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It's in the genes

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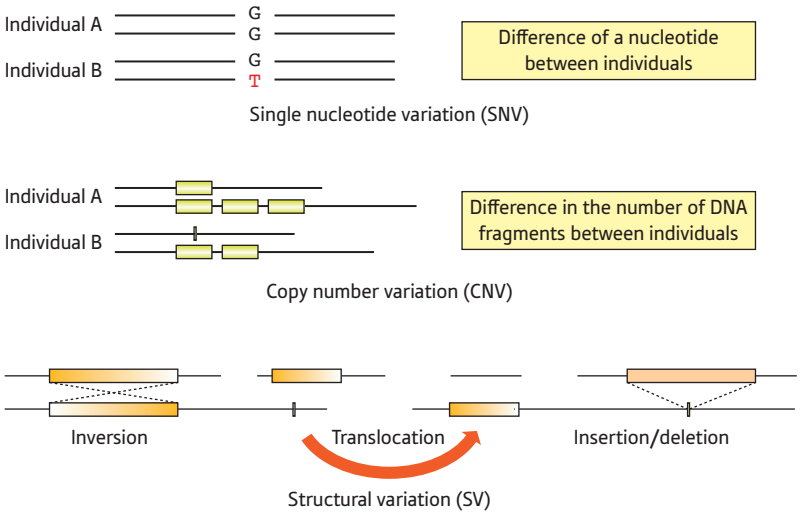


Figure 2: Individual human genomes can differ from one another in a variety of different ways. These can include single-nucleotide variations (top), where only one base is different; copy number variations (middle), in which a stretch of DNA is repeated to a different extent between individuals, or structural variations (bottom), which represent larger-scale rearrangements of chromosomal structure.

Tsunoda and colleagues observed a similar pattern when they compared NA18943 to six other previously characterized individual genomes. Of the nonsense SNVs identified within this collected dataset, 63% were 'singletons', or variants that occurred only once across all seven genome sequences. Further, the total collection of nonsynonymous SNVs contained significantly more singletons than were found among the set of non-protein-altering, synonymous SNVs.

Their analysis also revealed numerous regions where the NA18943 genome had been subject to insertions or deletions, more than 350 of which were predicted to markedly alter or disrupt the coding sequence of a gene. Notably, a significant percentage of these were detected within genes involved in olfactory or chemical stimulus perceptions, both of which are known to vary extensively between individuals.

Cause for a closer look

The researchers used a variety of established molecular biology techniques to verify the quality of these data from NA18943. Their findings collectively confirm that the genome of any given individual is likely to exhibit large numbers of rare, but functionally meaningful, variations relative to the general population or even individuals who are closely related from an evolutionary perspective. "We will have to sequence many more individuals within our population as well as across other populations around the world in order to obtain a clearer, more complete picture of the human genome," says Tsunoda.

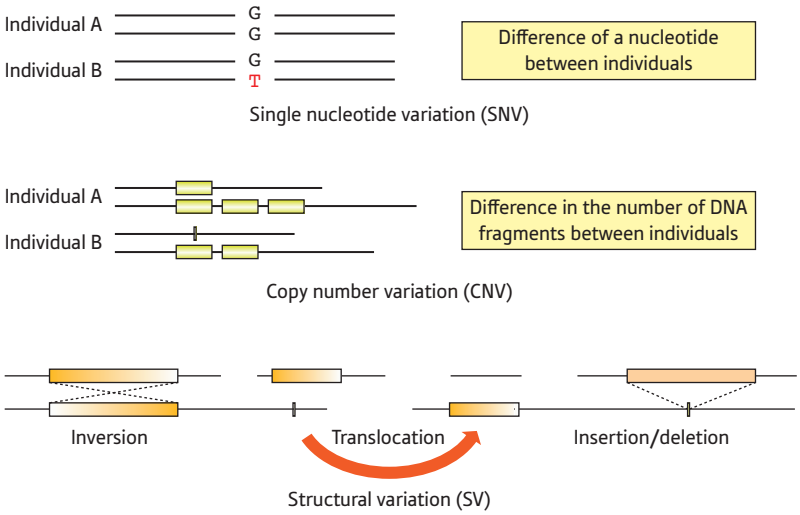
These findings could also have important ramifications for the conduct of studies into the genetic roots of human disease. Many such investigations are based on so-called 'genome-wide association studies' (GWAS), which use known SNVs as starting points for mapping sites in the genome that contribute to the pathology of complex conditions such as diabetes, rheumatoid arthritis or various forms of cancer. However, by over-emphasizing known SNVs, which are by definition more common in the general population, such studies may ignore many rare variants that offer better insight into disease pathology or are more prevalent among select populations, such as individuals of Japanese ancestry.

Tsunoda hopes this work will help steer future population-scale genetic studies as well as the group's ongoing tumor analysis efforts for the ICGC. "Our findings promote the potential of high-accuracy personal genome sequencing," says Tsunoda. "We have found that the variations that are functionally relevant to diseases may include lower frequency alleles that are not so common in the population as the SNVs that people are currently using for GWAS, and we may have to sequence individuals' genomes to look at such variations." ■

1. Fujimoto, A., Nakagawa, H., Hosono, N., Nakano, K., Abe, T., Boroevich, K.A., Nagasaki, M., Yamaguchi, R., Shibuya, T., Kubo, M., Miyano, S., Nakamura, Y. & Tsunoda, T. Whole-genome sequencing and comprehensive variant analysis of a Japanese individual using massively parallel sequencing. *Nature Genetics* **42**, 931–936 (2010).

About the researcher

Tatsuhiko Tsunoda was born in Tokyo, Japan, in 1967. He graduated with a degree in physics from the Faculty of Science at The University of Tokyo in 1989. He spent two years as a postgraduate studying elementary particle physics, and in 1995 obtained his PhD from The University of Tokyo's Department of Engineering. After researching computational linguistics as an assistant professor of Kyoto University until 1997, he started research on human genome sequence analysis as a research associate of Institute of Medical Science at The University of Tokyo. He subsequently worked on cancer gene expression as an assistant professor. In 2000, he joined the RIKEN Center for Genomic Medicine as head of the Laboratory for Medical Informatics. He holds PhDs in medicine and engineering, and his research focuses on statistical genetic analysis of human genome variations and gene expression analysis for medical research, including methodologies for personalized medicine.



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Quantum or not?

