Newsletter

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From KING SKYFRONT to the world - NanoMedical Innovation

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Fascinating! World's first mRNA therapeutics

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Combining mRNA and DDS, Aim for Treatment of Particular Disease

Expectations are growing for mRNA drugs that deliver messenger RNA (mRNA) to target cells and express necessary proteins there.We asked Dr. Keiji Itaka, Professor, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University and Dr. Shiro Akinaga, Chairman of the Board, CEO, AccuRna, Inc., who advance R&D of mRNA medicine in COINS Theme 3 and Dr. Takao Inoue, Head of laboratory, Laboratory of Oligonucleotide Therapeutics, Division of Molecular and Gene Therapy Products, National Institute of Health Sciences who supports those research and development from the side of regular science, about the current situation and goals of R & D.

Takao INOUE

Head of laboratory, Laboratory of Oligonucleotide Therapeutics
Division of Molecular target and gene therapy Products
National Institute of Health Sciences

Dr.Inoue graduated from Faculty of Pharmaceutical Sciences, The University of Tokyo in 1998 and completed Doctoral Course in Graduate School of Pharmaceutical Sciences, The University of Tokyo in 2003 (PhD). He worked as Assistant Professor at Graduate School of Pharmaceutical Sciences, The University of Tokyo from April, 2003 and was transferred to work at National Institute of Health Sciences in October 2011. Laboratory of Oligonucleotide Therapeutics was newly established at National Institute of Health Sciences in October 2013 and he was appointed as Head of Laboratory. In April 2015, he was seconded to AMED, responsible for allocating budget for regular science research (Section Chief of Office of Regulatory Science and Clinical Research Support). He returned to current position in June 2017. His areas of expertise are molecular biology, biochemistry and genetics.

Keiji ITAKA

Professor, Dept. Biofunction Research,
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Tokyo Medical and Dental University

Keiji Itaka, M.D., Ph.D. is a professor of Dept. Biofunction Research, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University. He graduated Dept. Of Medicine, The University of Tokyo in 1991, worked as an orthopedic surgeon. He started researches of DDS, gene therapy, biomaterials, and regenerative medicine with Prof. Kataoka in 1999, and got Ph.D. in 2003. Now he is focusing on development of mRNA medicine, some of which were awarded as Best Abstract Award in mRNA Health Conference in 2015 and 2016 and as Mizushima Award in the Japan Society of Drug Delivery System in 2016. He also participated in the launch of a new venture company, AccuRna Inc., in 2016.

Shiro AKINAGA

President and CEO, AccuRna, Inc.

Dr. Akinaga joined Kyowa Hakko Kogyo Co., Ltd, where he engaged in exploration and evaluation of anti-cancer drugs at pharmaceutical research center for 20 years and worked on many anti-cancer drug candidates. After that, he transferred to clinical development of anti-cancer drugs and was involved in the global development of many drugs, anti CCR4 antibody Mogamulizumab. was approved for marketing in Japan in 2012 based on the collaboration research with Professor Ryuzo Ueda, then professor at Nagoya City University. He became an international clinical development director in 2006. After the establishment of Kyowa Hakko Kirin Co., Ltd. in October 2008, he was a clinical development director of oncology group, Executive officer of global clinical development in 2011, fellow in 2013.He assumed the post of Board member at AccuRna Inc. in March 2017 and CEO in November 2018.

mRNA can be synthesized artificially. If it is put into practical use, a paradigm shift will occur in personalized treatment

■ Please introduce your research first.

Itaka: I am originally a clinical orthopedist. When I was working in a hospital, I also wished to engage in something new that might contribute to the future. Then, I knew a theme of DDS (Drug Delivery System), which appeared interesting and promising, and started the research career on the theme. I engaged in research of regenerative medicine using DDS in the orthopedic field, and I have been focusing on development of mRNA medicine in recent years (Figs. 1 and 2).

Akinaga: I joined Kyowa Hakko Kogyo Co. Ltd. (in that time), engaged in research of low molecular anticancer drugs as the senior researcher, and was promoted to the development position. In 2017, I retired from the former company and joined AccuRna Inc., where I have been the CEO since November 2018. Currently, I engage in drug discovery research using mRNA with Dr. Itaka and other members, along with nucleic acid medicine research into siRNA and ASO (Fig. 1).

Inoue: I joined the laboratory of The University of Tokyo just at the time when RNA interference (RNAi) was discovered in nematodes, and I engaged in basic research utilizing RNAi. Since I joined the National Institute of Health Sciences, I have engaged in regulatory science research to study how to evaluate/assess the quality and safety of nucleic acid medicines, such as siRNA and antisense, in order to put them into practical use. I also keep in mind the initiative role of our institute in creating innovative drugs as a national organization.

■ Explain the advantages of using mRNA as medicine. What is the current status of the research?

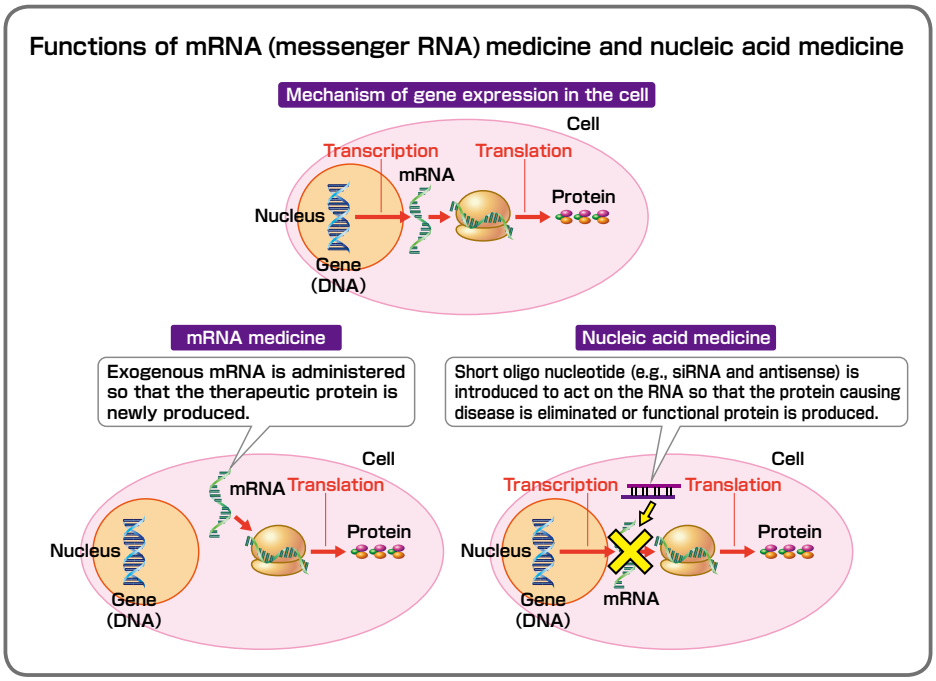
Itaka: In the treatment using mRNA , we administer mRNA encoding therapeutic proteins that can act as a key molecule for treating diseases. The advantages of mRNA are being synthesized relatively easily and being adaptable to personalized medicine by flexibly changing its sequence (Fig. 2). I think mRNA medicine has the potential to cause a paradigm shift in treatment. On the other hand, diseases involving various factors in a complex manner such as cancer, are difficult to treat with mRNA medicine alone. In cancer, mRNA should be co-administered with other therapeutic agents, such as anticancer drugs.

