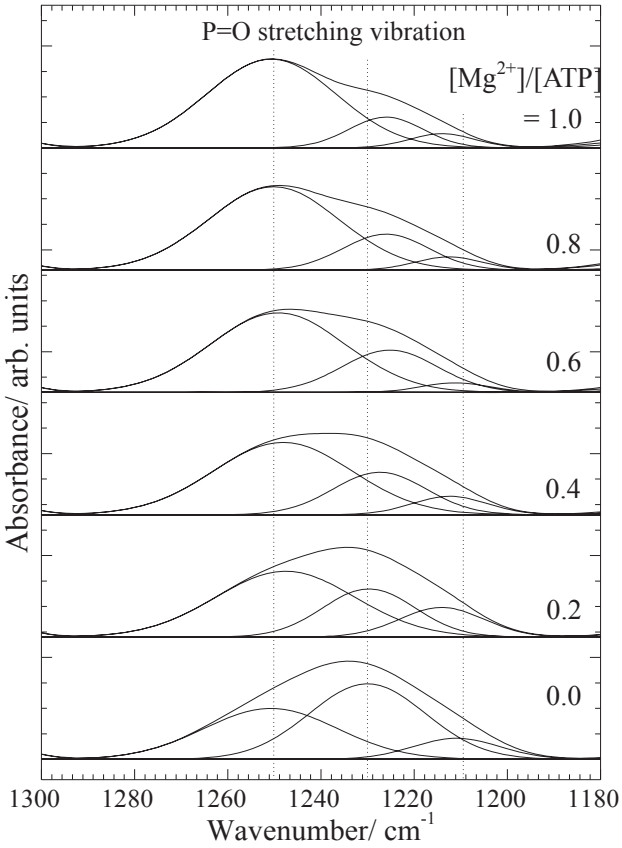

Fig.1 Transmission IR spectra of pure ATP and ATP-Mg complex in aqueous solution. P=O stretching vibrational peak exhibits blue shift by adding Mg(II) ions.

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P12

Electron microscopic observation of antigen and antibody

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The details of the antigen-antibody reaction were yet under discussion. Study of the microstructure is necessary to clarify the mechanism. Transmission electron microscope (TEM) is suitable for high resolution observation of microstructure. However, the contrast of biological soft matter is low because it is composed of light elements. Thus, an electronic staining with heavy metal is generally adopted. However, the generally stain is radioactive uranyl acetate[1]. Thus, there is a difficulty on treating uranyl stained specimen. In this study, phosphotungstic acid sodium (PTA) was chosen as nonradioactive stain, and we observed human serum albumin (HSA), anti-HEL monoclonal antibody and HSA-anti-HSA monoclonal antibody complex.

Preparing of HSA: HSA solution was adsorbed by the hydrophilized carbon film. HSA was positively stained by 4 mass% PTA.

Preparing of Anti-HEL monoclonal antibody: The antibody solution was adsorbed by the hydrophilized carbon film. The antibody was negatively stained by 1 mass% PTA.

Preparing of HSA-anti-HSA monoclonal antibody: HAy5[2] was selected as anti-HSA antibody. HSA and HAy5 were compounded and waiting for reaction over 30min. The complex solution was adsorbed by the hydrophilized carbon film. The complex was negatively stained by 1 mass% PTA.

HSA observation: The HSA was observed using STEM (Hitachi, HD-2300C) HAADF method of acceleration voltage 200kV. In Fig.1, observed HSA is compared to X-ray model[3]. The observed shape is a heart-shaped which is similar to X-ray model. The result was larger than X-ray model about 1-2 nm because the stain was adsorbed by the HSA surface.

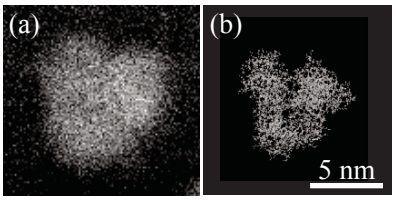

Fig.1 (a) Observed HSA (b) X-ray model[3]

Anti-HEL monoclonal antibody observation: The antibody was observed using TEM (JEOL, JEM-2000FX) of acceleration voltage 200kV. Fig. 2 shows the various shape of antibody. In (a), the Fab shapes were long form. On the other hand, in (b), the Fab shapes were round from and Fc shape is shorter than (a). Those observations show the flexibility of the antibody.

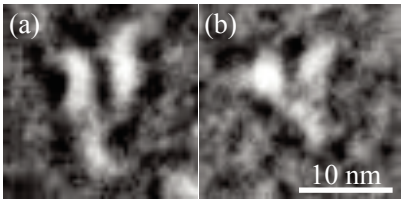

Fig.2 Antibodies observation

HSA-antibody complex observation: The complex was observed using TEM (JEOL, JEM-2000EX Ⅱ) of acceleration voltage 100kV. In fig.3, comparison between observed result and X-ray model[4] and the reaction site is lower left side of the HSA. Antigen-antibody reaction site presumably was observed.

In conclusion, HSA was heart-shaped, which was consistent with the shape estimated by X-ray crystallography. The antibody has the flexibility. Antigen-antibody complex was observed and reaction site presumably was confirmed. PTA is useful for biological soft matter TEM observation.

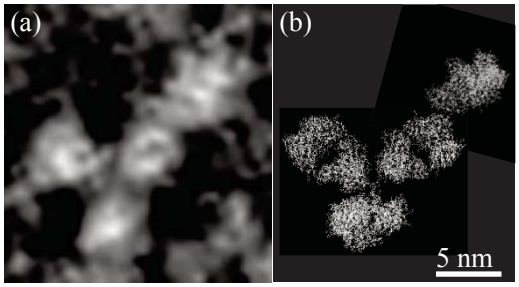

Fig.3 HSA (white arrow) – antibody (black arrow) complex (a) Observed (b) X-ray model[3], [4]

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In-vitro infrared spectroscopy for cancer diagnosis

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In-situ diagnosis method for cancer and degenerative disease is required on the fields in biology and medical science. Among them, "visualization" technology brings precise information and be implemented to medical devices. However, in-situ observation using conventional methods is difficult, because pretreatments, such as staining, are required for optical microscope observation.

In recent years, progress in imaging devices, such as high sensitive charge coupled device (CCD), facilitates 2 dimensional (2D) imaging using invisible light. Infrared (IR) imaging is one of them and sensitive for bonding states, which cannot observe using visible light. Thus, IR imaging suits for analyzing fine structural changes of biomaterial, such as protein, and it could be applied for cancer diagnosis. In this study, we performed IR measurement of a single cancer cell and compared to a normal cell. As this research required high quality IR spectrum of specimen to interpret the absorption peak, we employed transmission optical arrangement for measuring aqueous solution to avoid saturation of water large IR absorption coefficient.

Two types of cells originated from mouse, namely NIH3T3 (normal cell) and MCA205 (cancer cell), were adopted as specimens. Cells are cultured on CaF_2 window with poly-L-lysine coating and sealed with medium by another CaF_2 window, and the gap between two windows is maintained just 10 μm using Pt spacer. Infrared spectra were acquired using 10 μm square shaped aperture, i.e. each spectrum is corresponds to a single cell. Adherent cell to window are treated as living cells and floated sphere-shaped cell as dead cells. Background spectra were measured at brank medium area near the cell.

Figure 1 shows the phase contrast image of cells which were cultured on window. Long and dark shape are adherent cells i.e. living cells. We measured IR spectra of typical cells. Figure 2 shows IR spectra of four type cells. The significant difference between the normal cell and the cancer cell is the amount of intercellular CO_2. Furthermore, posthumous cancer cell contains lactic acid due to anaerobic glycolysis. Thus, IR absorbance measurement can distinguish single cancer cell from normal cells and applied for detecting circulating tumor cells (CTC).

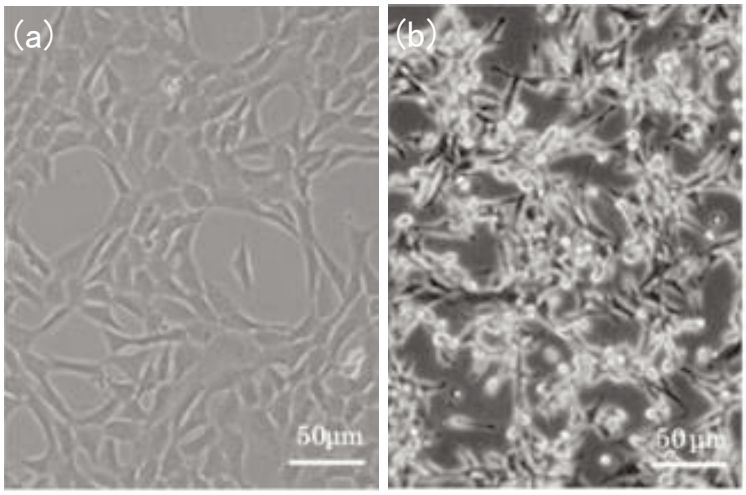

Figure 1 Phase contrast images of (a) normal cells and (b) cancer cells.