Mathematical equations can now resolve whether electron transport in nanostructures follows classical or quantum mechanical behavior

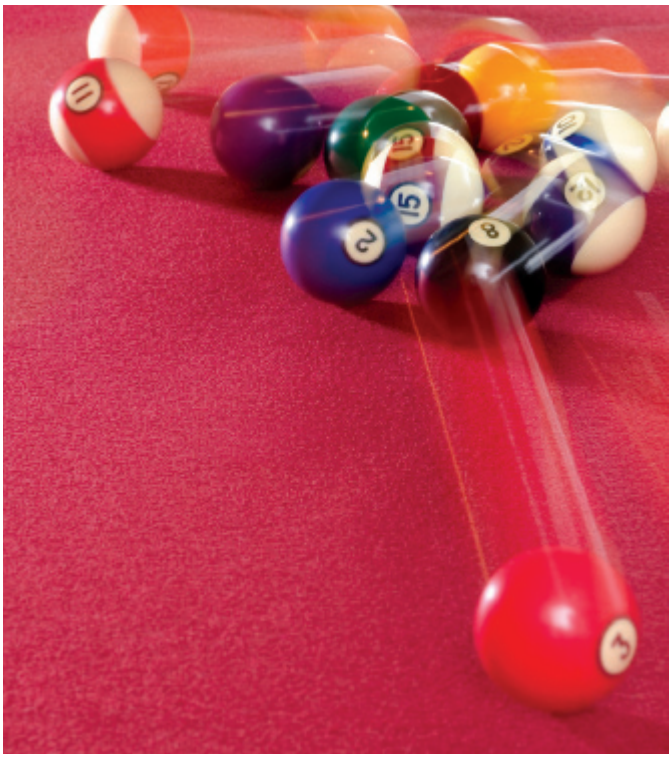

Figure 1: In a game of billiards, the path of each ball is determined by the classical laws of motion. At the microscopic scale, equivalent objects may be governed by quantum mechanics, such that their exact path of movement could take several directions.

Understanding the transport of electrons in nanostructures and biological molecules is crucial to understanding properties such as electrical conductivity or the biochemical behavior of molecules. However, determining whether the electrons are behaving according to the classical laws of motion or the quantum mechanical regime at the nanoscale is challenging because many nanostructures fall in a grey area between both regimes. Researchers from the RIKEN Advanced Science Institute in Wako, with colleagues from Germany and Taiwan, have now devised a set of mathematical equations that can distinguish classical from quantum mechanical behavior of electrons in nanostructures[1].

On a macroscopic scale, objects follow the classical laws of motion. Golf or billiard balls, for example, will follow exact, predictable paths. On a microscopic scale, objects such as electrons move according to the laws of quantum mechanics, where processes occur in a probabilistic manner (Fig. 1). Measuring the properties of quantum mechanical systems, however, is challenging.

"In microscopic systems, it is very difficult to perform ideal measurements without disturbing the system," explains Neill Lambert from the research team. As a consequence, measurements on quantum mechanical systems are difficult to distinguish from invasive measurements on classical systems, says Franco Nori from RIKEN and the University of Michigan, who led the research team. "It is important to be confident that experimental results are not originating from a classical effect, giving a false impression of quantum behavior."

As a model system, the researchers chose the transport of electrons through vanishingly small pieces of matter known as quantum dots. "Even measuring the current passing through a quantum dot represents an invasive measurement of the system," Lambert notes. To identify quantum effects, he and his colleagues developed a set of criteria expressed as a mathematical inequality relationship for experimental data from these quantum dots. Any excess over a critical threshold in the formula by a parameter represents a clear sign of quantum behavior. In their simulations the researchers found several regimes at low temperatures where quantum effects in the dynamics of electrons in the quantum dots should occur.

The inequality relation derived by the researchers is based on fundamental principles and therefore applies not only to the transport of electrons through quantum dots, but also to many open, microscopic electron transport systems, says Nori. He believes that it will soon be easier to determine whether electrons in nanostructures follow the rules of quantum mechanics or take the classical route of their billiard-ball counterparts. ■

1. Lambert, N., Emary, C., Chen, Y.-N. & Nori, F. Distinguishing quantum and classical transport through nanostructures. *Physical Review Letters* **105**, 176801 (2010).

Watching extreme lasers at work

Frame-by-frame observations of the ionization of argon atoms under extremely bright and energetic illumination could prove a boon to research

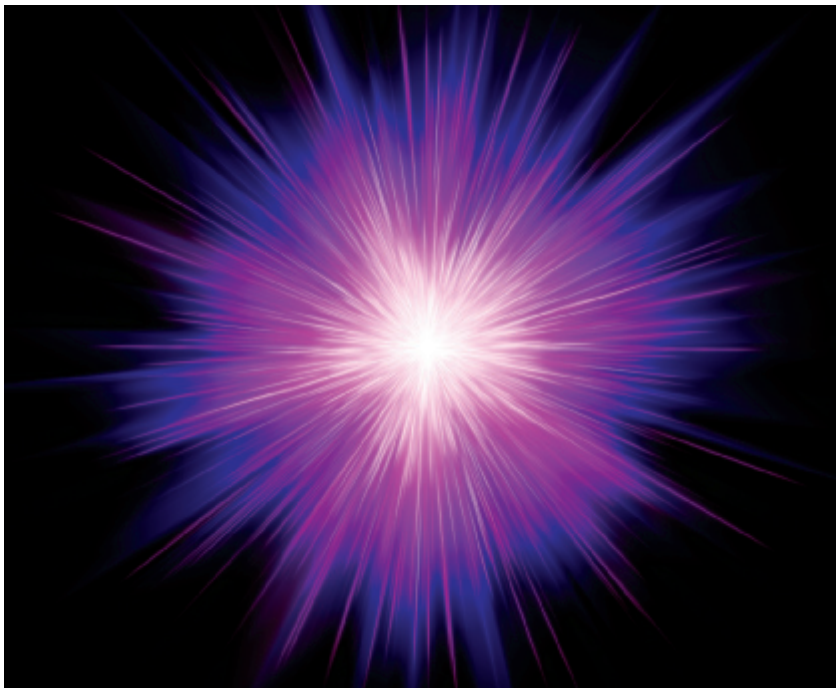

Figure 1: A bright and deeply ultraviolet laser beam can cause atoms to ionize.

Under extremely intense illumination materials may exhibit so-called nonlinear optical properties such as ceasing to absorb light beyond a certain brightness, or becoming highly ionized. Yasumasa Hikosaka, Mitsuru Nagasono and colleagues at RIKEN XFEL Project Head Office, Harima, and several other Japanese research institutes have now described the details of this ionization process by using very short bursts of bright laser light[1]. Their finding is relevant to a broad range of pure and applied research, including x-ray imaging of biological molecules, ultrafast optical switches, fusion and astrophysics.

The researchers focused on the behavior of argon atoms, which is easy to handle and well-characterized, under illumination by laser light about one hundred trillion times brighter than the noonday sun, and containing about seven times more energy per photon than the bluest light visible to the human eye. Previous work by other researchers showed that such intense, energetic light removes multiple electrons from target atoms, resulting in highly charged ions. While the mechanism of the ionization process was partially understood from observations of the yields and momenta of these ions, important details were missing.

Hikosaka, Nagasono and colleagues chose to observe the electrons emitted during the ionization process (Fig. 1), instead of the ions themselves. Not only do these electrons carry unique information about the ionization process, but they can be measured after each ultra-short laser pulse. Since the laser spectrum and power are constantly fluctuating, the fine details of the ionization process are averaged or 'smeared' during a continuous measurement. A shot-by-shot measurement, however, can account for laser fluctuations.

The experiment showed that the dominant ionization pathway of the argon atoms has two steps: first, a single laser photon is absorbed to create singly-ionized argon, and then two more photons are absorbed to create doubly-ionized argon. The researchers also found that the intermediate argon ion states had energy levels, or energy resonances, that induced this pathway.