Akinaga: mRNA medicine is roughly classified into vaccines and therapeutic agents. Clinical trials of the vaccines are underway mainly by Western venture companies and some have reached phase II trials in the cancer and infectious disease fields. In addition, AstraZeneca is conducting a phase II clinical trial in ischemic heart diseases. Since there are many other treatments for ischemic heart diseases, the meaning of the use of mRNA may be controversial.

Itaka: In either case, the research is still in the predawn stage, but the market will likely expand in the future. Because this is a novel treatment,
we believe that clinical application will be accelerated by targeting diseases that currently do not have treatment

Inoue: Mentioning oligonucleotide therapeutics in contrast to mRNA therapeutics, oligonucleotide therapeutics to remove the causative molecule for the disease is being studied well as of now. In 2018, for example, the antisense drug inotersen (trade name: Tegsedi) and the siRNA drug patisiran (trade name: Onpattro) were approved for inherited atypical transthyretin amyloidosis,*1 an inherited neurological disease with little or no therapies for them. Both oligonucleotide therapeutics degrade mRNA of the mutant transthyretin that forms amyloid aggregation in the blood. Such drugs have set off development and approval of oligonucleotide therapeutics in Japan and foreign countries. I feel that the basis to facilitate

Figure 1



practical use of mRNA medicine is being formed.

Itaka: Nucleic acid medicine and mRNA are inseparable like both wheels of a car.+ While treatment with antisense nucleic acid medicine reduces the expression of the endogenous gene in the cells, mRNA medicine produces proteins that are encoded by the mRNA.

Inoue: Since both mRNA therapeutics and oligonucleotide therapeutics are designed on the basis of the gene sequence, candidate compounds for them can be obtained overwhelmingly more quickly than low molecule drugs or antibody drugs, and multiple drug combinations will be easier in the future. They have clear principles to control gene expression at the RNA level and have high efficacy. They are also appealing in that they harm no genes compared to gene therapy or genomic gene editing.

Itaka: An article has already reported that mRNA medicine and nucleic acid medicine were both introduced in one single cell at the same time to enhance the therapeutic effect. Our group once had an idea to introduce a mixture of mRNA and siRNA in a nanomachine. However, the more complex the system, the more factors produce unexpected results to make it difficult to obtain approval.

Akinaga: Even if an idea for combining mRNA therapeutics and oligonucleotide therapeutics occurs, that seems difficult to be put into practice. That may be difficult to try.

Inoue: If a number of mRNA therapeutics or oligonucleotide therapeutics are approved, and expertise in quality control or safety evaluations are accumulated in the future, the combination of them will be popularized. I think regulations would be less strict for combinations of drugs similarly constituted by nucleic acids than those for combinations of drugs with completely different structures. The accumulation of experience in reviews and risk-benefit viewpoints are the keys, and at least the combinations of more than one mRNA therapeutics or more than one oligonucleotide therapeutics could be accepted in the future. Because it is clear that combinations of expression controls by mRNA or oligonucleotide greatly expand the therapeutic options, I have expectations of future progress in combination therapy.

DDS is used to deliver mRNA to cells in a stable form

Why is it necessary to combine with DDS?

Itaka: Since mRNA is very unstable in vivo, it is difficult to deliver to mRNA. Although RNA may be designed to function in the cell more easily, the priority is to deliver mRNA securely. For example, while studies on DDS of low molecular drugs focus on retention and stability in the blood, in DDS of mRNA, it is uncertain whether an increase in the amount of mRNA reaching the target simply improves the therapeutic effect. On the other hand, DDS is definitely essential, and the use of PEGylated carrier is effective for better protein expression even in local administration.

Akinaga: We think DDS should be used ingeniously in the combination of mRNA therapeutics and oligonucleotide therapeutics mentioned before. Combinations of mRNA and oligonucleotide could result in better effects than the separate use of them.

Itaka: In the future, the ideal formulation will be carrying more than one nucleic acid medicine or mRNA together in a nanomachine to achieve a better effect. As a strategy to take advantage of the properties of mRNA, we consider the direction to keep the target cells alive or enhance their functions to be more important, rather than the purpose of killing the target cells as like cancer treatment.

Akinaga: One of our social missions is to develop a drug with strong unmet needs and great meaning, such as that for a rare disease caused by an abnormality in a specific gene, rather than diseases with various therapies like cancers.

Itaka: Use of DDS is already an established concept now, and the research field of DDS has matured in a positive sense. In the current phase, we have to use currently available DDS securely and select what to set in while improving DDS methods. Although DDS is basically used for systemic administration, mRNA should first be administered locally and eventually move to the next step of systemic administration, considering the risk of manipulating the gene,

Figure 2

Characteristics and problems of mRNA medicine

- mRNA expresses protein in the cytoplasma.
  → It can be used in any cells in principle (e.g., nerve and cartilage).
- mRNA has no risk of being inserted in the host genome.
  → It can be used without the risk of oncogenesis.
- mRNA is very unstable in vivo.
  → A DDS is needed to hold mRNA stably and deliver it to the target cells.
- mRNA induces immune responses
  → A mechanism for regulating immunogenicity is needed (mRNA modification and DDS).

which is contrary to low molecular drugs.

■ Let us know the issues in research and development of mRNA therapeutics and future resolutions

Inoue: I would like more researchers to study mRNA therapeutics. The Society of DDS covers only a few topics of mRNA.

Itaka: I fully understand that mRNA is difficult to obtain substance patents by itself, and many hesitate to commercialize it. That is why it is necessary to combine DDS to patent it. I'd like to accumulate evidence of its efficacy first through actual use in patients, even if only slightly.

Akinaga: AccuRna owns the patents for DDS nano-micelles that were inherited from Dr. Kazunori Kataoka.

So, we'd like to select mRNA to be inserted through discussions with Dr. Itaka and start a clinical trial in two or three years as a goal, while paying attention to research in the improvement of nano-micelles at the same time. Indeed, I'd like to say conservatively that we will start clinical trials in five years but that would be taken to be lack of motivation, while starting within a year is unrealistic. So, I say that the goal may be two or three years. If I were a CEO of an American company, I'd be dismissed with such a sense of speed (laugh).

Itaka: No mRNA medicine has been used clinically yet. Although the first-mover does exist, the winner takes all attitudes are not applicable in the medical sector, contrary to the IT community. We'd like to proceed with the development consulting with the regulatory authorities about specific indications for mRNA medicine.

Inoue: Consultation should be held as early as possible referencing the regulation for previous items developed.

Akinaga: Japan has already been fallen behind Western countries in the development of mRNA vaccines. Foreign venture companies are operating well-designed mRNA businesses. If this situation remains unchanged, Japan will not be able to manufacture mRNA vaccines but will have to import them.

Itaka: The members of today's three-way conversation are a researcher, a person in charge of regulatory science, and the CEO of a venture company, symbolizing the current stage of mRNA medicine development. We have all reached the point of no return at respective standpoints (laugh). It is important to have realistic concepts premising actual progress toward clinical phase, instead of remaining in non-clinical phase to obtain results from animal experiments.

Inoue: The approval of oligonucleotide therapeutics has prepared the way from the research and development to clinical application. It is important to discuss how to respond to personalized medicine from now on.

Akinaga: Although looking for and responding to various unmet needs are important; I will first proceed with development to obtain approval selecting a disease more suitable for mRNA.