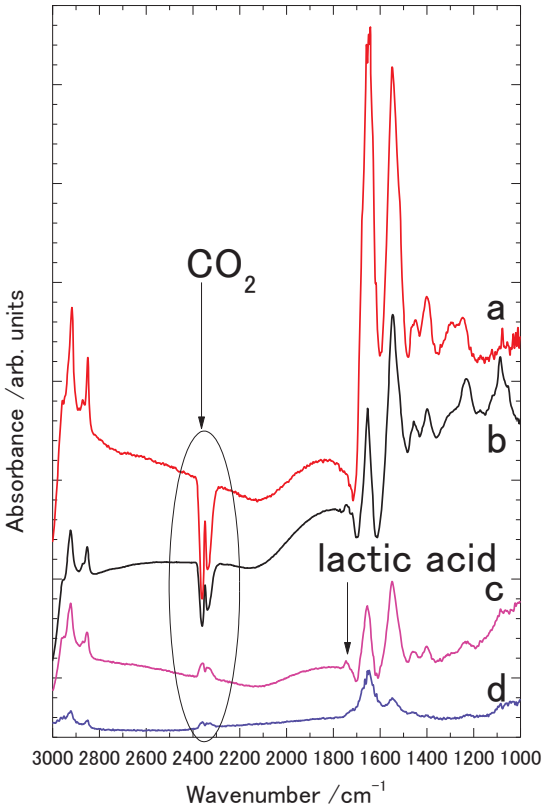

Figure 2 IR spectra of (a) dead normal cell, (b) living normal cell, (c) dead cancer cell and (d) living cancer cell.

Microfluidic device for capturing circulating tumor cells
-Separation by cell size and rigidity-

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Circulating tumor cells (CTCs) are tumor cells circulating in blood vessels, and detection or separation of CTCs is current topic. The CTCs are rare compared to usual blood cells, and there is no established method for CTC detection. It is said that CTCs tend to be larger, and several studied were performed for capturing large cells dispersed in blood using microfluidic device.

We have demonstrated recovery of cultured tumor cells dispersed in blood and enrichment of CTCs from tumor-bearing mouse blood by deterministic lateral displacement (DLD) microfluidic devices (fig.1)[1]. By the DLD device, cells were divided into large particle section and small particle section, and enrichment of CTC was observed in the large particle section. Although the enrichment of CTCs was demonstrated with tumor-bearing mice, the concentration of CTCs was lower than expected, and separation of blood cell and CTC was inadequate. It was presumed that blood cells became larger or number of large blood cell increased in the tumor-bearing mice.

It was also reported that CTCs are rigid than normal blood cells, and cell deformation in the DLD device was focused in order to improve the separation efficiency. Due to non-uniform flow velocity in the DLD device, cells deform in the DLD device by shearing force, and deformation of soft cells results in increase of the small section cell number, and sorting behavior was evaluated. Experimental results showed that number of small section increased by raising buffer flow velocity in the DLD device, while SP2/O (mouse myeloma cell) did not showed such sorting difference.

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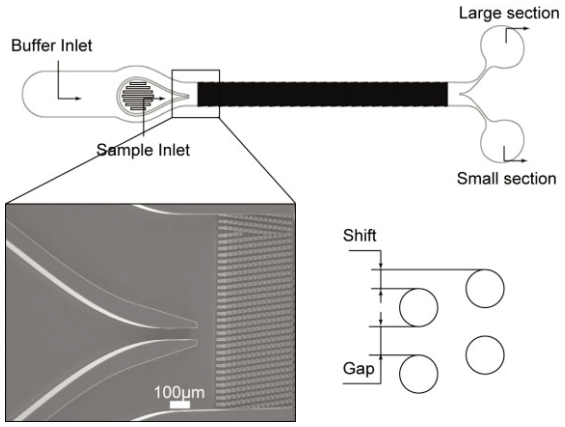


Fig.1 Separation ratio of lymphocytes

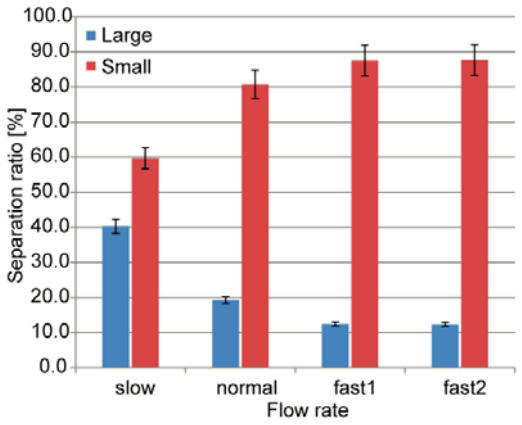
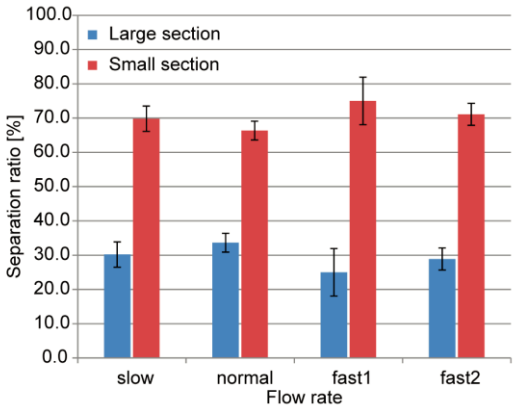


Fig.2 Separation ratio of lymphocytes



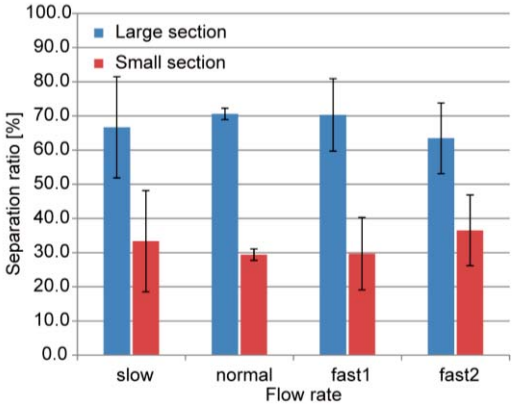


Fig.3 Separation ratio of tumor cell

Extraction of white blood cells with a microfluidic channel

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Blood cells are categorized mainly into white blood cell (WBC) and red blood cell (RBC). Number of WBC is 1/5000 to 1/7000 of the one of RBC. WBC plays an important role in immunity, and extraction of WBC was often performed. To extract WBC, centrifugal separation and hemolysis of the RBC are used, and most RBCs are spoiled. Damage to WBC by hemolysis is also concerned. Therefore, novel separation technique of blood cells which minimizes damage to cells is expected.

Recently, we have tested deterministic lateral displacement (DLD) devices for size sorting of cells, and found that RBCs and WBCs were divided though they have similar diameter. Then, we observed the behavior of RBCs in a DLD device. RBCs have biconcave shape and are flexible, and it was found that the RBCs behave as small particle in the DLD device by making standing attitude between the micro posts.

Therefore we thought that we might isolate RBC exactly in DLD microfluidic device if we could arrange RBC posture by some microfluidic devices. We assumed that posture of RBC can be controlled in some extent by modulating streamline in cell size scale.

Therefore, I thought that RBC posture changed by using the channel which section configuration changed(Fig.1).

In the result of the analysis, we were observed at the place can change RBC posture. As a result, RBC posture changed when I tested it using this device(Fig.2). However, there is little number of RBC which were able to change a posture, and further examination is necessary for RBC isolating.

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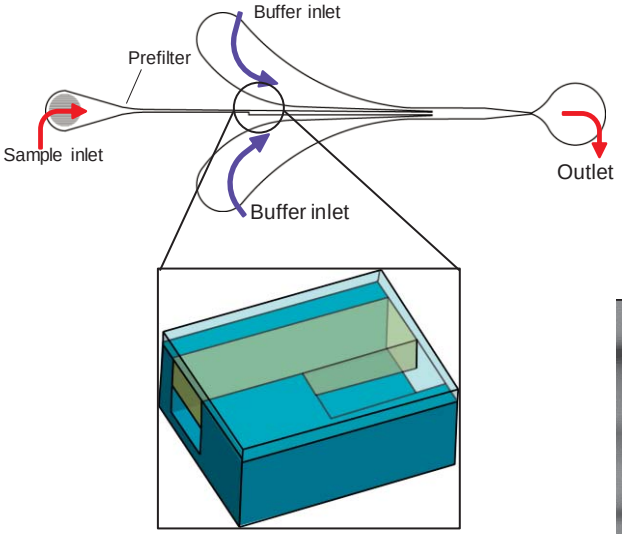

Fig.1 Channel form

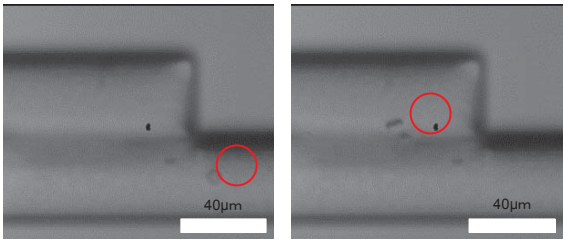

Fig.2 Change of posture

P16

in vivo NIR Fluorescence and Nuclear Medical Hybrid Bioimaging Probe

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Introduction

Rare-earth doped ceramic nanoparticles (RED-CNPs) are known to efficiently emit over 1000-nm near-infrared (OTN-NIR) fluorescence under NIR excitation. Recently, OTN-NIR fluorescence bioimaging (OTN-NIR FBI) by using the RED-CNPs has attracted attention, providing highly transparent *in vivo* imaging[1]. However the information obtained solely by OTN-NIR FBI is insufficient for the accurate quantification of the biodistribution while nuclear medical imaging (NMI) by using radioisotopes is a suitable method for quantitative studies. Previously, we have reported hybrid probes termed polyethylene glycol (PEG) modified ^{111}In-labeled YPO_4 nanoparticles (PEG-^{111}In-YPO_4 NPs) with the average size of 110 nm, applicable for both OTN-NIR FBI and NMI[2]. However, their retention in blood was low because PEG-^{111}In-YPO_4 NPs were trapped in the reticuloendothelial system (RES) after the injection into mice. The RES-trapping was expected to be avoidable by modifying surface with functional polymer such as PEG and making the particle size smaller. In this study, we intended to synthesize the hybrid imaging probes modified with PEG to avoid RES-trapping. To make the particle size smaller, we attempted to modify PEG, and label ^{111}In on the oleic acid coated $NaYF_4$ NPs (OA-$NaYF_4$ NPs) with the average size of 20 nm.