The research leverages the recent development of free electron lasers, which are uniquely capable of producing very bright, energetic and short pulses of radiation. The work also illustrates that energy resonances are key to multi-photon, multiple ionization processes, a finding that is likely to be relevant to a variety of research programs. Hikosaka says that the research team will continue to focus on the basic science, as well as applications: "Our goal is to develop and leverage a deep understanding of the mechanism and dynamics of non-linear processes in order to manipulate or control these processes and their final products." ■

1. Hikosaka, Y., Fushitani, M., Matsuda, A., Tseng, C.-M., Hishikawa, A., Shigemasa, E., Nagasono, M., Tono, K., Togashi, T., Ohashi, H. *et al.* Multiphoton double ionization of Ar in intense extreme ultraviolet laser fields studied by shot-by-shot photoelectron spectroscopy. *Physical Reveiw Letters* **105**, 133001 (2010).

From pollution to solution

'Green' catalysts transform carbon dioxide gas into valuable building blocks for organic synthesis

Chemists are helping to reduce heat-trapping carbon dioxide (CO_2) emissions, which are a global concern. For example, they are devising new catalytic systems that would enable waste CO_2 to be recycled as a non-toxic and practically free source of carbon for organic synthetic reactions. However, current CO_2 conversion techniques require toxic heavy metal catalysts or expensive, drawn-out procedures.

Now, Zhaomin Hou and colleagues from the RIKEN Advanced Science Institute in Wako have found a way to insert CO_2 directly into the framework of aromatic molecules, turning them into carboxylic acid derivatives that are widely used as pharmaceuticals, agrichemicals, and dyes[1]. Importantly, this transformation can be achieved economically and with negligible environmental impact, thanks to a low-cost copper complex bearing an organic ligand.

N-heterocyclic carbenes (NHCs) are molecules with near metal-like reactivity because of an electron-deficient carbon center. For the past two decades, scientists have used NHCs as organic replacements for metal catalysts and as 'spectator' ligands that attach to metal centers and influence their catalytic behavior. Hou and colleagues recently discovered that adding NHCs to copper, one of the most abundant metals in nature, created a complex that catalyzed CO_2 addition to boron esters[2]—a trick the team hoped to repeat with aromatic hydrocarbons.

The most efficient way to incorporate CO_2 into benzene-like molecules is by replacing one of the carbon–hydrogen (C–H) bonds on the outer ring; unfortunately, these bonds are notoriously unreactive. To overcome this problem, the researchers turned to benzoxazole: this double-ringed aromatic compound has a C–H bond situated between nitrogen and oxygen atoms, making it easier to chemically activate this position.

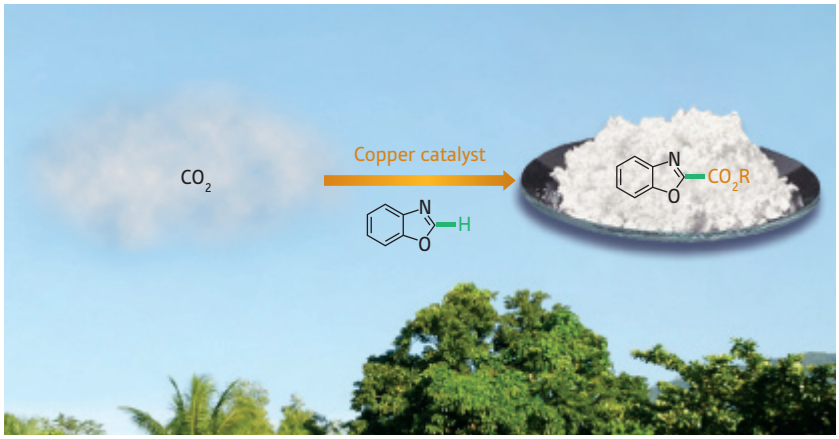

Figure 1: Copper catalysts are at the heart of an environmentally sustainable process that converts carbon dioxide gas into useful organic materials.

With just a pinch of the NHC–copper catalyst complex, the team found they could convert a mixture of CO_2 and several different benzoxazole-based molecules into solid carboxylic acids and esters in excellent yields (Fig. 1). Carefully characterizing the crystal structures of several intermediate compounds revealed that CO_2 inserted in between a copper–carbon bond formed at the benzoxazole C–H site, followed by a dissociation step that regenerated the catalyst.

According to Hou, the NHC ligand was essential in enabling CO_2 capture. "The electron-donating ability of NHC could make the C–H activation and CO_2 insertion steps easier, while its steric bulk brings stability to the active catalyst species," he notes. The researchers now hope to extend this technique to less reactive C–H bonds by fine-tuning the catalyst complex and optimizing reaction conditions. ■

1. Zhang, L., Cheng, J., Ohishi, T. & Hou, Z. Copper-catalyzed direct carboxylation of C–H bonds with carbon dioxide. *Angewandte Chemie International Edition* **49**, 8670–8673 (2010).
2. Ohishi, T., Nishiura, M. & Hou, Z. Carboxylation of organoboronic esters catalyzed by N-heterocyclic carbene copper(I) complexes. *Angewandte Chemie International Edition* **47**, 5792–5795 (2008).

Wired up and ready to glow

Linking silicon and carbon double bonds into an extended network with bulky molecules produces air-stable and photo-responsive crystals

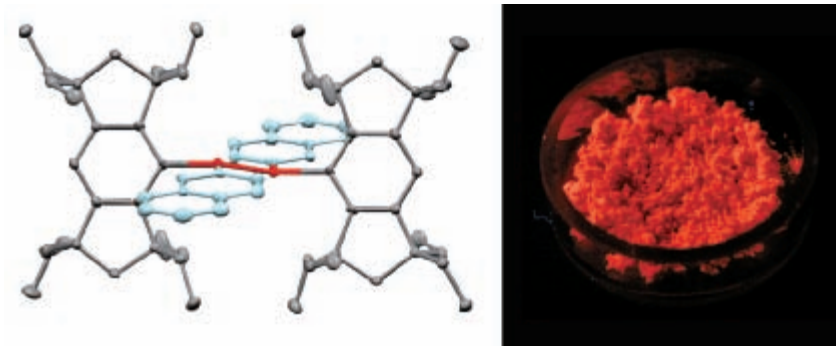

Figure 1: The x-ray crystal structure of a new compound with silicon–silicon double bond and naphthyl groups (red/blue atoms) held in place by bulky 'Eind' ligands (grey atoms) (left). This stable material formed from this compound emits a fluorescent glow after ultraviolet irradiation (right).

Thirty years ago, no one believed that elements other than carbon, nitrogen, and oxygen could form double bonds at room temperature. But the discovery of 'kinetic protection' ligands—large, bulky molecules that trap heavy atoms into multiply-bonded arrangements—forced a textbook rewrite. Researchers soon found that unsaturated bonds in newly synthesized substances such as disilenes, which have double silicon–silicon connections, generated chemical reactivity unlike any materials seen before.

Kohei Tamao and colleagues from the RIKEN Advanced Science Institute in Wako and Kyoto University have now discovered a way to make disilenes into thermally stable, light-emitting crystals by combining them with aromatic hydrocarbons[1]. The key to their approach is a protecting ligand known as 'Eind' that is rigid enough to lock carbon and silicon atoms into a wire-like network.

Atoms make double bonds by sharing electrons through banana-shaped regions of space known as 'pi orbitals'. If enough pi orbitals exist in a molecule, they can overlap and create conjugated pathways that allow for easy movement of electrons—properties that make such materials extremely responsive to light.