Inoue: If mRNA therapeutics is recognized more in the future, pharmaceutical companies and researchers will enter this field in Japan as well. To take the initiative, I will proceed with preparation of the regulations as soon as possible and facilitate the developments from the view of public communication such as holding symposiums.

(Facilitator: Ayumi Kojima, science writer)

Terminology

＊1 Hereditary atypical transthyretin amyloidosis

A type of amyloidosis where a protein amyloid is deposited in systemic organs to cause various symptoms. In atypical transthyretin amyloidosis, an amyloid precursor protein with one amino acid mutated is produced mainly in the liver and deposited systemically. The disease is treated by liver transplantation.

Development of a Neuroprotective Drug Based on mRNA Medicine

Focus on development of a clinically applicable drug for treating ischemic CNS diseases

Ischemic CNS disease suddenly deprives the body of the function to control body movements, and the lost function is never recovered. Although development of a neuroprotective drug and neuro-regenerative medicine for ischemic damage may be an ardent wish of humans, there are a number of obstacles in the development of drugs targeting the CNS. We have demonstrated the neuroprotective effect of messenger RNA (mRNA) medicine using polymer micelles against ischemic neuronal death. Believing that mRNA medicine may overcome the CNS-specific challenges, we are working on the further development of therapies.

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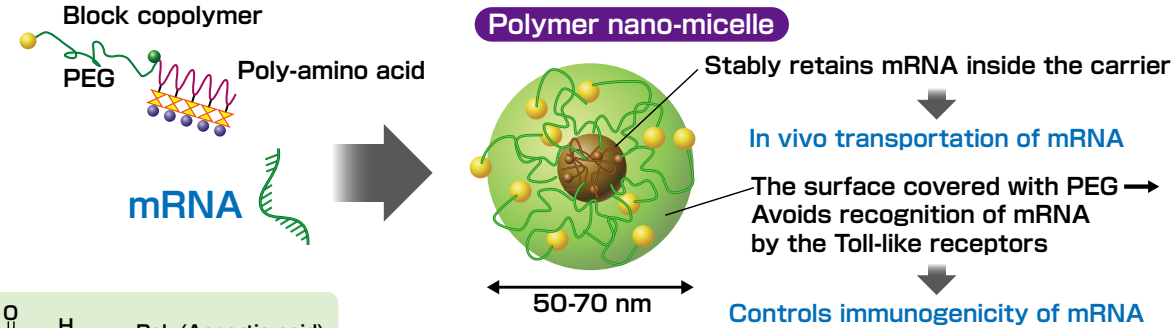
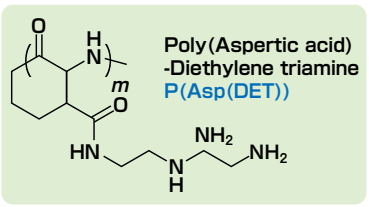
Ischemic CNS diseases, such as cerebral infarction, are mainly characterized by their sudden onset in any person, as well as their neurological sequelae lasting for a lifetime once established. Major therapies that have been shown to have efficacy in cerebral infarction are only hyperacute recanalization therapy[*1] and rehabilitation in the early phase of onset. Unfortunately, none of therapies that humans have long sought, such as drugs to protect neurons[*2] from cell death and regenerative medicine for established sequela, have achieved clinical application at present. Although not a few substances were expected to have neuroprotective effects against ischemic CNS diseases, they all had unsuccessful results in clinical trials due to huge obstacles, such as CNS-specific problems in drug delivery and adverse reactions. Hence, our research group focused on the mRNA medicine using polymer micelles (Fig. 1) that locally produces high protein expression, and we conducted a therapeutic research to verify the neuroprotective effect of the mRNA expressing brain-derived neurotropic factor (BDNF).[*3] BDNF is a protein abundant in brains with physiological neuroprotective effects. While it is secreted not only from the neurons but from the glial cells,[*4] particularly in ischemic conditions, local decreases in its expression are also known to be involved in the vulnerability of neurons against ischemia. Although protein drugs were expected as neuroprotective drugs, excessive administration was needed to deliver them from the peripheral blood or cerebrospinal fluid (CSF) to the lesion, posing a problem of adverse reactions. This research used the rat global ischemia model that produces transient severe ischemic damage. Particularly in the hippocampus,[*5] only the neurons in the CA1 region slowly result in cell death after a grace period of about two days from the ischemia, which is a characteristic cell death process called delayed neural death. As the route for local expression of mRNA in the hippocampus, we adopted administration in the ventricle near the hippocampus, which is filled with CSF. Administration of BDNF-expressing mRNA using polymer micelles via this route of administration obtained continuous BDNF expression in the hippocampus for two to four days. As a result, the neuroprotective effect against delayed neural death in the hippocampal CA1 was confirmed in the immunohistochemical-stained rat hippocampal slices. Furthermore, a behavioral test reflecting short-term memory indicated its efficacy as well (Fig. 2). This therapeutic experiment also obtained an interesting result that BDNF was majorly expressed in the astrocytes[*6] that compose the largest proportion in the CNS.

This result demonstrated that the therapy to locally express the BDNF-expressing mRNA in the ischemic brain lesion using polymer micelles is promising as neuroprotective therapy, which was difficult to develop due to the CNS-specific problems in drug delivery and adverse reactions. The following characteristics of mRNA medicine delivered via polymer micelles are suitable for ischemic CNS diseases: 1) transient gene expression, 2) no serious adverse reactions because it is not incorporated in the host genome, and 3) no need for a promoter that allows unlimited cell species for expression. We would like to apply it to regenerative medicine research as well.

Figure 1. A nanomachine enclosing mRNA medicine



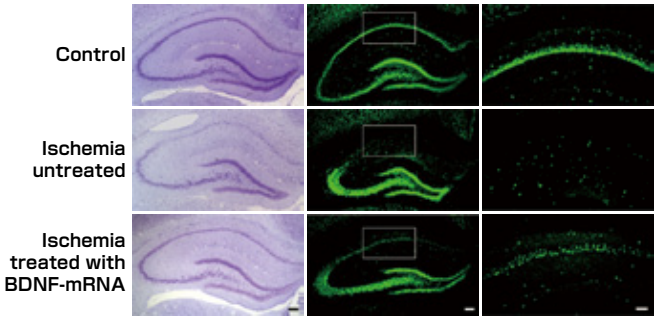


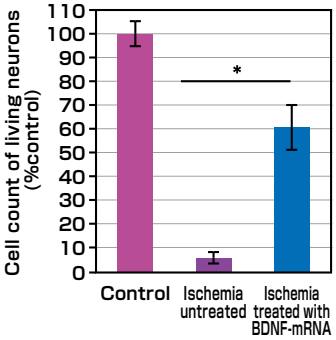
Exemplary structure of poly-amine acid
= P[Asp(DET)]

When block copolymer synthesized from polyethylene glycol (PEG) and poly-cation is mixed with mRNA, they are self-assembled to from polymer micelle with the surface covered with PGE that contains mRNA aggregated in its central portion.

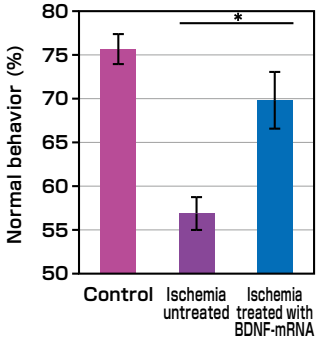
Figure 2. Neuroprotective effect of BDNF mRNA against delayed neural death

a. Neuroprotective effect against delayed neural death in rat hippocampal slice





b. Improvement in short-term memory in behavioral evaluation using Y maze



Terminology

＊1 Hyperacute recanalization therapy
A therapy to recanalize the artery occluded with emboli. It is used in limited cases within several hours after onset. Two modalities are indicated for this treatment: intravenous administration of thrombolytic agents and endovascular treatment with thrombus retrieval devices.