Experimental

Aproximately OA-$NaYF_4$ NPs with the size of 18±3 nm were synthesized by a hydrothermal method. 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (DSPE-PEG) and the OA-$NaYF_4$ NPs were dispersed in chloroform and added to distilled water, ethanol or methanol. The obtained emulsion was well stirred to evaporate chloroform and excess polymer in the solution was removed by a centrifugal washing. Finally, a chloroform suspension of 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (DSPE-PEG) and OA-$NaYF_4$ NPs with the average size of 20 nm was stirred until chloroform was evaporated after addition of distilled water, ethanol or methanol. Particle size and dispersion stability of the samples were investigated. OA-$NaYF_4$ NPs modified with DSPE-PEG-1,4,7,10-Tetraazacyclododecane-1,4,7,10 -tetraaceticacid (DSPE-PEG-DOTA) in methanol was ^{111}In-labeled.

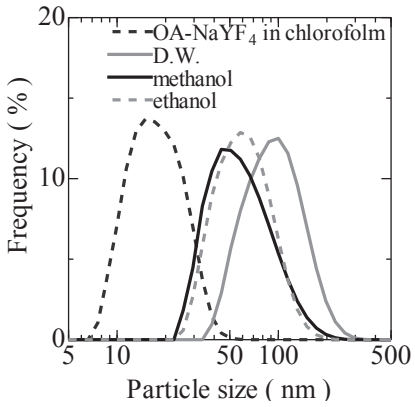

Fig. 1 Particle size distribution in D.W. of OA-$NaYF_4$ NPs modified with DSPE-PEG in D.W., ethanol and methanol respectively. That of OA-$NaYF_4$ NPs in chloroform was also shown.

Results and Discussion

Fig. 1 shows the particle size distribution of the OA-$NaYF_4$ NPs modified with the DSPE-PEG in distilled water. The size of the OA-$NaYF_4$ NPs modified with the DSPE-PEG in the ethanol and methanol were smaller than that in the distilled water. Because of the difference of the polarity between the chloroform and an alcohol is smaller than that of between the chloroform and water, aggregation in the reaction solvent was suppressed by using the alcohols. Fig. 2 shows the relative turbidity of the OA-$NaYF_4$ NPs modified with the DSPE-PEG in physiological saline. The relative turbidity of the OA-$NaYF_4$ NPs modified with the DSPE-PEG in methanol or distilled water was higher than that in ethanol. In case of the use of the ethanol, hydrophobic interactionity was weaker and the density of the DSPE-PEG layer decreased, because the difference of polarity between chloroform and ethanol was small. Next, smaller hybrid imaging probes with PEG were successfully fabricated by ^{111}In-labeling of OA-$NaYF_4$ NPs modified with DSPE-PEG-DOTA. This probe can be expected to avoid RES-trapping.

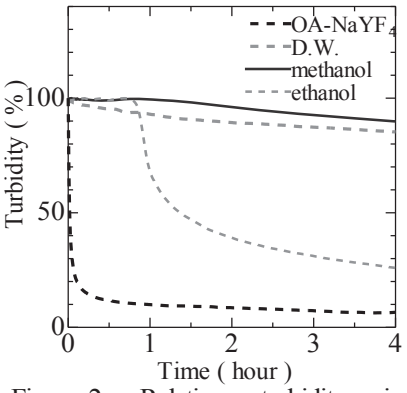

Fig. 2 Relative turbidity in physiological saline of OA-$NaYF_4$ and OA-$NaYF_4$ modified with DSPE-PEG in D.W., ethanol and methanol.

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Molecular Mechanism of SQAG action as a radiosensitizer

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SQAG is an abbreviation for a kind of natural sulfolipid, Sulfo-Quinovosyl-Acyl-Glycerol, which is a general component of plants. In previous studies, SQAG was suggested to suppress the tumor angiogenesis and have a property of a radiosensitizer [1-3]. Specifically, our studies revealed that SQAG treatment temporarily induces the remodeling of tumor microenvironments, which is known as 'vascular normalization', and reduced oxygen (hypoxia) domains in tumor turns to be re-oxygenized, and shrinks the tumor effectively by combining radiation treatment [1-3]. However, the molecular mechanism of SQAG action remained unclear. In this study, we examined how much effect SQAG had on HIF-1 signaling pathway. Western blot analysis using cultured cancer cell lines exhibited that SQAG inhibits the expression of HIF-1α in hypoxia, and then inhibits the expression of VEGF, FGF, and Ang-2. This leads to the efficient inhibition of angiogenesis in tumor tissues, leading to vascular normalization. The mTOR and ERK1/2 are involved in the expression of HIF-1α, but SQAG was not. On the other hand, SQAG significantly increased the expression of VHL (Von Hippel–Lindau), a tumor suppressor protein in tumor tissues. Accordingly, an increase in VHL may promote the degradation of HIF-1α in tumor tissues where the oxygen concentration is relatively high. Furthermore, it has been suggested that SQAG, in hypoxic conditions, may not directly inhibit HIF-1α by increasing the expression of VHL, but may reduce the expression level of HIF-1α by regulating negatively NFκB.

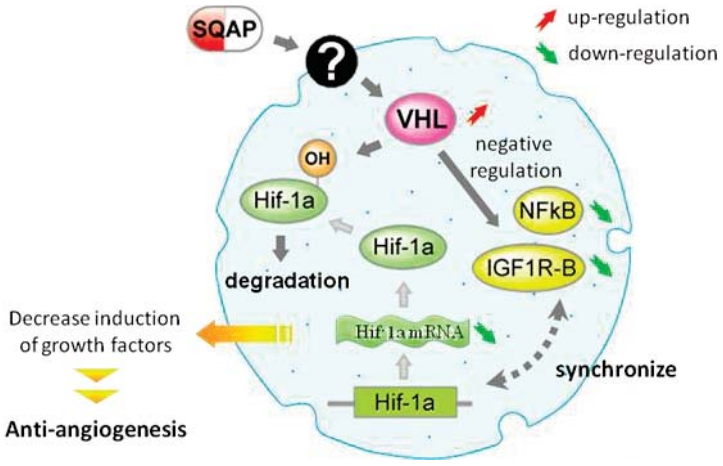

Figure. Illustration of molecular mechanism of SQAG action.

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P18

Ceramic Nanophosphors for Near-infrared Fluorescence Bioimaging Applications

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Rare-earth-doped ceramic nanophosphor (RED-CNP) materials are promising near-infrared (NIR) fluorescence bioimaging (FBI) agents that can overcome problems of currently used organic dyes including photobleaching, phototoxicity, and light scattering. Here, we report a NIR–NIR bioimaging system by using NIR emission at 1550 nm under 980-nm excitation which can allow a deeper penetration depth into biological tissues than ultraviolet or visible light excitation. In this study, erbium-doped yttrium oxide nanoparticles (Er^{3+}:Y_2O_3) with an average particle size of 100 and 500 nm were synthesized by surfactant-assisted homogeneous precipitation method. NIR emission properties of Er^{3+}:Y_2O_3 were investigated under 980-nm excitation. The surface of Er^{3+}:Y_2O_3 was electrostatically PEGylated using poly (ethylene glycol)-*b*-poly(acrylic acid) (PEG-*b*-PAAc) block co-polymer to improve the chemical durability and dispersion stability of Er^{3+}:Y_2O_3 under physiological conditions. *In vitro* cytotoxic effects of bare and PEG-*b*-PAAc-modified Er^{3+}:Y_2O_3 were investigated by incubation with mouse macrophage cells (J774). Microscopic and macroscopic FBI was demonstrated *in vivo* by injection of bare or PEG-*b*-PAAc-modified Er^{3+}:Y_2O_3 into C57BL/6 mice. The NIR fluorescence images showed that PEG-*b*-PAAc modification significantly reduced the agglomeration of Er^{3+}:Y_2O_3 in mice organs such as liver and enhanced the distribution of Er^{3+}:Y_2O_3 (Figure 1).

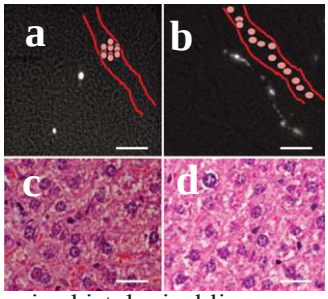

Fig. 1 Fluorescence emission from mice histological liver samples after 24 h injection period with (a) bare and (b) PEG-*b*-PAAc-modified Er^{3+}:Y_2O_3 nanoparticles (250 nm); (c) and (d) are bright field images of liver histological samples respectively (scale bar: 25 μm).