Incorporating disilenes into organic conjugated systems could produce enhanced photo-activity, but positioning two types of double bonds in one geometric plane for maximum pi orbital overlap is difficult. In 2007, Tamao and his team solved this problem by developing Eind ligands to protect a disilene–benzene compound[2]. Eind groups have a stiff framework of three fused hydrocarbon rings that encapsulate the pi network and force it into a planar geometry by stacking perpendicular to it, which enabled the disilene–benzene product to be isolated as a fluorescent orange solid.

In their latest work, the researchers investigated the effects of extended organic pi conjugation by adding naphthalene and fluorene groups—aromatic molecules nearly twice the size of benzene rings—to an Eind-protected disilene. X-ray analysis revealed that the resulting red crystals also had planar silicon–carbon conjugation, a structure that produced intense fluorescent light emissions after ultraviolet irradiation (Fig. 1). "We were excited to see such strong solid-state emissions even at room temperature," says Megumi Kobayashi, a co-author of the study.

Furthermore, these novel compounds showed unprecedented thermal stability, a critical requirement for future optical applications. "Usually, compounds having unsaturated silicon bonds are reactive and air-sensitive," says Tsukasa Matsuo, another co-author, "but our red disilenes are air-stable for almost one year." The team is currently working on improving the solubility of disilene–aromatic molecules to help develop longer and more light-sensitive double bonded materials. ■

1. Kobayashi, M., Matsuo, T., Fukunaga, T., Hashizume, D., Fueno, H., Tanaka, K. & Tamao, K. Air-stable, room-temperature emissive disilenes with π-extended aromatic groups. *Journal of the American Chemical Society* **132**, 15162–15163 (2010).
2. Fukazawa, A., Li, Y., Yamaguchi, S., Tsuji, H. & Tamao, K. Coplanar oligo(*p*-phenylenedisilenylene)s based on the octaethyl-substituted *s*-hydrindacenyl groups. *Journal of the American Chemical Society* **129**, 14164–14165 (2007).

Light games with DNA

The toolbox for imaging DNA now comes with an artificial DNA fluorescent base that can be 'switched off'

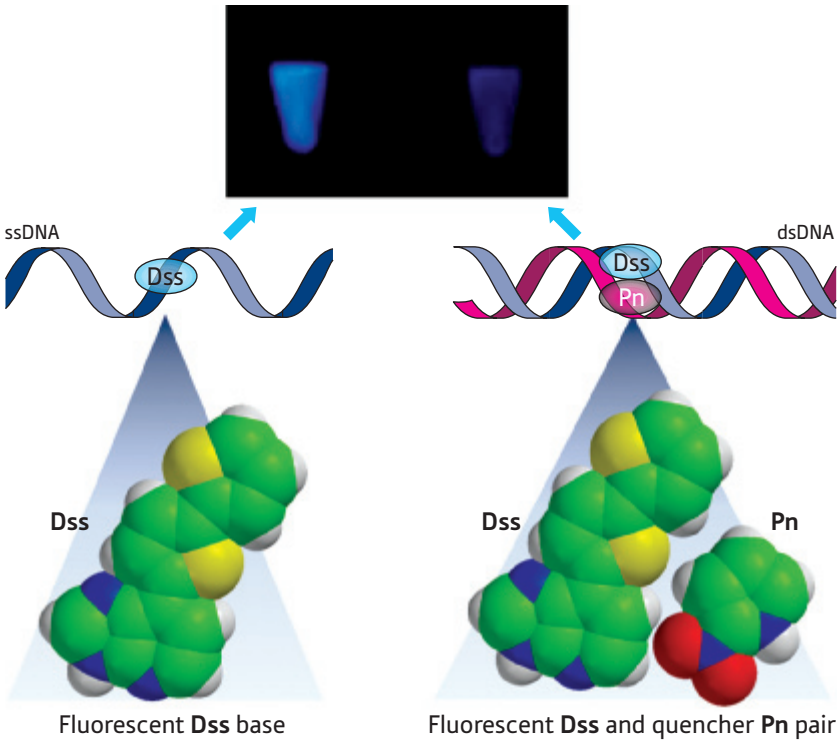

Figure 1: Schematic illustration showing fluorescence of the Dss base (left) and the Pn base (right) quenching the fluorescence upon hybridization.

The diagnosis of hereditary diseases and the identification of genetic fingerprints hinge on high-sensitivity DNA imaging biotechnologies. These imaging tools detect specific genes in cells using fluorophores—fluorescent tags that can illuminate DNA structures—and quenchers that interact with these tags to prevent them from emitting light, effectively working as an 'off switch'.

In a development that expands the detection toolbox and the genetic alphabet, a team led by Ichiro Hirao from the RIKEN Systems and Structural Biology Center, Yokohama, has now designed an artificial base pair between a fluorophore (Dss) and quenchers (Pn and Px)[1]. This method incorporates the pair into complementary DNA strands using polymerases and demonstrates that either Pn or Px can decrease the fluorescence of Dss upon hybridization (Fig. 1).

Hirao and his team previously developed artificial base pairs involving Dss because of its strong fluorescence, which could illuminate DNA and RNA structures. "This time, we can put out the candle lit by Dss using the quencher as its pairing partner at will," he says.

Hirao notes that this ability is unique because fluorescent dye Dss and quencher Pn face each other on their respective ssDNA strand, forming an artificial DNA base pair that also works in biological systems. He says that this close proximity results in strong 'contact quenching' of the fluorophore.

Usually, researchers have attached fluorophores and quenchers to natural bases through a linker that mediates so-called fluorescence resonance energy transfer (FRET) between dyes. However, this process lacks efficiency compared to contact quenching. Also, according to Hirao, unlike the Dss–Pn system, typical fluorophore–quencher pairs cannot be introduced at specific positions in DNA strands using polymerases, limiting their applications.

After establishing that the pairs were compatible with natural DNA synthesis techniques, Hirao's team integrated the Dss–Pn pair in the stem of molecular beacons—hairpin-shaped single-stranded DNA (ssDNA) structures that fluoresce upon hybridization with DNA targets. They found that the beacons detected the targets with high sensitivity and differentiated ssDNA containing one mismatched base.

Next, the researchers tested the performance of Dss–Px in polymerase chain reaction (PCR)—a powerful DNA amplification technique. Dss-bearing ssDNA fragments became less fluorescent upon assimilation of Px into synthesized DNA chains, allowing the team to monitor the amplification process in real time.

"One of our present tasks is to apply this system to *in vivo* cell experiments," says Hirao. "If it is possible, we will be able see the on–off of a specific gene expression." ■

1. Kimoto, M., Mitsui, T. Yamashige, R., Sato, A., Yokoyama, S. & Hirao, I. A new unnatural base pair system between fluorophore and quencher base analogues for nucleic acid-based imaging technology. *Journal of the American Chemical Society* **132**, 15418–15426 (2010).