＊2 Neurons
Neurons serve as the entity of information processing, the primary function of the nervous system through intracellular conduction of membrane potential and intercellular chemical transmission. Neuronal death causes disorders in various neurologic diseases.

＊3 Brain-derived neurotropic factor (BDNF)
A neurotrophic factor protein that regulates various physiological functions, such as learning and development. BDNF is expected to be used as medicine because it is deeply involved in maintenance of neural survival. However, it has not been applied clinically due to various obstacles to its delivery to the CNS in a form of protein drug.

＊4 Glial cells
A collective term referring to the cells constituting the nervous system other than the neurons. Although they were considered to be glue cells that merely sustain the neuron in the past, now they have been revealed to have various functions.

＊5 Hippocampus
A region in the brain involved in memory and space perceptional function. Because the hippocampus is very vulnerable against ischemic damage, it has had a number of findings accumulated as a target of studies on ischemic neuronal death.

＊6 Astrocytes
A type of glial cells that accounts for the majority in the CNS. Its various functions gather great scientific interest.

Regulation of Gene Expression Involved in Tissue Formation: from Basic Research to Therapeutic Application

Understanding the mechanism of gene expression[*1] involved in cell formation seems to be the key to regeneration or repair of the tissue. In recent years, the regulatory mechanism of gene expression by transcription factors[*2] important in the formation or maintenance of the tissues is being clarified in genome-wide manners. In the therapeutic application of such basic knowledge, a strategy to intracellularly express mRNA of "therapeutic transcription factors" that regulate expression of genes effective for treatment seems to be promising. I proceed with the study to identify the mechanism of gene expression that can be fed back therapeutically and to contribute to the development of therapy for musculoskeletal disorders.

Shinsuke OHBA

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Mammalian skeletons are generated by chondrocytes that form the cartilage and osteoblasts that form the bone. Cartilage formed during the fetal stage regulates skeletal growth until puberty and plays important roles in locomotion all our lives.

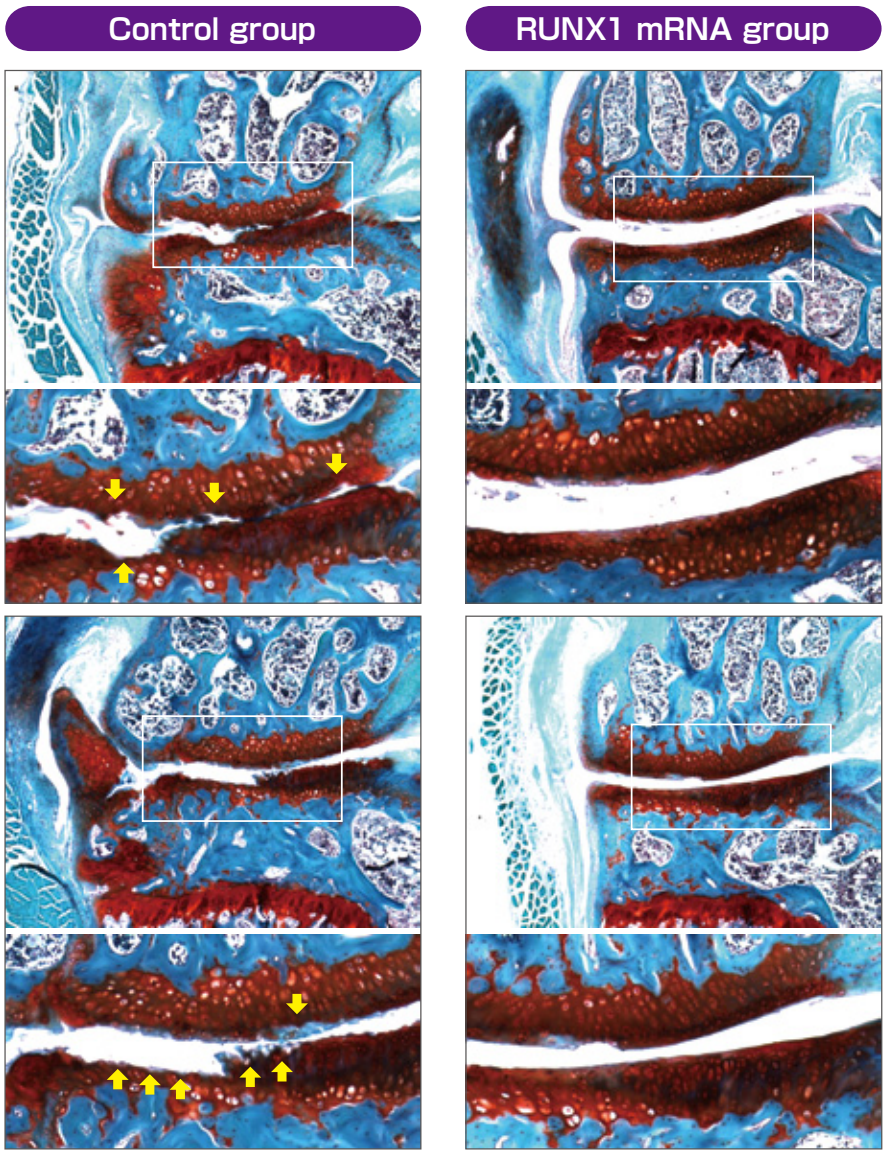
In tissue formation, the transcription factors should function normally to correctly express the genes involved in formation/maintenance of the cells constituting the tissue. Understanding such mechanisms is the shortest way to develop tissue regenerative/repairing therapy.

We have been working on the identification of the regulatory mechanism of gene expression important for cartilage/bone formation using mouse genetics or molecular biological approaches. As a result, we discovered that the transcription factor RUNX1[*3] is expressed in the adult articular cartilage and induces expression of the gene of type II collagen, which is a major component of the cartilage. Furthermore, RUNX1 expression disappeared in patients with osteoarthritis, where functions of joints as cushion and hinge are affected due to degeneration of the articular cartilage (Reference 1). We are currently aiming to identify the regulatory mechanism of gene expression in chondrocytes and osteoblasts by taking advantage of genome-wide analyses using next-generation sequencers. We have identified the modes of actions of transcription factors SOX9[*4] and SP7[*5] in the whole genome and obtained genome-wide evidence of their importance in skeletal formation (References 2 to 4).

The above basic findings suggest that intracellular expression of therapeutically effective transcription factors (therapeutic transcription factors) leads to direct and efficient therapies. In gene expression, the a specific portion of genomic DNA encoding genetic information of a protein is copied as messenger RNA (mRNA) and functions as the blueprint for synthesis of the protein. Application of mRNA is therefore expected as a new nucleic acid medicine. Hence, in cooperation with Professor Keiji Itaka, Professor Kazunori Kataoka, and coworkers, I examined whether delivery of the therapeutic transcription factor RUNX1 into the joint via nanomachine can prevent cartilage degeneration and suppress the progression of osteoarthritis. Polymer micelles encapsulating RUNX1 mRNA were administered into the knee joint of osteoarthritis model mice once every three days for a month. Progression of osteoarthritis was suppressed in the articular cartilage of the RUNX1 mRNA group compared to the control mRNA group (Fig. 1: Reference 5), accompanied with enhanced expressions of type II collagen, which is a major cartilage matrix protein, transcription factor SOX9, which is essential for cartilage formation and proliferating cell nuclear antigen (PCNA), which is a marker of cell proliferation (Reference 5).