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Design and Synthesis of 8-Quinolinyl Sulfonate Derivatives That Undergo Photolysis with Visible Light and Application to Photocleavable Linker

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It is well known that circulating tumor cells (CTCs) are related to invasion and metastasis of cancer. Therefore, selective capture and detection of CTCs leads to prophylaxis of cancer disease and selection of the proper remedy. Accordingly, considerable efforts have been devoted to the development of device that could recollect and analyze specific live cells that exist in very small amount in blood.

Our effort has been focused on the development of devices for the capture and recollection of live target cells (Figure 1).[1] The platform will be silicon (Si) wafer that is modified with an anti-EpCAM antibody (anti–EpCAM–IgG (EpCAM: epithelial cell adhesion molecule)) through a photocleavable 2-methyl 8-quinolinyl sulfonate (8-MQS) linkers.[2] First, blood samples are flown into the device, and CTCs are trapped on silicon wafer. Second, photoirradiation promotes the photocleave of 8-MQS unit so that the trapped CTCs are released and recollected. For the treatment of collection and concentration of living cells with low damages, the photoirradiation at longer wavelength and more efficient cleavage reactions than our previous linkers are desirable.

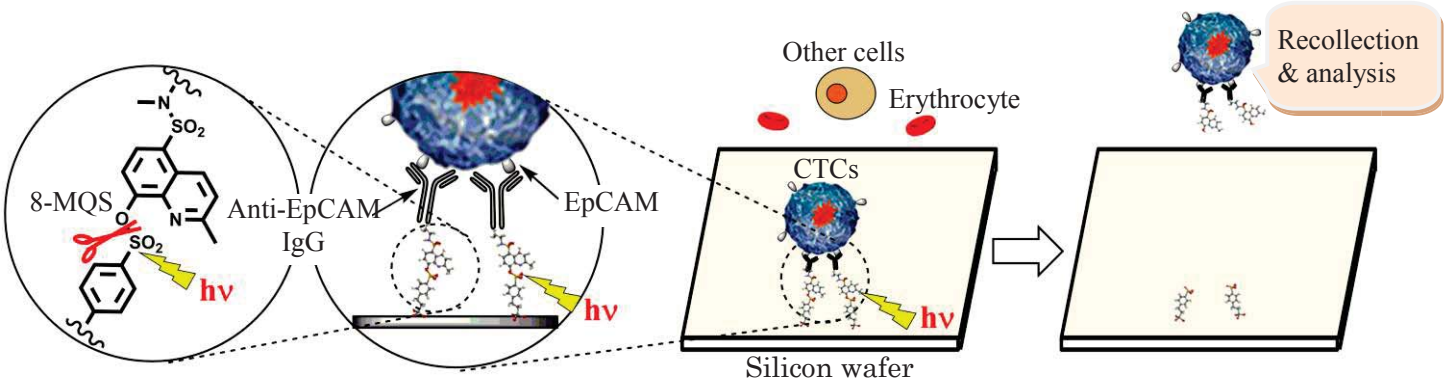

Figure 1. Our concept of the device for capture and release of CTCs

In this presentation, we report on novel photocleavable 8-MQS derivatives. For instance, it has been found that the compound **4** has absorption maximum at 383 nm, and can be cleaved upon photoirradiation with blue light (465 nm) more effectively than our previous derivatives **1-3** (Figure 2).[2c]

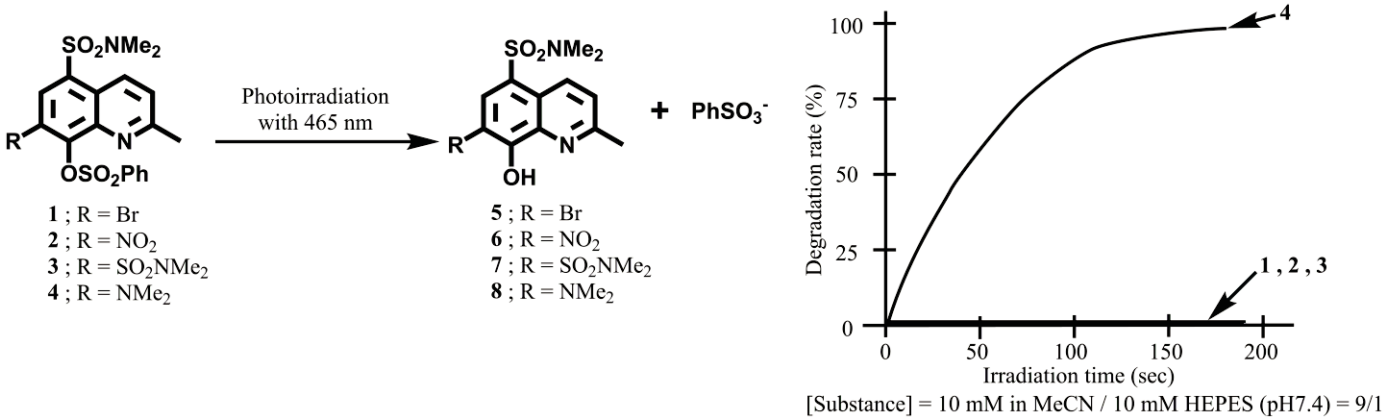

Figure 2. Photocleavage reaction of 8-MQS derivatives with visible light (465 nm)

References

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P20

A model of new cancer immunotherapy using antigen-specific B cells selected *in vitro*

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Monoclonal antibodies have recently been considered as most promising therapeutic drugs for human diseases such as cancer or autoimmunity. Currently most of them are produced as recombinant humanized antibodies based on monoclonal mouse antibodies, which potentially involve problems such as antigenicity in human body, laboriousness for generation and functional uncertainty of the mosaic antibodies. Alternatively, vaccination of patients with appropriate tumor antigens would potentially generate tumor-specific antibodies produced by long-lived plasma cells (LLPCs), but such attempts have so far not been very successful.

Here we propose a new cancer immunotherapy, where tumor-specific precursors of LLPCs are selected from patient's blood B cells *in vitro* and returned to the same patients, from which anti-tumor antibodies are produced. We first tested this method in a mouse model. We have established a B-cell culture system to induce *in vitro* germinal-center-like B cells (iGB cells) from mouse naïve B cells. After transfer into non-irradiated mice, the iGB cells became plasma cells and produced IgG antibodies for more than a month in the bone marrow of the recipient mice. When transferred into mice bearing melanoma cells expressing a surrogate tumor antigen, the iGB cells producing antibodies against the same antigen suppressed lung metastasis and growth of such melanoma cells and prolonged survival of the recipients. In addition, we have developed a novel culture system called FAIS to selectively expand antigen-specific iGB cells utilizing the fact that iGB cells are sensitive to Fas-induced cell death unless their antigen receptors are ligated by membrane-bound antigens. The selected iGB cells efficiently suppressed lung metastasis of melanoma cells in the adoptive immunotherapy model as stated above.

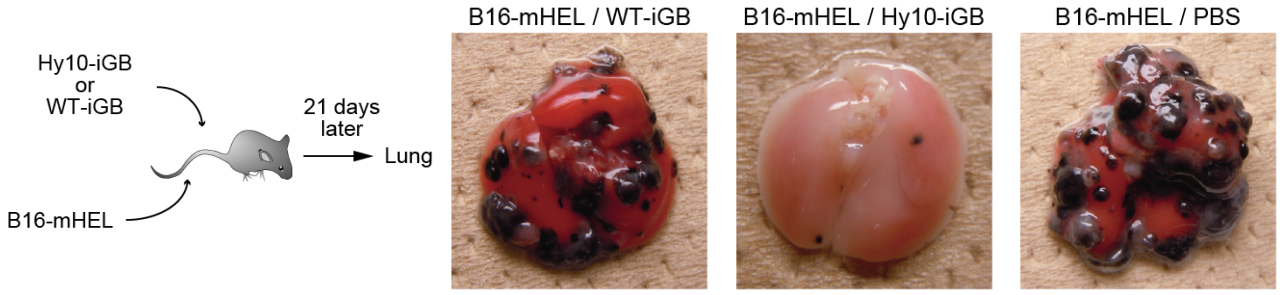

Figure 1. HEL-specific iGB cells inhibit lung metastasis of B16 melanoma cells expressing HEL in mice