A clear view through trees

Large tree-like sugar clusters provide potential *in vivo* probes for cancer cells

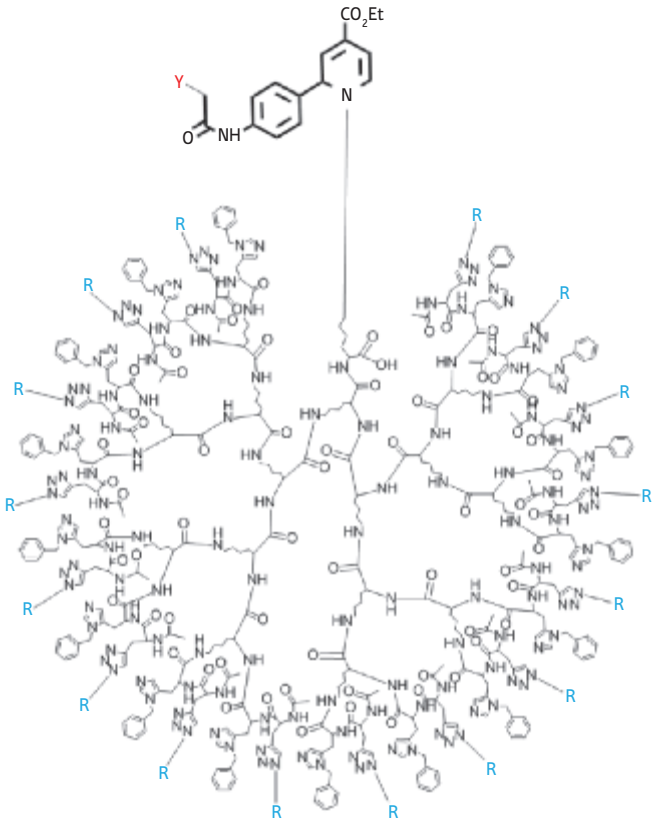

Figure 1: The structure of the tree-like cluster with 16 sugar branches.

Challenges in isolating and synthesizing protein-bound sugar molecules called N-glycans, which help stabilize insulin levels and modulate antibody-dependent immune responses among many other important processes in the body, has limited the investigation of their function and interaction with cultured cells and dissected tissues. Now, a team led by Yasuyoshi Watanabe and Satoshi Nozaki from the RIKEN Center for Molecular Imaging Science (CMIS), Kobe, has developed the first series of fluorescent and radioactive probes to track these molecules in living animals, which may eventually be used to track tumors[1].

According to Nozaki, N-glycans, which contain sialic acid residues, always form clusters *in vivo* allowing them to maximize their interactions and selectivity towards N-glycan-binding proteins and other biomolecules. "It is rather rare that a single molecule of N-glycan shows significant biological activity," he says.

To recreate these *in vivo* conditions, the researchers worked in close collaboration with Katsunori Tanaka from Osaka University to attach up to 16 sugar molecules to branched lysine oligopeptides, creating the largest tree-like oligosaccharide cluster ever prepared (Fig. 1). After linking the clusters to fluorescent and radioactive labels, they injected the resulting probes into the tail vein of immunodeficient mice.

Positron emission tomography (PET) imaging showed that the number of glycans in the clusters determined their lifetime *in vivo*. Four- and eight-sugar clusters rapidly disappeared through the kidney in just one hour. Clusters containing 16 N-glycans, however, remained in the body for over four hours before being eliminated through the kidney and the gallbladder—a desirable feature when studying how N-glycans travel in living subjects.

Furthermore, the team discovered that differences in the way the sialic acids are connected to the N-glycans influenced cluster behavior and build up in specific organs. The so-called (2–6)-linked sialic acids stabilized the clusters in serum, leading to their accumulation in the liver through interactions with specific protein receptors. In contrast, their (2–3)-linked congeners rapidly cleared through the bladder. Also, fluorescence imaging revealed that clusters bearing both types of linkages were most fluorescent in the spleen, suggesting their capture by a part of the immune system called the reticuloendothelial system.

The researchers hope to use these clusters as molecular probes for tumors. They are also planning to prepare clusters consisting of three to four different glycans in order to enhance the selectivity of the probes toward tumors and specific organs. "Nobody has done it, but the data shows that we can achieve it," says Nozaki. ■

1. Tanaka, K., Siwu, E.R.O., Minami, K., Hasegawa, K., Nozaki, S., Kanayama, Y., Koyama, K., Chen, W. C., Paulson, J. C., Watanabe, Y. & Fukase, K. Noninvasive imaging of dendrimer-type N-glycan clusters: *in vivo* dynamics dependence on oligosaccharide structure. *Angewandte Chemie International Edition* **49**, 8195–8200 (2010).

Towards a radical treatment for leukemia

Fumihiko Ishikawa

Unit Leader
Research Unit for Human Disease Model
RIKEN Research Center for Allergy and Immunology

When viruses or bacteria enter the body, they are eliminated by white blood cells. Yet white blood cells, which are so instrumental to our immune function, can become cancerous and proliferate abnormally and uncontrollably, resulting in a loss of the ability to produce normal blood cells. This disease is called leukemia, and it can occur in people of almost any age, from infants to the elderly. Several types of this intractable disease exist, including acute myelocytic leukemia, which has a higher incidence in adults. About three in every 100,000 people are thought to develop the disease. A small number of leukemia patients can now be cured completely through anticancer drug treatment or bone-marrow transplants, but acute myelocytic leukemia has a particularly high relapse rate, leading to death in many cases. In 2007, Fumihiko Ishikawa and the members of his Research Unit for Human Disease Model at the RIKEN Research Center for Allergy and Immunology (RCAI) discovered that the major cause of this high relapse rate lies in leukemia stem cells, which are resistant to anticancer drugs. In 2010, they presented new research results on two approaches to killing leukemia stem cells.

Development of immunologically humanized mice

After graduating from the Faculty of Medicine at Kyushu University in 1997, Ishikawa began to work as a clinician in charge of leukemia treatment in the First Department of Internal Medicine at Kyushu University's Faculty of Medicine. "I used to share wonderful experiences with patients and their families when our treatment with anticancer drugs greatly improved patients' symptoms. They were indeed amazing experiences for a doctor, but I thought that we should not be satisfied with this."

Some patients experience a recurrence of leukemia even when their symptoms have been greatly improved. Acute myelocytic leukemia has both high recurrence and mortality rates. "Patients and their families are leading their lives in fear of a recurrence of the disease. This led me to think that basic research is indispensable to prevent the disease from recurring, and that finding a radical treatment for the disease is of utmost importance."

In 1998, Ishikawa moved to the Medical University of South Carolina in the USA, where he started his studies on humanized mice in order to analyze the human immune system using mouse models. "Mice are used in many studies to understand biology *in vivo* and find effective ways to overcome diseases. For example, researchers create model mice and develop particular diseases in them in place of human patients. However, the findings from mouse studies are not always applicable to medical care or drug discovery. This is why I wanted to attempt to recreate the human immune system in a mouse."