As described above, we are developing the platform, where the mechanism of gene expression involved in the formation and maintenance of the tissue is identified, and the basic findings are applied to treatment via mRNA delivery with nanomachines. I will continue working on identification of mechanism of gene expression and therapeutic transcription factors that can be fed back to treatment to contribute to the development of therapies for musculoskeletal disorders.

Figure 1



The control group shows surface curing-up, degeneration and abrasion (yellow arrows) of the articular cartilage (stained red) , which are suppressed in the RUNX1 mRNA group. Magnified images of the rectangular parts are shown in the bottom of respective panels.

Terminology

＊1 Gene expression
Gene expression refers to protein synthesis based on genetic information. It is roughly divided into transcription (synthesis of mRNA through the actions of transcription factor and basic transcription machinery) and translation (protein synthesis based on genetic code copied in mRNA).

＊2 Transcription factor
A factor that recognizes and binds to a specific DNA sequence to regulate the transcription process of genetic information on the DNA to mRNA.

＊3 RUNX1 (runt-related transcription factor 1)
A transcription factor encoded by RUNX1 gene that belongs to RUNX gene family. RUNX1 is known to be involved in cartilage formation and act as the transcription factor essential for hematopoiesis.

＊4 SOX9
SOX9 is one of the SRY-related HMG-box (Sox) family proteins and acts as a transcription factor. Proteins with highly conserved HMG box is collectively called the Sox family.SOX9 has important roles in skeletal formation as well as development of the gonads, hair follicles, nerves, heart, pancreas, intestines, and inner ears.

＊5 SP7
A transcription factor that belongs to the SP family. SP7 is strongly expressed in the osteoblasts and their precursors. SP7 knockout mice have no bone developed systemically.

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Light to Cancer Immunotherapy! Development of basic technology of cancer vaccine[*1] using a new formation of mRNA medicine

As exemplified by the Nobel Award to Dr. Tasuku Honjo, cancer immune therapy is gathering attention in recent years, although the therapy is effective in only a few patients. More patients could obtain benefits from immune therapy by concurrent use of a cancer vaccine with the existing immune therapy. Now we have successfully developed the basic technology for a cancer vaccine using a novel nucleic acid medicine RNA.

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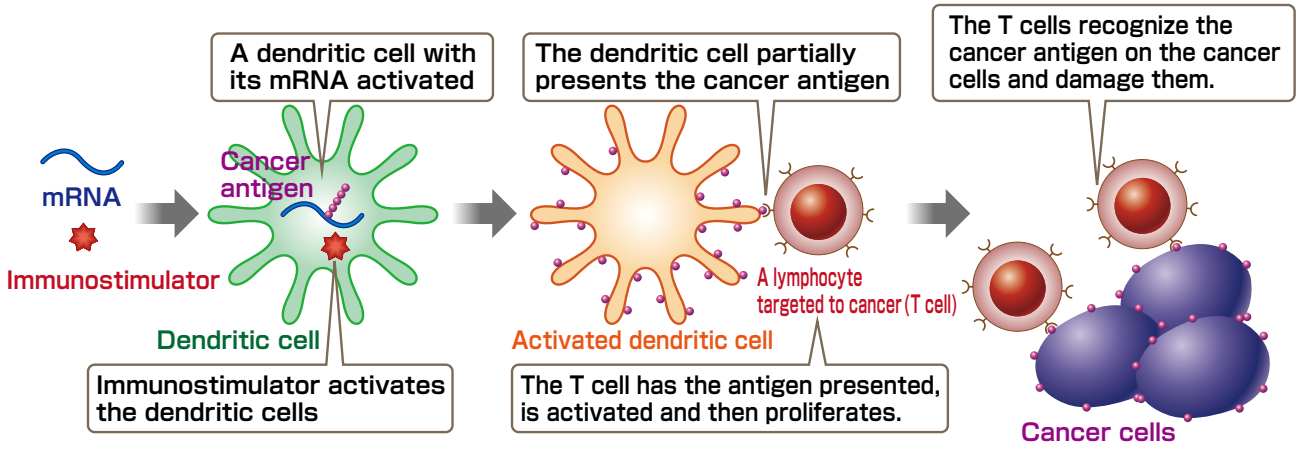
Cancer immune therapy removes the cancer cells with the patient's own immunity. It is characterized by the long-lasting effect capable of controlling relapse of cancer, contrary to the existing surgical therapy, chemotherapy, and radiotherapy. It is gathering attention also because of the Nobel Award to Dr. Honjo for the development of the drug to activate immunity against cancer in 2018. However, this drug is effective in only a few patients. On the other hand, animal experiments and clinical studies have revealed that more patients can benefit from immune therapy through concurrent use of a cancer vaccine to induce immunity against the proteins that exist only in the cancer cells (caner antigens).[*2]

Conventional vaccines obtain immunity against pathogens by administering proteins derived from the pathogens in advance. A cancer vaccine obtains immunity to attack cancer cells by administering cancer antigens in advance. Cancer vaccines corresponding to individual patients should be manufactured rapidly here because cancer antigens differ by patient, contrary to pathogenic antigens. For such personalized treatments, a method to administer to patients messenger RNA (mRNA), a novel nucleic acid medicine designed to have the cancer antigen be produced in the patient's body, allowing production of a cancer vaccine rapidly at low cost (Fig. 1). In this research, we developed the basic technology to enhance the effect of this mRNA vaccine.

For effective action of the vaccine, in vivo production of cancer antigens is necessary as well as concurrent immune activation. Thus, an immunostimulator[*3] is administered in combination with the vaccine (Fig. 1). However, there have been problems that this immunostimulator weakens the activity of mRNA, that it exerts no effect because of its in vivo distribution at a site different from that of the mRNA, and that it induces adverse reactions. For these problems, we developed immuno-stimulating mRNA where mRNA has its own immuno-stimulating effect. In specific, when mRNA is bound to its counterpart RNA (complementary RNA),[*4] a double strand RNA structure is formed by a pair of RNA chains, which activates the immunity (Fig. 2). The mRNA simultaneously produces the cancer antigen. In this study, we optimized the design from the various double strand structures and improved the vaccination effect in animal experiments. Its effect was also demonstrated in an experiment in human cells. In this method, the mRNA action is conserved, and the mRNA and the immunostimulator will not be distributed in different sites. Furthermore, this vaccine is decomposed in several days after the administration, posing no concerns about adverse reactions.

From now on, I will study whether the mRNA developed in the present research actually has a suppressive effect on cancer growth in animal studies and seek its clinical application by using concurrently with the drug developed by Dr. Honjo. I will work on the research to make my dream of developing drugs useful for patients come true.

Figure 1. mRNA vaccine against cancer



*Dendritic cell ··· Exists in the skin, airway, and intestines that contact the outer environment. This cell takes up foreign matter and presents the antigen to the lymphocytes

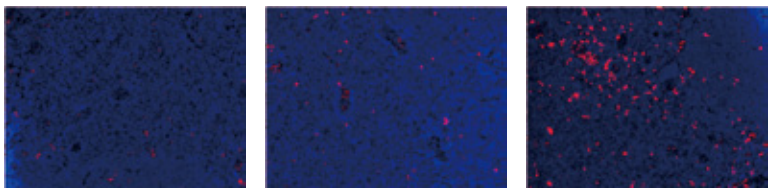
*T cells ····· A type of lymphocytes that attacks the cancer cells and cells infected with the pathogen and regulates the functions of other immune cells.