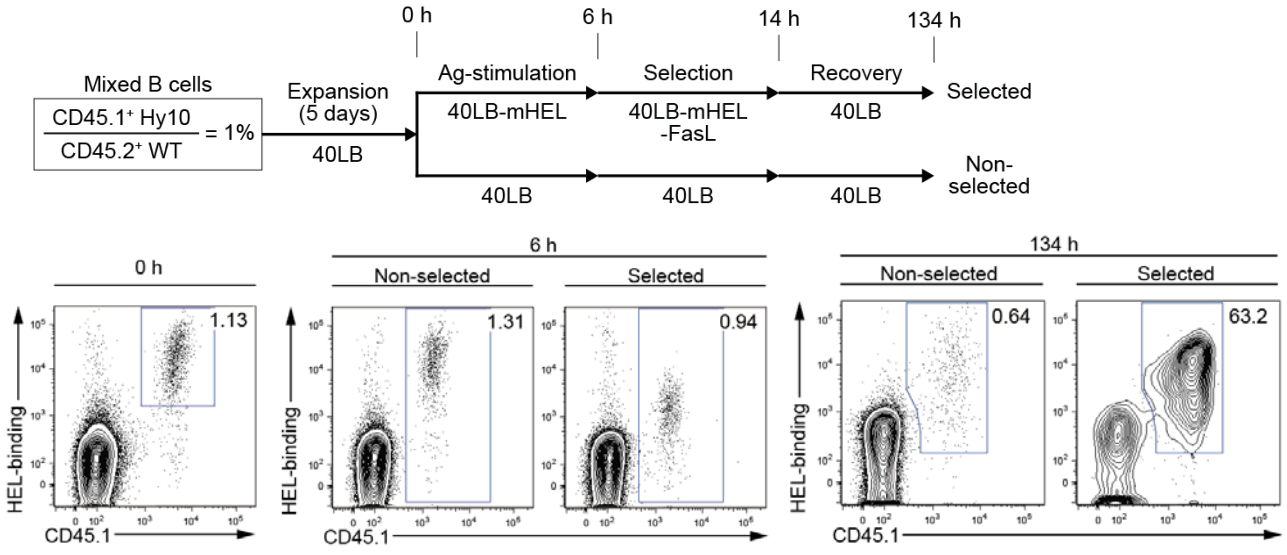

Figure 2. A B-cell culture system to selectively expand Ag-specific iGB cells

Effects of Water on Solvation Layers of an Aprotic Room-Temperature Ionic Liquid on Silica

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Room-temperature ionic liquids (RT-ILs) are a relatively new class of solvents. They exhibit unique physicochemical properties such as excellent thermal stability, negligible volatility, and non-flammability, etc. These physicochemical properties make them "ideal green solvents" in many industrially important reactions. RT-ILs have also gained importance in the fields of colloid and interface chemistry [1]. Recent atomic force microscopy (AFM) data have demonstrated that solvation layers consisting of ion pairs are spontaneously formed at solid/RT-IL interfaces [2]. On mica the protic RT-IL (ethylammonium nitrate, EAN) forms an interfacial innermost layer in a slightly disordered wormlike morphology, and an upper layer formed on the innermost layer gives less ordered and more undulating appearance. In the case of the aprotic RT-IL (1-ethyl-3-methyl imidazolium bis(trifluoromethylsulfonyl)imide, EmimTFSI), the imidazolium cations adsorb onto mica in a more isolated fashion. This difference is attributed to the hydrogen bonding network of the protic RT-IL, to promote self-assembly at the solid/liquid interface as well as in the bulk liquid [3].

Our research in this area has been directed toward understanding the effects of water on the interfacial properties of RT-ILs. In the field of inorganic material chemistry, the preparation of metal oxide particles in RT-ILs has attracted much attention because of their unique morphologies and crystalline structures. The metal oxide particles are synthesized through the reactions of hydrolysis and polycondensation of metal oxide precursors with water dissolved in RT-ILs. We expect, therefore, that in these reactions the interaction between water and metal oxide surfaces plays a key role in determining the unique morphologies and crystalline structures of the particles. In addition, surfactants usually act as a "structure directing agent" when preparing metal oxide particles, and hence the shape and size of the products are expected to be changed by the presence of surfactant assemblies formed in RT-ILs. From this point of view, the knowledge regarding the interfacial properties of RT-ILs with water in the absence and presence of surfactants will contribute to a better understanding of the preparation mechanism of metal oxide particles in RT-ILs.

In this study, we have performed AFM force curve measurements in order to assess the effects of water on the solvation layers of the aprotic RT-IL (EmimTFSI) formed on the silica surface. **Figure 1** shows the resulting force curve data obtained in the absence and presence of added water. The water concentration was set at 2.0 wt%, which is below the solubility limit of water in EmimTFSI. In the absence of added water (0.05 wt%), we detected the oscillating forces indicating the formation of solvation layers consisting of EmimTFSI ion-pairs. The addition of water gradually disrupted the oscillating forces (thereby the solvation layers) with increasing water concentration, and resulted in the disappearance of the oscillating forces at 2.0 wt%. Instead, an attractive interaction was observed from ca. 4 nm at this concentration. We assume that this attractive force originates from van der Waals interaction or bridging interaction between water layers formed on the silica and cantilever surfaces. This means that the solvation layers consisting of the EmimTFSI ion-pairs are replaced by the water layer. The formation of the water layer was also supported through the depletion analysis in the colloidal silica/EmimTFSI dispersion system.

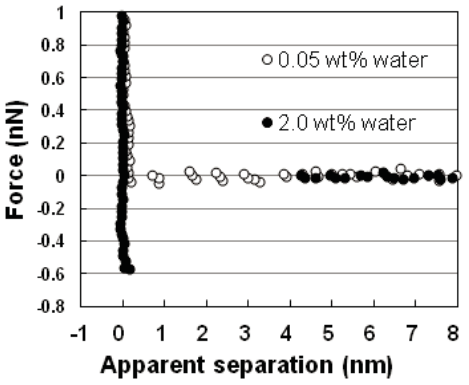

Figure 1. Force curve data measured in EmimTFSI with and without added water.

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Fabrication of Micro Half Cylindrical Grooves on Quartz Substrate

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Affinity capture of target cells using antigen-antibody interaction is widely used in biological science. We have also attempted to capture specific cells using the affinity capture in a microfluidic channel. In the study, antibody coated quartz substrate was prepared. Target cells expressing specific antigen were adsorbed on the substrate, and adhesion force was estimated. Moderate capturing force was observed, but some cells which should be captured were not captured. In those experiments, flat substrate was used, and preliminary experiments with a substrate having hemispherical depressions showed better capturing efficiency. Cells are basically spherical, and it was presumed that contact of cell surface to antibody coated surface was prompted by hemispheric shape with appropriate diameter. But it was concerned that cell removal by strong flow would be difficult once cells fit into the depressions. In order to prompt the contact of cell surface to the substrate, in this study, we attempted to fabricate half cylindrical grooves on a quartz substrate as shown in figure 1.

Figure 2 shows fabrication process. To etch quartz substrate, hydrofluoric acid was used, and tough mask was required. There was no appropriate photoresist for the hydrofluoric acid etching, and poly-silicon was employed as the mask material for the etching. Poly-silicon was deposited on the quartz wafer, and usual photolithographic patterning was performed to make thin line mask pattern. To etch the poly-silicon, dry etching with SF_6 was used. Then, isotropic hydrofluoric acid wet etching was performed, and half cylindrical grooves were formed. To obtain precise semicircle cross section, narrow line shape opening was required as the mask for the wet etching. After process optimization, line opening with 1μm width was achieved with a laser drawing machine whose spatial resolution was 3μm in its specification. Figures 3 and 4 show the grooves made with the above process. It was found that depth of the groove was proportional to the etching time at least until 8μm. Further affinity capturing study will be performed with the substrate with the grooves.

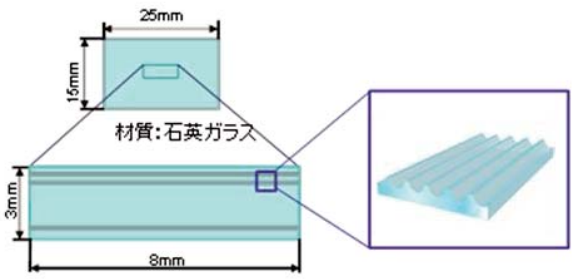

Fig.1 Overview of the quartz substrate.

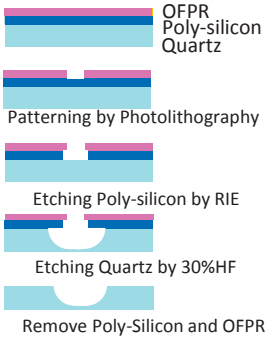

Fig.2 Fabrication procedure.

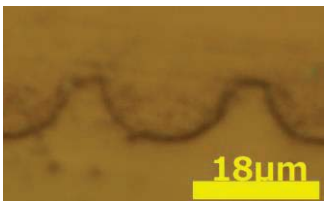

Fig.3 Cross section of substrate

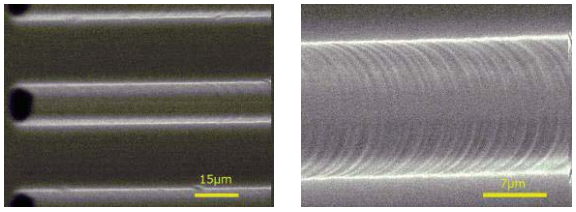

Fig.4 image observation by SEM

Application of Liposome-Encapsulated Rare-Earth Doped Ceramic Nanoparticles for OTN-Near-Infrared Fluorescence Bioimaging

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Introduction

Over 1000 nm near-infrared (OTN-NIR) light is highly transparent for living tissues and expected to be applied to fluorescence bioimaging (FBI)[1)]. Rare-earth-doped ceramic nanophosphors (RED-CNP) are known to emit efficient NIR fluorescence under NIR excitation and are expected as a good candidate for the NIR-FBI phosphor. In order to apply them as the FBI probes, prevention of nonspecific adsorption, activation for specific interaction to the target cells and the dispersion stability under physiological conditions are required. Liposome is bilayer vesicle composed of phospholipids that have a similar composition with biological membrane and has high biocompatibility. A variety of functional polymers and targeting ligands can be introduced on the surface of liposome easily. Thus, liposome encapsulated RED-CNP (CNP-Lipo) are expected to be highly functional FBI Probes. Furthermore, RED-CNP can be introduced into inside of a cell by membrane fusion (lipofection). In this study, we synthesized two kinds of FBI probes with the aim of the application of CNP-Lipo. One is the NIR-Lipo modified with single chain antibody (scFv) (scFv-CNP-Lipo) which has specific interaction onto target cells, and the other is the cationic CNP-Lipo which can be delivered into the cancer cells by lipofection and be applied as tumor formation observation probes.