The research theme was quite challenging. In 1988, a research group led by Stanford University in the USA published a pioneering study on reconstituting human immunity in mice. As the engraftment levels of human cells were not that high, investigators attempted to improve the *in vivo* assay in varous ways. Ishikawa's attempt to create an immunologically humanized mouse took a different approach. "When human cells are transplanted into a mouse, the cells are rejected by the mouse's immune system. To avoid rejection, we need to create

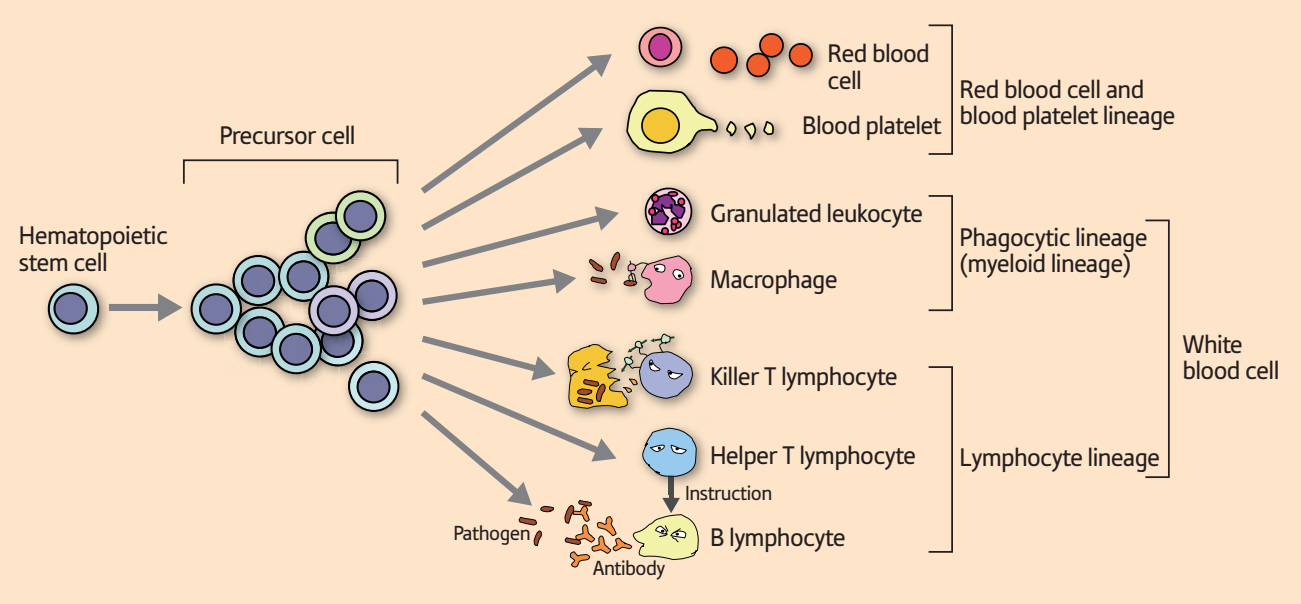

Figure 1: Major blood cells that differentiate from hematopoietic stem cells.
Hematopoietic stem cells differentiate into various types of blood cells. Acute myelocytic leukemia develops when phagocytic-lineage cells become cancerous.

an immune-deficient mouse, or an immune-suppressed mouse. Then we can transplant hematopoietic stem cells from humans into the mouse to create various blood cells, including human-derived white blood cells."

There are many types of blood cells, including white blood cells, red blood cells and blood platelets. White blood cells are responsible for the immune response and can be grouped into phagocytic cells, which ingest and consume foreign bodies, and lymphocyte cells, which attack foreign bodies (Fig. 1). The various types of blood cells all are produced by hematopoietic stem cells. "The exact location of hematopoietic stem cells was not clearly known, which made it difficult to extract them from surrounding tissue. I searched for molecules that could serve as markers for hematopoietic stem cells, and in doing so moved our own research forward."

In 2002, Ishikawa had a chance to meet Leonard Shultz from the Jackson Laboratory in the USA. "Doctor Shultz is an authority on immune-deficient mouse development. We got along well because of our shared desire to overcome leukemia and a belief that the key to achieving this goal is to search for the pathogenesis of the disease by studying immunologically humanized mice. We have been collaborating in our research ever since." Leonard Shultz has been supporting the research activities of the Ishikawa's laboratory through the creation of various new strains of immune-compromised mice and constructive discussion.

Ishikawa returned to Japan in 2002 and again started to work as a physician scientist in the First Department of Internal Medicine at Kyushu University's Faculty of Medicine. He also teamed up with young medical doctors and graduate students in the First Department and pursued his studies on immunologically humanized mice. "We worked not only on developing a technique for extracting hematopoietic stem cells from humans selectively, but also on a technique for transplanting those stem cells into newborn immune-deficient mice by injecting the cells into the mouse's blood vessels. We also had discussions with Dr Shultz to decide what kind of immune-deficiency mice should be developed in order to devise treatments for human diseases including recurrent leukemia. In 2005, we successfully created a prototype of an immunologically humanized mouse." In their model immunologically humanized mouse, human-derived white blood cells account for 80–90% of the white blood cells.

Pointing the finger at leukemia stem cells

It was Masaru Taniguchi, director of the RIKEN RCAI, who drew special attention to the center's studies on immunologically humanized mice. In 2006, Ishikawa started his own research unit at the RCAI and began to work on creating leukemia humanized mice, that is, mice with human acute myelocytic leukemia.

Leukemia has been thought to be caused by the continuous proliferation of leukemia cancer cells. In recent years, however, it has become known that the large numbers of leukemia cells found in cases of acute myelocytic leukemia are produced by just a small number of leukemia stem cells.

"We extracted leukemia stem cells from the bone-marrow fluid of an

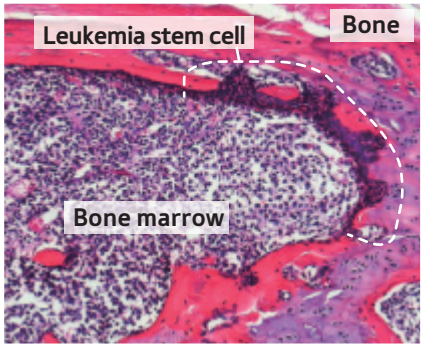

Figure 2: Leukemia stem cells that survive treatment with anticancer drugs.
Treatment of leukemia humanized mice with anticancer drugs can kill many leukemia cells, but 70–80% of leukemia stem cells survive, concentrating in the niche between the bone and bone marrow.

acute myelocytic leukemia patient and transplanted them into newborn immune-deficient mice. The mice subsequently developed symptoms similar to human acute myelocytic leukemia." Ishikawa and his team successfully created leukemia humanized mice in 2007 by applying the same technique as used for generating immunologically humanized mice.

"The mice developed leukemia after receiving about 1,000 leukemia stem cells, but did not develop the disease when injected with just the leukemia cells themselves, even when we transplanted over a million such cells. This result clearly showed that the stem cells are the pathogenesis of leukemia. Various studies on mice now suggest that leukemia stem cells result from some abnormality occurring in the genes of hematopoietic stem cells or precursor cells that eventually differentiate into various blood cells."

Ishikawa and his team have also succeeded in clarifying the reason for the recurrence of leukemia. "Most leukemia cells other than stem cells are eliminated when we administer anticancer drugs to leukemia humanized mice during studies that recreate leukemia treatment for human patients. However, we found that 70–80% of leukemia stem cells survive treatment, and these continue to go on producing large numbers of new leukemia cells. This is how we discovered that the recurrence of leukemia is caused by the leukemia stem cells. The anticancer drugs used currently are ineffective against leukemia stem cells." The team also found that the leukemia stem cells that survived the treatment with anticancer drugs gather in the boundary region (niche) between the bone and the bone marrow (Fig. 2).

It is controversial whether cancer stem cells could be the pathogenesis for cancers other than leukemia. "It is well-accepted, however, that leukemia stem cells are the pathogenesis of acute myelocytic leukemia in adult patients because all of the different types of blood cells are derived from hematopoietic stem cells. Our research results strongly support this fact."