Figure 2. Design of a double strand mRNA and vaccine effect

When the whole length of mRNA was bound to its complementary RNA, immunostimulation was obtained from the double strand RNA structure, although production of the antigen protein from the mRNA was lost.

When the complementary RNA was bound only to the sequence called poly A at the end of mRNA, immunostimulation was obtained while production of the antigen protein from the mRNA was maintained as well, and this design was therefore adopted.

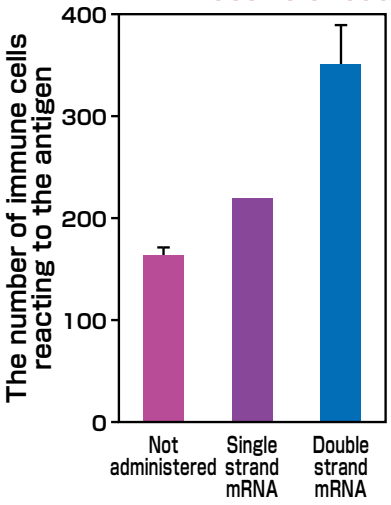
Proliferation of the dendritic cells in the lymph node



(Top): mRNA was administered to mouse lymph node and the activated dendritic cells were stained red 24 hours after. The blue portion indicates the nucleus. Proliferation of the activated dendritic cells was noted in the double strand mRNA group.

(Right): An mRNA to develop a model antigen was administered to the mouse lymph node and the number of cells reacting to the antigen was counted seven days after. The number of cells increased in the double strand mRNA group with increasing vaccine effect confirmed.

Vaccine effect



Terminology

＊1 Cancer vaccine
A drug to be used in the therapy where a cancer antigen is administered to induce immunity against the antigen and attack the cancer cells.

＊2 Cancer antigen
A substance that does not exist in normal cells but in cancer cells and serves as a marker for the immune cells to attack the cancer cells.

＊3 Immunostimulator
A substance to enhance the vaccine effect by inducing an inflammatory reaction in the vaccine administration site. It is also referred to as an adjuvant. Aluminum salts are currently used as immunostimulators.

＊4 Complementary RNA
RNA consists of four bases: adenine (A), guanine (G), cytosine (C), and uracil (U). A and U mutually bind and G and C mutually binds each other as well. An RNA chain consisting of bases that mutually bind to the bases of another RNA chain is called complementally RNA.

References

S. Uchida, N. Yoshinaga, K. Yanagihara, E. Yuba, K. Kataoka, K. Itaka, Designing immunostimulatory double stranded messenger RNA with maintained translational activity through hybridization with poly A sequences for effective vaccination. Biomaterials 150 162-170 (2018)

Listen to COINS' Members

What w

Works on research every day to provide drug delivery system (DDS) for nucleic acid medicine for the world.

Miki MASAI

Researcher, Research and Development Division, AccuRna Inc.

Nucleic acid medicine leads to global KAWASAKI

Since 2016, I have worked for the Innovation Center of NanoMedicine, the core organization of COINS. At first, it was surprising for me that a leading-edge institute exists in an industrial region. Although I have lived in Kawasaki since early childhood, I did not know about the Innovation Center of NanoMedicine or even the construction of a new research town called King SkyFront either. The former factory sites have been converted into a new town completely different from the previous one.

I currently engage in research of nucleic acid medicine and study every day for the goal of treating cancers, such as brain tumors, rare diseases, as well as various diseases in the future. Nucleic acid medicine is the next-generation medicine using nucleic acid, such as DNA and RNA. This field has markedly progressed in recent years, such as a short-chain nucleic acid called siRNA that was approved as a pharmaceutical product in the US in 2018. However, nucleic acid is very unstable and is still difficult to deliver to the disease target securely, posing a lot of problems. Thus, our company fuses the excellent technology of Dr. Kazunori Kataoka, the research supervisor of COINS, with nucleic acid technologies from pharmaceutical companies and universities around the world, seeking to create a new nucleic acid medicine and launch it to the world.

My secret goal in the future is to launch in-body hospital and nucleic acid medicine that COINS is seeking to the world, making Kawasaki famous as global Kawasaki.

With colleagues

Treatment of a brain disorder such as Alzheimer's disease has been found impossible because it is not possible for drugs to enter the brain. I am using nanocarriers for actively invade the brain and protecting neurons for treatment that could stop the progression of Alzheimer's disease.

Daniel Gonzalez Carter

Research Scientist

Challenge big health problems with a small solution

Last year I had the great opportunity to join the Kataoka lab in the Innovation Center of NanoMedicine. I was really excited to take up this opportunity to work in Japan not only because of the great work and scientific innovation being done at the Kataoka lab, but also to be able to experience the culture of Japan. I have always enjoyed travelling and experiencing new places. After leaving my home country of Mexico at the age of 15, I lived in various countries around the world, including the United States, France and England. I've also had the opportunity to explore some very amazing places, like the southern wetlands of Brazil, the rainforests of Costa Rica, and cycling across the Shetland Islands. Therefore, coming to the land of the rising sun was a really exciting prospect.

Having now lived almost two years in Japan, I find that the experience itself has aided my scientific research. In order to overcome the problems facing our research, scientists have to be creative and able to think outside the box. This is best achieved if the mind is properly stimulated. The culture clash I have experienced living in Japan has definitely kept my mind stimulated, whether it is simply trying to decipher kanji characters in the bus or navigating the complex etiquette of this culture. I will definitely remember my stay here with great fondness for the rest of my life and I believe will greatly aid me in my scientific advancement.

e treasure

Takuya ISHII Manager of Kawasaki Research Insititute, Div. of Pharmaceutical Research, SBI Pharmaceuticals, Co., Ltd.

Studies minimally invasive diagnosis/treatment of cancer with a combination of ALA and light

Beyond the imagination

I thought "I wish the cancer emitted light!" while I was observing an operation in the Hospital Training Program in my school days, where I wanted to be a clinical engineer in the future. At that time, I did not imagine that this would be put into practical use in years, or even that I would work on the research of this subject as a researcher of a pharmaceutical company engaging in the research. Visualization of cancer is one of the great issues of the diagnosis and treatment of cancer.

5-aminolevulinic acid (ALA) is a natural occurring amino acid and a precursor of biosynthesis of porphyrin that is important in the biological activity of animals and plants. Porphyrin is the source of chlorophyll in plants and heme in animals.

On the other hand, administration of ALA to cancer cell causes the accumulation of porphyrins that are metabolite of ALA and can emit red fluorescence when irradiated with blue light. This property has already been put into practical use as a cancer imaging technology with ALA, which is called photodynamic diagnosis (PDD) in neurosurgery and urology. Furthermore, cancer can be treated with photodynamic therapy (PDT) that irradiates cancer cells with red light after administration of ALA. Linking these technologies finally enables minimally invasive diagnostic and therapeutic systems that integrate diagnosis and treatment with the combination of ALA and light.

We moved from Kobe to Kawasaki (iCONM) in 2018 and have participated in COINS since then. As the environment for experiments was eventually established, we will accelerate the research, and I will contribute using ALA to achievement of smart life care society proposed by COINS.