Experimental

The CNP-Lipo modified with N-hydroxysuccinimide groups (NHS-CNP-Lipo) were prepared by microencapsulation. The CNP-Lipo modified with scFv (scFv-CNP-Lipo) were prepared by an reaction of NHS-CNP-Lipo with scFv at pH 8.3 for 12 h at room temperature, where a molar ratio of NHS : scFv was 10 : 1. The scFv-CNP-Lipo were mixed with the target cells and the non-target cells. The cells were observed using NIR microscopy.

The cationic CNP-Lipo were prepared by microencapsulation. The cationic CNP-Lipo were mixed with LLC cells under serum-free medium for 3 hours, then, serum added to medium and incubated 37 °C for 1 day. The cells were suspended in Matrigel at 8.0×10^7 cells/mL concentration and were injected 100 μL into mice through subcutaneous injection. The mice were observed *in vivo* under the NIS-OPT imaging system.

Results and Discussion

Figure 1 shows the NIR images of the scFv-CNP-Lipo mixed with the cells. The NIR-Lipo could be observed on the target cells, while they could not be observed on non-target cells. This result indicates that we successfully prepared the scFv-CNP-Lipo which has specific interaction onto target cells without being adsorbed on non-target cells. Figure 2 shows the NIR images of mice after subcutaneous injection of LLC cells lipofected with the RED-CNP. The fluorescence intensity in the images decreased with time. The decrease is due to the decrease of the fluorescence intensity per cells caused by the cell divisions and the volume increase of the whole cancer tissue, which in turn causes the decrease of the special density of the RED-CNPs by the growth of the cancer tissue. Thus, the growth behavior of the cancer tissue was successfully observed by the in vivo OTN-NIR FBI.

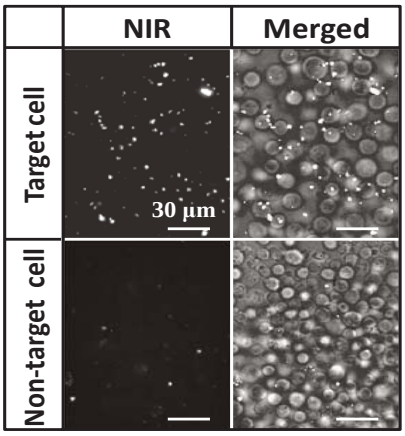

Figure 1. NIR images of the scFv-CNP-Lipo mixed with target and non-target cells.

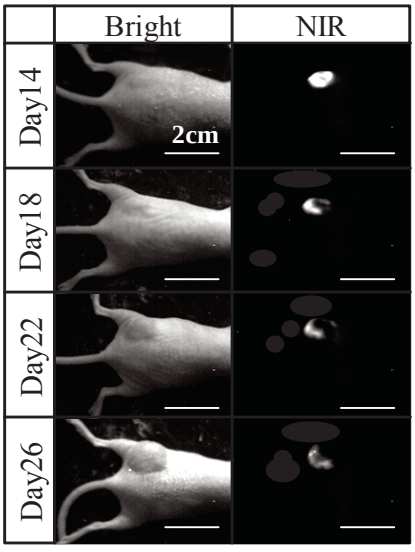

Figure 2. *in vivo* NIR fluorescence images of mice after subcutaneous injection of cells lipofected with the RED-CNP.

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P24

Ridaifen-B induces apoptosis of cancer cells using various signaling pathways

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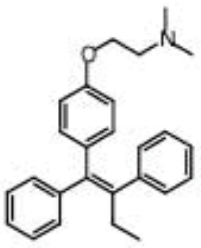
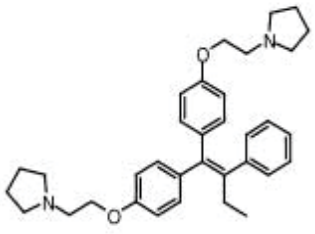
Tamoxifen is an antagonist of estrogen receptor, which is used widely as an anticancer drug that inhibits proliferation of breast cancer cells and induces apoptosis. However, recent studies have brought out that tamoxifen induces apoptosis even in estrogen receptor-negative cells. To enhance the effect of tamoxifen as an anticancer drug, we synthesized tamoxifen derivatives, ridaifens. We have explored the effect of ridaifens on the proliferation of various tumor and normal cells. In the present study, human hepatoma cell line, HuH-7, and primary cultures of rat hepatocytes were treated with ridaifen-B to examine whether it has any effects on the proliferation of these cells. In addition, the signaling pathway of the effect induced by ridaifen-B was also investigated.

Rat normal hepatocytes were harvested from 8 week-old female rat livers. The primary culture of rat normal hepatocytes and HuH-7 cells were incubated in 96-well and 24-well cell culture plates at 37℃ for 24h. Each cell was treated with ridaifen-B for 48 h, then cell numbers were counted by using wst-1 and hemocytometer independently. It was shown that ridaifen-B decreased the viability of HuH-7 cells in a dose-dependent manner, whereas it didn't decrease the viability of rat normal hepatocytes. Western blotting analysis indicated that ridaifen-B may induce the apoptosis of HuH-7 cells through the alteration of caspase 3 expression levels, and increases the caspase 3 activity. On the other hand, it has no effect on the primary cultures of rat normal hepatocytes. However, cell-counting using hemocytometer showed different result from that by wst-1. This shows that ridaifen-B might have multiple pathways to affect cancer cells. We are now conducting further study on intracellular signaling pathways to elucidate the mechanism of apoptosis of HuH-7 cells induced by ridaifen-B.



tamoxifen



Ridaifen-B
(RID-B)

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Binary Classification of Compounds by Learning from Docking Software Results and Chemical Information

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This paper proposes a new approach to classification of binding of compounds with proteins for drug design virtual screening. Currently, docking software programs often use real numbers as docking scores; but due to their predictive accuracy, it is difficult for biologists to use such scores in realistic experiments. In contrast, our approach utilizes binary classification that indicates whether a candidate compound is docked to a target protein. This leads to automatic screening of compounds without the input of biologists in drug design. The present method provides consensus use of the scores of existing docking software, yielding higher accuracy of binary classification. In this paper, we discuss several implementations of the proposed method based on Support Vector Classification and Regression. We have created a classification model from docking scores and chemical information. The experiment demonstrates that our method outperforms the existing docking software in classification.

Device for capturing circulating tumor cells
- Combination of dimensional difference and specific adsorption -

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Two step capturing of circulating tumor cells (CTC) was attempted. Capturing CTCs is one of current topics in cancer research, and many studies have been performed. We demonstrated that CTC was enriched by selecting large cells in a blood, but the concentration of CTC was still quite small even after the selection. In this study, we used the size selection system as shown in figure 1 in the first step. Then, affinity capture using specific adsorption between antibody and antigen was performed in the second step. SP2/0-EGFP cells (mouse myeloma cells) were spiked into blood, and the blood was sorted by the two step capturing system. Figure 3 shows an example scene in the first step size selection. Then, the selected large cells were poured into the second step settling channel which has antibody coated wall on the bottom. Figure 4 shows that most of the captured cells showed GFP fluorescence.

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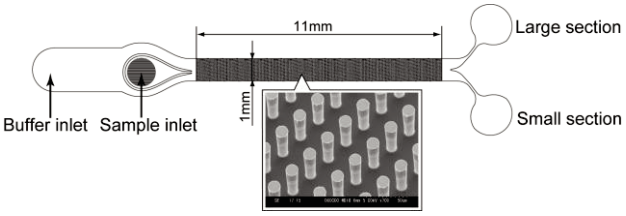


Fig.1 Microchannel for size sorting

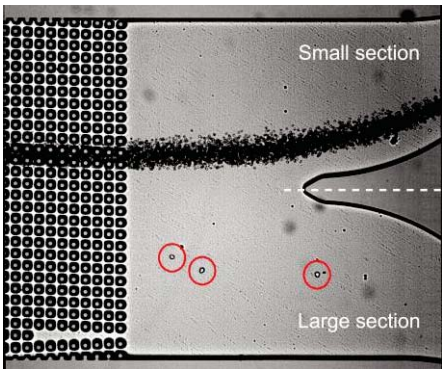
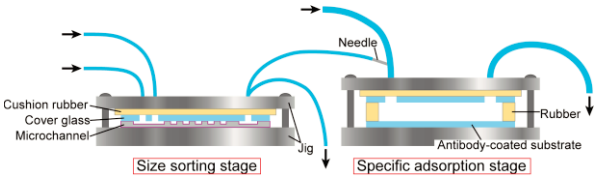