Killing leukemia stem cells

Investigations in Ishikawa's research unit then turned to examining the reason why anticancer drugs are ineffective against leukemia stem cells. Cancer cells, unlike normal cells, generally undergo repeated cell division and proliferation. In 2010, however, Ishikawa and his team found that leukemia stem cells in the bone marrow of leukemia humanized mice did not undergo this cell cycle of cell division and proliferation. "Researchers have developed anticancer drugs that target the cancer cells that divide and proliferate at high rates. These drugs can be considered to be ineffective against leukemia stem cells, which have a paused cell cycle."

Ishikawa tried administering a protein called cytokine to stimulate cell division and proliferation in leukemia humanized mice. Yoriko Saito, a senior researcher in the Ishikawa Unit, demonstrated through confocal imaging that treatment restarted the cell cycle of leukemia stem cells (Fig. 3) and that subsequent treatment with anticancer drugs killed many of the leukemia stem cells. "It has been known empirically at clinical sites that a combination of cytokine and anticancer drugs is effective." Although promising, however, this approach does not perfectly kill off all leukemia stem cells. "The effect depends on the patient," says Ishikawa. "Since there are various kinds of cytokines, one approach could be to find the best combination of cytokines for individual patients. We think that separating leukemia stem cells from their niche site between bone and bone marrow could also be effective because it may be that certain molecules in the niche act on the leukemia stem cells to stop their cell cycle. Separating leukemia stem cells from the niche may restart the cell cycle."

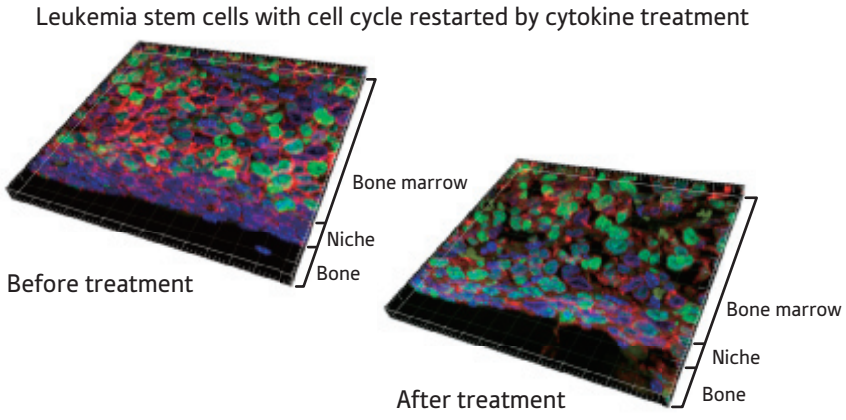

Figure 3: Composition of bone marrow in leukemia humanized mice.
Before cytokine treatment (left), cells with an active cell cycle (green) are absent from the niche, where leukemia stem cells concentrate. After cytokine treatment, the cell cycle is restarted in niche cells (blue, cells within the bone marrow; red, white blood cells).

In cooperation with Osamu Ohara, group director of the RCAI Laboratory for Immunogenomics, Ishikawa and his team have also been targeting leukemia stem cells directly. "We compared the genes of leukemia stem cells with those of normal hematopoietic stem cells and determined which genes are activated only in the leukemia stem cells. We also used various approaches to narrow down the genes to 25 molecules that express themselves only in leukemia stem cells." These molecules include those that express themselves on the surface of leukemia stem cells and enzymes essential for the leukemia stem cells' functioning and survival. Effective approaches can be devised based on this discovery, such as developing drugs that can combine with the molecules on the surface of the leukemia stem cells, or those that can inhibit the functions of the enzymes (Fig. 4). "We will have found a complete cure for leukemia if such drugs can kill the leukemia stem cells."

In April 2010, RIKEN started its Program for Drug Discovery and Medical Technology Platforms aiming to support studies on drug discovery. "Our studies have also been selected as research themes that are to be supported. For example, we can take advantage of immunologically humanized mice or leukemia humanized mice to recreate various clinical conditions on an individual patient basis and try out the effects of candidate compounds under development or various treatments on these mice. We can also take advantage of these humanized mice to determine the best drugs or treatments for individual patients, which could lead to the development of personalized medicine. This program will surely advance studies towards a radical treatment for leukemia."

Ishikawa and his team are also improving their leukemia humanized mice. "In conventional leukemia humanized mice, the molecules in the niche where leukemia stem cells are concentrated are mouse-derived. Thus, we are attempting to replace the mouse-derived molecules with human-derived molecules to develop a new generation of leukemia humanized mice."

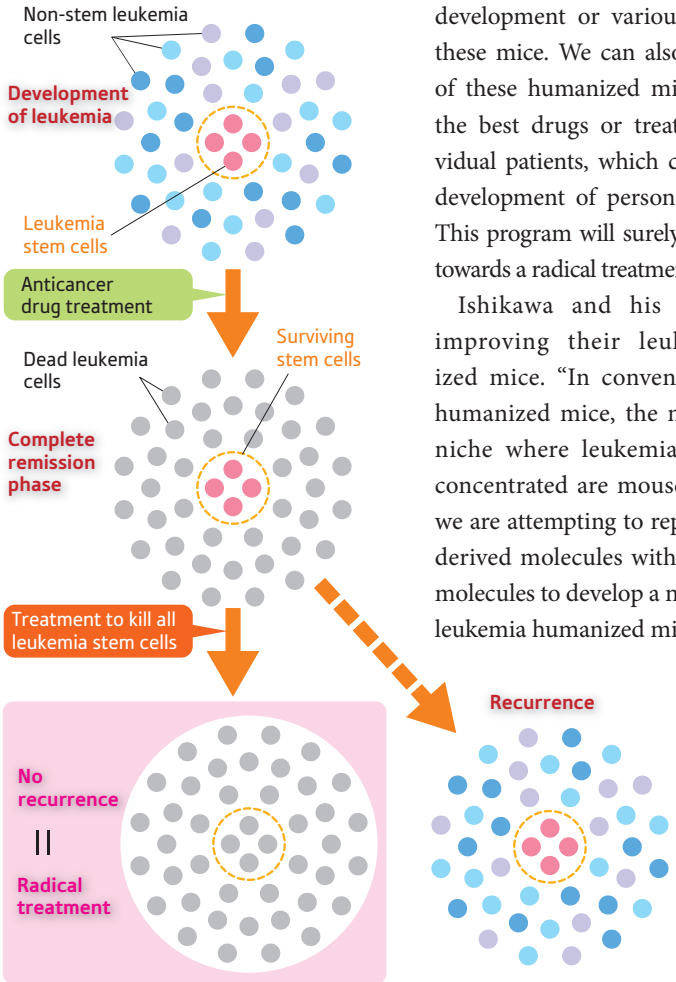

Figure 4: A possible cure for leukemia.
Treatment with anticancer drugs can kill non-stem leukemia cells, leading to a symptom-free state called the complete remission phase. The stem cells survive, however, because they are resistant to anticancer drugs, and continue to produce leukemia cells, leading to recurrence of the disease. Leukemia will be completely cured if all the leukemia stem cells are killed off, such as by starting the cell cycle of leukemia stem cells and administering anticancer drugs, or by using drugs that exclusively target leukemia stem cells.