The landscape of Kobe from Rokko Mountain. It was a beautiful seaport town.

I develop mRNA as regenerative medicine to treat problems like spinal cord injury.

Samuel Crowley Visiting Scientist (Tokyo Medical and Dental University)

Family History and New Technology

I was raised in a small town called Savannah, Missouri in the middle of the United States of America. The city had a population of less than 5000 people. My family has owned a farm near the town since the 1830s, where we raise cattle. I hope to eventually return to the farm and help my family with the work.

I received my bachelor's degree in Chemistry from Missouri Western State University in St. Joseph, Missouri in 2009. I received my Ph.D. in Medicinal Chemistry from the University of Iowa College of Pharmacy in 2015. It was in Iowa where I first began working with nanomedicine and mRNA delivery. I came to Japan in 2015 to join Keiji Itaka's group and continue to use mRNA as a therapeutic. Specifically, I have been using mRNA to treat Spinal Cord Injury in mice. I have also been exploring alternative types of mRNA that will hopefully extend the time that the mRNA can produce protein after delivery.

While in Japan I hope to visit Hokkaido. While Tokyo is a nice city, it is far more crowded than I am used to, and seeing Hokkaido will remind me of home.

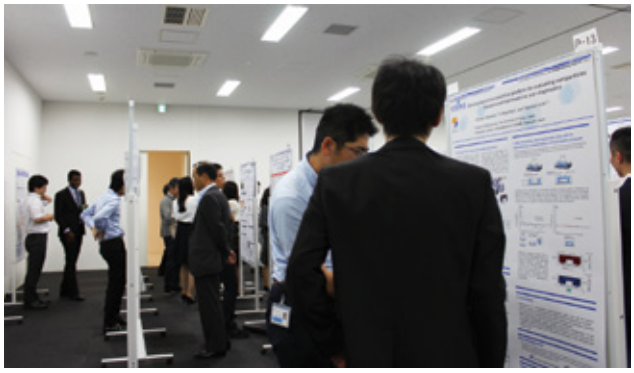
My family's farm

ACTIVITY REPORT

10th General Meeting ～Bring unity toward the final phase III～

10th General Meeting was held at the Kawasaki Life Science and Environment Research Center (LiSE) on Tuesday, June 19, 2018.

Since this fiscal year is the final year of Phase II (Heisei 28 -30), with the realization of "in-body hospitals" in mind, we confirmed the progress of research, social implementation and plans for phase III (Heisei 31-33) with awareness of the differentiation / advantage from the existing technology. In the panel discussion, we shared the determination to the phase III and also discussed on the tasks for promoting the project. In addition, COINS research promotion office introduced the challenge of COINS members to "research group cloud funding" which is a new effort at iCONM. Moreover, Mr. Yasuhiro Otomo, Deputy Director, Medical New Technology Strategy Office, Nitto Boseki Co., Ltd. that newly participated in COINS after moved in iCONM introduced the company. In poster session, there were 31 presentations and very active discussions were carried out among the researchers. Finally, Mr. Toshio Asano, Asahi Kasei Corporation, Mr. Hiroshi Misawa, Medical Technology Association of Japan and Mr. Masafumi Nogimori, Mr. Haruhiko Manabe, JST gave us feedback that we should always aware of step-up to social implementation, hoping to work with speed while catching up market and other people's trends. The meeting was very productive to further strengthen the tie of the researchers. This time, in cooperation with the National Institute of Health Sciences, we were invited to visit a facility just opened in March this year at King SkyFront as iCONM. Also because it was still charged up after the visit to NIHS, it became very fruitful opportunity that we could feel the breakthrough of COINS in phase III as there were active comments on regional collaboration and industry-university cooperation as future development.



Poster session scene

Mr. Masafumi Nogimori ,JST delivers feedback.

Topics January – June 2018

- 1.7.2018【News】Dr. Kazunori Kataoka, COINS Research Leader, appeared on TBS TV "Saturday Morning". In-body hospitals and Nanomachines were introduced.
- 1.11.2018【News】The article about the research of Dr. Yasutaka Anraku, Project Professor, Department of Bioengineering, Graduate School of Engineering, The University of Tokyo was posted on page 5, Nikkei Sangyo Shimbun. The title was "Nanoparticles give light for mental illness medicine, BBB-crossing nanomachines enter the brain via a systemic route - lead by leaders of next generation".
- 1.17.2018【News】This is summary of COINS research team was posted on website of Nature Communications. The title was "Development of "BBB-crossing nanomachines which enter the brain via a systemic route by efficiently reach neurons for glycemic control".
- 1.24.2018【News】Dr. Kazunori Kataoka, COINS Research Leader's seminar scene was introduced on Okayama University's website. The title was "President's Top Seminar was held by our invited speaker, Dr. Kazunori Kataoka, Vice-Director of Kawasaki Institute of Industrial Promotion".
- 1.25.2018【Activity】9th COINS General Meeting was held.
- 1.28.2018【Appoint】Dr. Kazunori Kataoka, COINS Research Leader was appointed to Associate Editor of ACS Nano published by American Chemical Society.
- 2.5.2018【Activity】COINS Seminar #29 was held at iCONM. Lecturer: Prof. Tatsuya Shimizu, Director, Institute of Advanced Bio-Medical engineering and Science Tokyo Women's Medical University / Title: Current status and outlook of regenerative medicine research using cell sheets
- 2.16.2018【Activity】COINS Seminar #30 was held. (Article →p.16) Lecturer: Prof. Sei Kwang Hahn, Department of Materials Science and Engineering at POSTECH, KOREA / Title: Smart Photomedicines Using Multi-Functional Nanomaterials
- 3.2.2018【Activity】COINS Seminar #31 was held. 1) Lecturer: Koji Suzuki, Director JSR Corporation, Research & Development Division, JKiC / Title: overview of JSR-Keio University Medical and Chemical Innovation Center (JKiC) 2) Lecturer: Hideyuki Saya, Professor, School of Medicine, Keio University / Title: Development of therapeutic strategies targeting cancer heterogeneity and microenvironment
- 3.10.2018【News】Dr. Kazunori Kataoka, COINS Research Leader's lecture at Yomiuri Techno Forum was introduced on YOMIURI ONLINE. Title was "Micro capsule, Target cancer cell ….Research treatment".

- 3.21.2018【News】Dr. Kazunori Kataoka, COINS Research Leader appeared on TV Tokyo "Yugata satellite".
- 3.27.2018【News】iCONM & COINS, our main project was introduced on Kanagawa Shimbun on P.14. Title was "Kawasaki Mirai Shimbun vol.4 Creation of new town for the future / Power to create a future / Future Keihin Coastal area aims for".
- 4.1.2018【News】Interview of visiting scientist, Dr. Shigehito Osawa (Assistant professor, Tokyo University of Science) was posted on Kawasaki city Shiseiyouran2018. Title was "People of Kawasaki Passion x Unity"/No.3 Technology Hope to create medicine from King SkyFront
- 4.24.2018【Activity】COINS Seminar #32 was held. (Article→p.16) Lecturer: Dr. Takao Inoue, Chief of laboratory, National Institute of Health Sciences Title: "Trend of development and regulation of oligonucleotide therapeutics"
- 4.27.2018【News】The interview of Visiting Scientist, Dr. Yasutaka Anraku (Assistant professor, The University of Tokyo) was introduced on Kawasaki Shisei Dayori. Title: The future starting from the coastal area Under the research on nanomachines that deliver the drug to brain is on the way
- 5.14.2018【News】The interview of Director General, Dr. Kazunori Kataoka (Professor, The University of Tokyo) was introduced on Shuukangendai. Title was "Photo reportage: Mankind overcomes『cancer』by Nanomedicine"
- 5.14.2018【Activity】COINS Seminar #33 was held. Lecturer: Dr. Mariko Shiba, Director, National Cerebral and Cardiovascular Center Title: "Development of antisense drug targeting refractory dyslipidemia"
- 5.21.2018【News】Research Leader, Dr. Kazunori Kataoka (Director General of iCONM, Professor, The University of Tokyo)' s interview was posted in academist Journal. Title was "Make Kawasaki City the hub of Health kombinat! – Aim to realize a "In-body hospitals" where nanomachines go around the body –
- 5.25.2018【Activity】iCONM opened Twitter and Facebook.