Fig.3 Size sorting stage



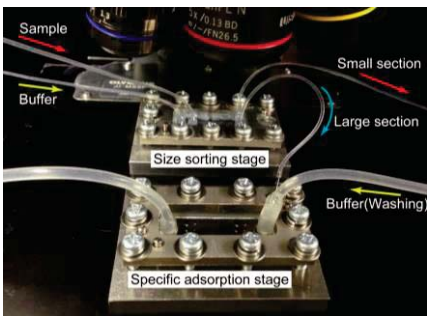


Fig.2 Two stage device

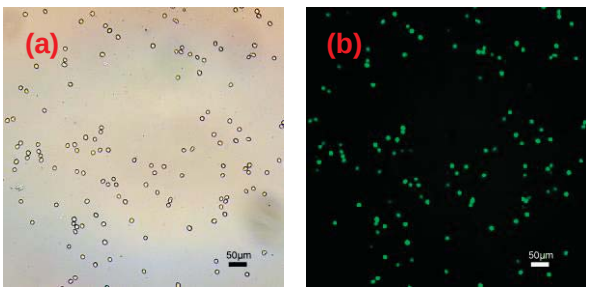


Fig.4 Specific adsorption stage
(a) Captured cells (b) Fluorescence of tumor cells

Identification of Necrotic DNA Fragmentation

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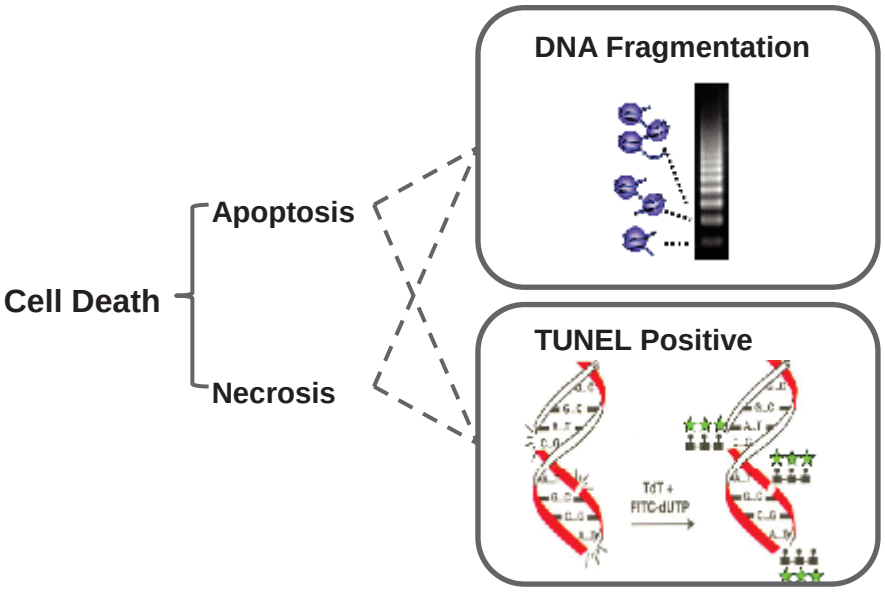
Studying cell death, apoptosis and necrosis, has been the major interest in the field of cell biology. It will be also essential for understanding cancer biology and cancer therapy. Despite substantial efforts in the past, the mechanism and physiological significance of cell death have not been still fully understood. Especially, compared to the study of apoptosis, the study of necrosis has been much delayed. Then, we focused on necrosis in this study.

Apoptosis and necrosis can be distinguished from each other morphologically and biochemically. Apoptosis is characterized morphologically by the formation of apoptotic bodies, membrane blebbing, nuclear breakdown, and chromatin condensation, and biochemically by the formation of internucleosomal DNA fragmentation, or DNA ladder, and TUNEL (fluorometric terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling) positive signals. Especially, DNA ladder and TUNEL are widely accepted biochemical criteria for determining apoptosis. Recently, however, this notion has been challenged by the reports showing that the DNA ladder can be detected in cells undergoing necrosis and TUNEL positive necrotic cells are detected. The notion of necrotic DNA fragmentation, however, has some uncertainty and has not been widely accepted. The uncertainty partly stems from the poor understanding of its molecular mechanism, including the identification of the responsible endonuclease, and its physiological significance. Here, we showed that DNase γ, a Mg^{2+}/Ca^{2+}-dependent endonuclease, is responsible for the DNA laddering during necrosis caused by the physical destruction of cells through freeze-thawing or membrane disruption with saponin or digitonin, by tissue ischemia, or by TNFα-receptor signaling under caspase-free conditions and that DNase γ generates TUNEL-positive 3'-OH DNA ends. Thus, DNA fragmentation and TUNEL positive are not always the specific criteria of apoptotic cell death, and necrotic DNA fragmentation should be taken into consideration. We also found that the DNase γ-dependent necrotic DNA fragmentation is essential for the clearance of dead cells. This suggests that DNA fragmentation is not a mere criterion but is critical for maintaining homeostasis *in vivo*. It will be interesting to study how necrotic DNA fragmentation can be correlated with cancer.



DNA fragmentation and TUNEL positive are detected in both apoptosis and necrosis

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Design and Synthesis of C_3-Symmetric Cyclometalated Iridium Complexes Having DR5 Binding Peptides for Selective Staining and Cell Death Induction of Cancer Cells

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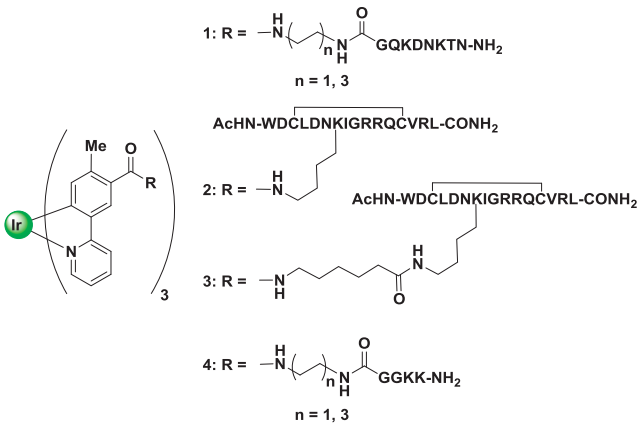
TNF-related apoptosis-inducing ligand (TRAIL) is categorized into TNF superfamily and induces the apoptosis in various tumor cells and tissues through the cell-extrinsic pathway, independently of p53.[1] TRAIL forms a C_3-symmetric homotrimeric structure by Zn^{2+}-centered self-assembly of monomeric unit and is capable of interacting with five cell surface receptors such as death receptors (DRs). Among known death receptors, death receptor 4 (DR4) and DR5 containing death domains send an apoptotic signals that activate caspase-8 (-10) to accelerate effector caspases (e.g. caspase-3, -6, and -7) and finally induce apoptosis.[1] Since TRAIL has very small influence on normal cells and tissues, antibodies and artificial compounds having TRAIL-like activity have been proposed as promising agents for cancer therapy.

In this presentation, we report on design and synthesis of peptide conjugated C_3-symmetric tris-cyclometalated iridium (Ir) complexes **1-3** and **4** (as a reference compound). The complex **1** has heptapeptide (QKDNKTN) that is important amino acid sequence of TRAIL (residues: 199-205) to interact with DR5.[2] The complexes **2** and **3** have cyclic peptide that specifically recognize DR5.[3] These complexes are expected to have agonistic activity for apoptosis induction of cancer cells and function for luminescent imaging of extra- and intracellular events.

For the purposes mentioned above, the Ir complexes **1-4** were synthesized utilizing regioselective substitution reactions reported by us[4] and coupled with the corresponding peptides. Affinity of these Ir complexes toward DR5 was evaluated by 27-MHz quartz-crystal microbalance (QCM) competitive assay, and self-assembly of DR5 with **3** was also observed on fast-scanning atomic force microscopy (FS-AFM). The green luminesce spots of **2** and **3** were observed on the cell membrane of Jurkat cells, and these spots showed overlap with staining area of anti-DR5 monoclonal antibody. The results of these experiments and cell viability assay of **1-4** will also be presented.



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Evaluation of Radioprotective Ability of Zinc-Chelator Derivatives