Finding a radical treatment for leukemia through research on leukemia stem cells

In parallel to his studies at RIKEN, Ishikawa is communicating with clinicians. "We cannot make use of our research results or advance our own research without listening to the opinions of clinicians. This is why we are conducting our research in cooperation with Shuichi Taniguchi's hematology division at Toranomon Hospital, which boasts outstanding achievements in the treatment of leukemia."

Ishikawa and his team are moving ahead with their research with a strong will to achieve a cure for leukemia as soon as possible. "However, leukemia is the intractable disease among intractable diseases. It has yet to be beaten despite a long history of medical research. We need to focus not only on drug discovery and clinical applications, but also on the essence of leukemia stem cells that cause the disease. We will then be able to find a new rational approach toward an effective treatment. We need effective approaches from different perspectives in order to overcome the difficult challenge that leukemia presents." ■

Fumihiko Ishikawa

Fumihiko Ishikawa was born in Fukuoka, Japan, in 1972. He graduated from the Medical School of Kyushu University in 1997, and after residency training in the Department of Medicine, he began research on human immunology and hematology at Kyushu University and the Medical University of South Carolina, USA. Based on his success in the creation of a humanized mouse system, he joined RIKEN as unit leader at the RIKEN RCAI in 2006. Ishikawa and his group have been conducting research on clarifying unknown aspects of human immunity and diseases.

Karolinska Institutet–RIKEN Joint International Doctoral Course

An international doctoral course on the 'Functional architecture of the cell nucleus' was held at the RIKEN Yokohama Institute on 24–30 November 2010 as a joint initiative between the RIKEN Omics Science Center and the Department of Cell and Molecular Biology at the Karolinska Institutet in Sweden. The course aimed to provide both training for doctoral students from Swedish and Japanese universities in molecular biology and at the same time an opportunity to make important contacts with other students and researchers in the field. Lectures were given by leading researchers from Europe including Christer Höög from the Karolinska Institutet who is a member of the Nobel Assembly and 2010 announcer of the Nobel Prize in Physiology or Medicine, top graduate schools in Japan and RIKEN. Organized by Matti J. Nikkola from the Karolinska Institutet and Carsten O. Daub from RIKEN, the joint course builds on a long history of collaboration in research between the two organizations.

Researchers and students who paticipated in Karolinska Institutet–RIKEN Joint International Doctoral Course, including Carsten O. Daub (front row, third from left), Christer Höög (front row, center), Yoshihide Hayashizaki (front row, third from right) and Matti J. Nikkola (front row, right).

International students from Swedish and Japanese graduate schools

The course was open to doctoral students from the Karolinska Institutet and Japanese graduate schools, and also to any doctoral student affiliated with a Japanese research institute. Students of many different nationalities were represented among the group of 22 students that were accepted. In this truly international environment, the students studied topics such as evolution of the cell nucleus, meiosis and nuclear export. Courses were conducted in English, and the call for participants and advertisement for the course were made in both English and Japanese. "The poster on the graduate school bulletin board stood out among all the other posters in Japanese," commented one Egyptian student from The University of Tokyo's graduate school. "I saw it and immediately ran to my PC to fill out an online form and apply for the course."

Speaking English as a second language

The students were divided into six groups, each assigned scientific questions such as: "With the clinical use of induced pluripotent stem (iPS) cells, one of the biggest problems is quality control. A certain population of iPS cells is tumorigenic. Why can't we still completely control the reprogramming step?" Throughout the week-long course, students worked together in groups, and for the course examination they presented the results of this group work to other students and to the examiners. It was impressive to see how well the students communicated in English, even though this was not a mother tongue for most of them. Presentations were dynamic, and all students successfully passed the final course examination.

Building professional networks

The course also successfully motivated students to build new networks. "I joined this course because my professor recommended it," said one Japanese student, "but I found it quite exciting to create a presentation so quickly with people I had just met for the first time. It was a very positive experience for me." The course was also highly praised by other students. "I decided to join this course because I was interested in the subject," said a student from the Karolinska Institutet. "I really enjoyed everything I learned. I doubt there are many schools in Europe or elsewhere that offer the same level of courses and lecturers." Yoshihide Hayashizaki, director of the RIKEN Omics Science Center, was similarly pleased with the course's success. "I am very glad to see young researchers developing and building networks that will help them in their future career." The next course in this series will be held at the Karolinska Institutet in Stockholm, Sweden, next autumn. ■

Hiroyuki Osada
Antibiotics Laboratory
RIKEN Advanced Science Institute
Wako, Saitama, Japan

Dear Osada-sensei,

I hope you are keeping well during the winter months in Japan. As I have just finished writing my doctoral thesis, I have looked back in retrospect and realized that my three-year stay at RIKEN starting from the spring of 2007 has been a life-changing experience.

The amazing journey began when I was fortunate enough to have been accepted as a RIKEN Asia Program Associate to conduct research for my doctoral thesis at your laboratory. I was initially worried whether I would be able learn anything new, as the word 'antibiotics' is usually associated with Fleming, penicillin and the beginning of the twentieth century, but my skepticism was laid to rest when I saw before my eyes how the lab grew from strength to strength with each passing season under your visionary leadership.

Apart from the day-to-day lab work under the supervision and kind mentoring of Prof. Nobumoto Watanabe, I count myself lucky to have been in the right place at the right time to witness the establishment of the Chemical Biology Department, from the organization of staff and resources to the planning and opening of the Chemical Biology building itself.

Even from this bystander's point of view, I had a first-hand experience of the famous Japanese work ethic and research approach that I had only heard of before coming to Japan. It is true that the antibiotics lab members work hard, but they also play hard. I recalled many fond memories of sharing time with everyone—playing softball and badminton, the seasonal and celebratory lab parties, and especially being involved in organizing lab gatherings during my last year.

I am personally grateful that you and the other lab members took the trouble to use more English in the lab meetings, as this exposed me to the various types of research being conducted by different teams in your lab. Because of this, I was able to deepen my understanding of the multidisciplinary field of chemical biology.

I will always be humbled by the kindness and respect you showed for each and every member of the lab, and the effort you made to be available to everyone, especially through the weekly students' lunch that you attended.

I must also thank you and RIKEN for the opportunity to travel to different parts of Japan to participate in scientific meetings and to present my research findings. This greatly enriched my experience. One of the memorable meetings that I attended was the Noyori Summer School in 2009, where RIKEN's president, Prof. Noyori, was present to share his wisdom and experience with the younger scientists at RIKEN.

Upon my return to Malaysia, I was frequently asked to describe my experience at RIKEN, and thinking back, besides RIKEN's top-notch research facilities and support staff that allowed high-quality research to take place, I also learned so much more about life and how to be a better scientist from you, Watanabe-sensei, Sudo-san, Kobayashi-san and many other lab members too numerous to mention.

I can only imagine that your lab will grow further in strength and I wish you and everyone good health and success.

Terima kasih.
Gratefully yours,

Eugene Ong
School of Biological Sciences
Universiti Sains Malaysia

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RIKEN, Japan's flagship research institute, conducts basic and applied experimental research in a wide range of science and technology fields including physics, chemistry, medical science, biology and engineering. Initially established as a private research foundation in Tokyo in 1917, RIKEN became an independent administrative institution in 2003.

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