Twitter →　Facebook→

- 5.28.2018【Activity】COINS researcher Mr.Takuya Miyazaki is challenging crowd-funding as part of research towards the realization of "In-body hospitals". Title was "Treat pancreatic cancer at pinpoint".
- 6.10.2018【Activity】COINS Seminar #34 was held. Lecturer: Mr.Toshio Nagae, Pharma Business Consultant, PeptiDream, HMT / Representative, Audit & Supervisory Board Member Title: "Value Optimization of JP-origin Drugs/DDS, Issues and Alternative Solutions"
- 6.12.2018【News】COINS Research Leader, Dr. Kazunori Kataoka (Director General of iCONM, Professor, The University of Tokyo)' s interview was posted on Nikkan Gendai. Title was "Featured healthcare that supports 100 years of life - Realization in as early as 3 years. Virus-sized medical robot treats cancer and dementia = Eventually "In-body hospitals".
- 6.19.2018【Activity】10th COINS General Meeting was held. (Article →p.14)
- 6.24.2018【News】Research Leader, Dr. Kazunori Kataoka (Director General of iCONM, Professor, The University of Tokyo) appeared on TV program BS Fuji "Galileo X". He talked about the potential of In-body hospitals for Alzheimer's disease. Title was "Prevention of dementia".
- 6.25.2018【Activity】Sabina Quader, iCONM scientist (COINS theme 1 & 2) challenged crowd-funding Theme was "Glioblastoma Multiforme treatment".(Article →p.15)
- 6.26.2018【Award】Mr. Naoto Yoshinaga (Trainee of iCONM, The University of Tokyo) received The Best Oral Presentation Award from the 34th Annual Meeting of the Japan Society of Drug Delivery System.

Challenge cloud funding

～ Part of activities to realize "in-body hospitals" such as human resource development and outreach ～

For further accelerating research to realize "in-body hospitals", It is important that researchers take initiative to work even more active.Especially for young researchers who will be responsible for the future, they need the ability to think about the next research theme by themselves, communicate their significance in an easy-to-understand manner, receive various support and opinions, and advance research alongside them. As an implementation of human resource development, we started cloud funding. This time, Dr. Sabina Quader, Senior Research Scientist and Mr. Takuya Miyazaki, D3, Department of Engineering, Graduate School of Engineering, The University of Tokyo launched a site at academic cloud funding site "Academist (Academist)" with the aim of developing nanomachines for cancer that is difficult to treat such as pancreatic cancer and brain tumor. Although they were both puzzled by the fact that this was the first time, they had many meetings with the person in charge of the Academist and seriously thought about how to disseminate information to tell the people of the world what they wanted to do. As a result, the site received many comments of approval and expectation. The two challengers got more learning than the success or failure of acquisition of research funds, and it was a good opportunity for let more people know about "in-body hospitals" as well.

Dr. Sabina Quader's site

Dr. Miyazaki's site

COINS Seminar Report

～ From King SkyFront (Region) towards Construction of Global collaboration ～

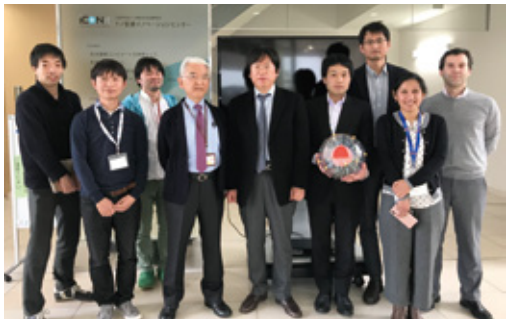
〈COINS Seminar #30〉

On Monday, February 16, Professor Sei Kwang Hahn (Department of Materials Science and Engineering) of Pohang University of Technology (POSTECH) in Korea gave a lecture. Under the theme of Smart Photomedicines using Multi-Functional Nanomaterials, he introduced development status of the latest Smart Photo Medicine for further biological and medical application. Many researchers from various universities and research institutes joined on the day, and discussions after the lecture and business card exchange were actively performed. The seminar was very successful.

POSTECH is a high-level research institute where pioneering research in the field of science and technology are conducted, and at the same time it is a remarkably growing university as it became the top ranking in world university rankings

Prof. Sei Kwang Hahn

Professor Hahn's technology is also important in the realization of "in-body hospitals" and the discussion among the researchers which was held before the seminar was also heated up. We anticipate that further collaboration between POSTECH and iCONM will progress with this association.



Anticipate collaboration with Prof. Hahn

〈COINS Seminar #32〉

On Tuesday, April 24, Mr. Takao Inoue, Director, Division of Molecular Target and Gene Therapy Products, National Institute of Health Sciences (Nucleic Acid Pharmaceutical Affairs Division) gave lecture at the Kawasaki Life Science and Environment Research Center (LiSE). (Reference: NL p.2–5 talks). There were many participants from companies, universities, research institutes, etc., and we could see that it was on going high-profile.

In the lecture on "Development Trend of Nucleic Acid Pharmaceuticals and Current Status of Regulatory Improvement", outline of basic characteristics, classification, action mechanisms, development trends, etc. of nucleic acid drugs and concept of quality / safety evaluation peculiar to nucleic acid drugs were introduced. Active discussion continued until at the reception party.

Currently, the National Institute of Health Sciences and iCONM are located in the same Kawasaki City King SkyFront area. We hope that this seminar is also a starting point that collaboration will progress with respect to the medicine of nucleic acid medicine and mRNA.

Prof. Takao Inoue

Editor's Note

Those who read the past issue of "NanoSky" (vol. 2–5) may have felt a little odd in the contents of this vol.6.

The themes of COINS that we have covered so far have the strong image of using the DDS function of nanomachines to deliver drugs to the target pinpoints and attack the cells / tissues and treat. However, the theme 3 of this journal is completely opposite.

DDS is essential, but rather than pursuing its emphasis on medicating the mRNA of its contents, it is oriented toward treatment based on normalization and activation of existing cells, not attacking on cells.

Theme 3 has been working on mRNA drugs ahead of the rest of the world and not only promoting research, but also establishing outreach and venture in cooperation with other organizations, social implementation is progressing in parallel at the same time.

It may be because of that reason, the tone at the talks (p. 2–5) was modest or realistic at the place, but we could feel their enthusiasm for sending out to the world to contribute for society. It seems that there are still few people with the same intention in the country yet, but we hope "NanoSky" will be able to contribute that it will grow into a big business which will save people in need and even the Japanese economy.

(Editor-in-chief Takashi Sugimoto)

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