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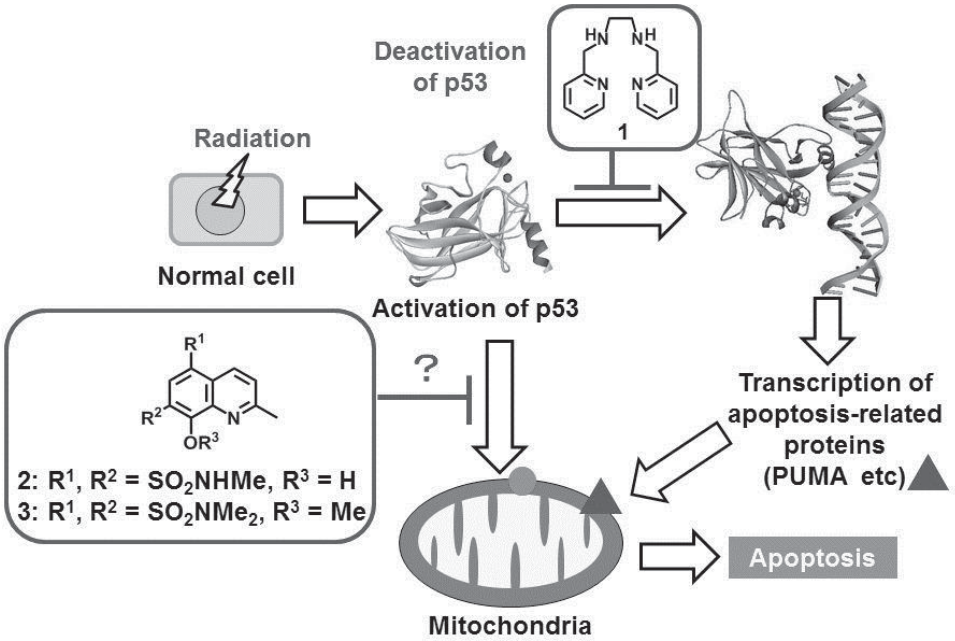
Radiation therapy and chemotherapeutic agents mainly target the DNA of growing cancer cells. Such therapies frequently have adverse side effects on normal tissues and cells, including p53-induced apoptosis. p53 protein, which is one of the important transcriptional factors, can activate its downstream targets in sequence specific manner to induce apoptosis. In contrast, many types of cancers tend to have a lower incidence of p53-mediated apoptosis, because the function of their p53s is often suppressed or lost during cancer development. Chemical agents that suppress p53-mediated apoptosis would be expected to prevent the damage of normal tissues during treatments of p53-deficient tumors.[1)] p53 protein contains zinc ions as structural factor in itself, and the zinc binding site in the p53 is essential for DNA transcription, and thus chelation can cause structural alterations, resulting in the inactivation of the p53 protein. Therefore, we expected that removing the zinc ion from p53 protein would be an effective means of inhibiting p53-mediated apoptosis induced by radiation.

In this presentation, we evaluated zinc-chelator derivatives such as Bispicen (N,N'-bis(2-pyridylmethyl)-1,2-ethanediamine) and 8-quinolinol as novel radioprotectors. The effect of some zinc chelators on intracellular p53 activity was examined p53-dependent apoptosis in irradiated MOLT-4 cells that are p53-active leukemia cells. The results of the dye-exclusion test suggest that Bispicen **1** and several 8-quinolinol derivatives such as **2** potently suppressed apoptosis induced by γ ray radiation. In addition, 8-methoxyquinoline derivatives such as **3**, whose affinity to zinc ion is very low, exhibit high radioprotective effect with low cytotoxity. The mechanistic study based on circular dichroism (CD) spectroscopy and electrophoretic mobility shift assay (EMSA) reveled that conformational changes of p53 are induced by **1**.[2)] On the other hand, it is very interesting that the mechanism of radioprotective effect **2** and **3** are different from that of **1**.



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Design and Synthesis of Sulfoquinovosylacylglycerol Hybrid Molecules as Tumor Theranostic Agents

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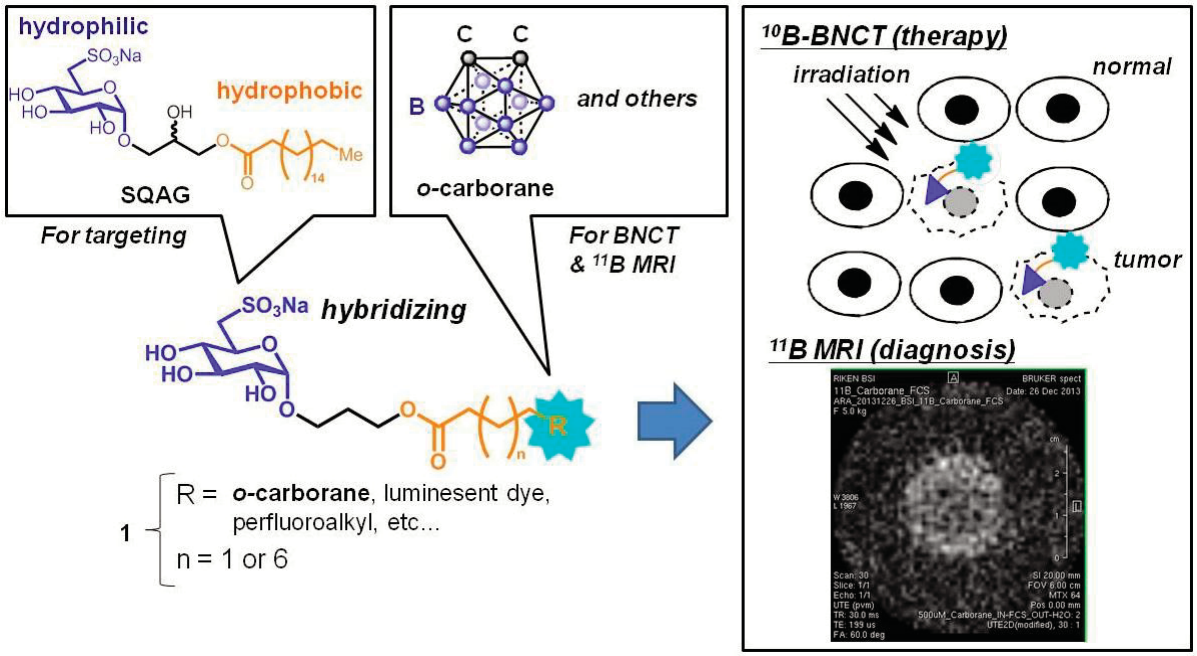
Sulfoquinovosylacylglycerol (SQAG) is a natural products that was isolated from See algae and identified by Prof. Sakaguchi and Prof. Sugawara.[1] SQAG is an amphiphilic glycolipid bearing 6-sulfo-quinovose and long-chain fatty acid linked via a glycerol linker. Recently, it has been found that SQAG has anti-tumor and radio-sensitizing activities.[2] SQAG and its analogues are accumulated at tumor cells, but with low cytotoxicity against normal cells. Based on this knowledge, we designed and synthesized SQAG analogues connected with biofunctional units for diagnosis and therapy of cancer. For instance, boron(^{10}B/^{11}B)-cluster "*o*-carborane" was introduced into the fatty acid part of SQAP, because *o*-carborane derivatives are recognized as a potent compound for boron neutron capture therapy (BNCT) and magnetic resonance imaging (MRI), respectively.

Synthesis of SQAG analogues **1** having different alkyl chains have been achieved from commercially available penta-*O*-acetyl D-glucoside. Physical behaviors of **1** and **2** such as critical micelle concentration (CMC), vesicle size and ζ-potential were measured. Intracellular uptake and cytotoxicity of these molecules were also evaluated by ICP-AES and MTT assay, respectively. In this presentation, these experimental data and the results of ^{11}B MRI experiments will be presented.



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Nano-drug Delivery System Containing Metalloporphyrin (Part 5): Investigation with Various Active Targeting Liposome Systems

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We have found that nano-drug delivery systems containing metalloporphyrins (MP/n-DDS) are non-cytotoxic and toxic hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) from a superoxide anion radical ($O_2^{\bullet -}$) are efficiently generated through the metal ions such as iron and manganese catalyzed dismutation and the Fenton-like reaction [1]-[3]. In this paper, we report for the investigation with iron and manganese porphyrins-loaded liposomes modified with active targeting sites such as glucose, folate and transferrin groups as a novel design of anticancer MP/n-DDS and their comparison of anticancer properties.

The MP/n-DDSs used were 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), Pluronic F68^{TM} (block-copolymer of polyetylene glycol-polypropylene glycol-polyetylene glycol, PEG) and glucose-, folate- and transferrin-modified polyetylene glycol-1,2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine (Glc-, FA- and Trf-PEG-DSPE) containing ion complexes composed of iron and manganese porphyrin and sodium stearate. The MP/n-DDSs were prepared by ultrasonic irradiation of aqueous solution containing phospholipids, surfactants and ion complexes. The particles of MP/n-DDSs modified with Glc-, FA- and Trf-groups were spherical, highly dispersed (ϕ < 200 nm) and stable in water and phosphate-buffered saline for several days at 37°C from the results of DLS particle size distributions and FF-TEM images. This would provide at least an opportunity of physiology-based targeting of MP/n-DDSs modified with Glc-, FA- and Trf-groups to these pathological areas via the active and passive targeting effects.

An alamar blue exclusion assay of the modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method was used to detect the cytotoxicity induced by the MP/n-DDSs. Colon 26, HeLa and L1210 as the tumor cell was treated with the MP/n-DDSs modified with and without Glc-, FA- and Trf-groups. An effective concentration of drug required to produce 50% lethal dose against cell (IC_{50}) was determined as shown in Table 1. Damage to such impregnated tumor cells was observed in active targeting systems. We demonstrated that the MP/n-DDSs modified with Glc-, FA- and Trf-groups as active targeting sites present a potential model for the design and development of novel anticancer drug.

Table 1 *in vitro* antitumor capacities of various MP/n-DDSs modified with Glc-, FA- and Trf-groups.

MP/n-DDSs and tumor drugs	MP	IC_{50} (μ M)	Cancer cells
Glc-modified MP/n-DDS	FeHpD	47.3	Colon 26
MP/n-DDS		77.0	
FA-modified MP/n-DDS	FeT4MePyP	9.5	HeLa
		40.1	L1210
MP/n-DDS		9.7	HeLa
		41.2	L1210
Trf-modified MP/n-DDS	$MnT4Me_2SuP$		Colon 26
sulfide-binding site / PEG 2000		28.7	
/ PEG 5000		28.5	
amido-binding site / PEG 2000		32.7	
/ PEG 5000		30.7	

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