RIKEN RESEARCH

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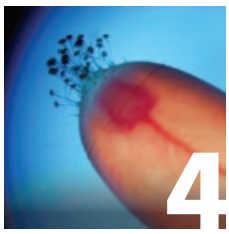
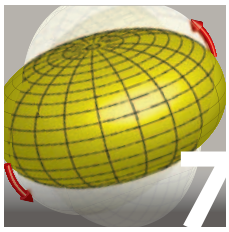
Melting point

900 MHz NMR facility
in Yokohama

Table of contents





RIKEN RESEARCH

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Biology

A vertebrate family reunion

By demonstrating the close 'relatedness' of two primitive jawless fish, scientists begin to assemble a more accurate depiction of the early history of vertebrate evolution

Figure 1: Adult hagfish, *Eptatretus burgeri*, seen from dorsal view.

Roughly 500 million years ago, our ancestors began developing a physical characteristic that is almost universal among modern vertebrates: jaws. In fact, the hagfish and the lamprey are the only known living vertebrates that do not belong to this 'gnathostome' majority. These jawless 'cyclostomes' are instead generally considered to be holdovers from the earliest days of the vertebrate lineage.

Yet hagfishes and lampreys bear striking physiological differences, calling into question the closeness of their relatedness and their relative positions on the 'evolutionary tree'. New research from Shigeru Kuratani's team at the RIKEN Center for Developmental Biology now provides striking evidence that these two fish should indeed be grouped together, based on a shared process of craniofacial development that is distinct from gnathostomes[1].

The hagfish (Fig. 1) lacks several features that define the vertebrate body plan, with a head that looks strikingly different from the lamprey in cross-section, leading some scientists to posit that hagfishes represent a more primitive digression from the vertebrate lineage. This would mean lampreys are a separate, more direct antecedent of gnathostomes, undermining the idea that both species belong to a single offshoot group of cyclostomes.

"It is a recent trend to view each animal lineage's developmental program as diverging and differing from each other," says Kuratani, "and not viewing one specific program as ancestral to the others." To resolve this debate, Kuratani and colleagues began a deep analysis of hagfish development.

Back to the beginning

One major developmental difference observed in hagfishes pertains to an essential endocrine gland known as the adenohypophysis. In lampreys and other vertebrates, this organ develops from an embryonic tissue called the ectoderm, while previous analyses have suggested that the hagfish adenohypophysis arises from an entirely different embryonic tissue—the endoderm. However, Kuratani was concerned about the foundations of that conclusion. "Those observations were partly based on ill-fixed samples with lots of tissue distortion, and from very few specimens," he says.

Historically, hagfish eggs have proved remarkably difficult to obtain. By working with local Japanese fishermen, however, Kuratani's team has been able to collect enough adult hagfishes to allow tissue to be cultivated in the laboratory, providing unprecedented access to freshly laid hagfish eggs (Fig. 2). This achievement has given the team valuable insights into the embryology of this enigmatic species.

Through close examination of craniofacial formation at precise timepoints in hagfish development with the aim of identifying similarities and differences relative to the lamprey and a representative gnathostome species, the cloudy catshark, Kuratani's team has made a number of important observations. For example, during an embryonic stage known as the neurula, the endoderm and ectoderm are brought into direct proximity with one another at a structure known as the oropharyngeal membrane, which ultimately forms

the mouth. However, the researchers identified another membrane in the hagfish neurula that appears to be unique to this group of species, which they termed the secondary oropharyngeal membrane (SOM). They concluded that previous investigators used the wrong 'landmark', mistaking the SOM for the oropharyngeal membrane, and thereby incorrectly positioned the origin of the adenohypophysis within the endoderm. Further embryonic analysis confirmed that this gland originates in the hagfish ectoderm, as with the shark and lamprey.

Kuratani and his colleagues identified other, clear similarities in lamprey and hagfish craniofacial development. In both animals the nostril and adenohypophysis originate from a single source tissue, remaining adjacent in the mature animal. In comparison, these structures arise from two separate tissues in gnathostome species, and mature gnathostomes typically have two nostrils (rather than the one observed in cyclostomes) positioned further apart from the adenohypophysis, which is in turn connected with the mouth and throat.

Uncovering an ancestral connection

These and other findings from Kuratani's team strongly support a model in which hagfishes and lampreys belong to a common 'branch' of the evolutionary tree. "With these findings, people will be convinced that these animals are evolutionarily very close to each other," says Kuratani, "and it also helps us identify homologous organs and structures in adult animals, which is very hard with simple dissection."

Their results also offer some other interesting insights. For example, the physiological oddities that have helped confound the positioning of hagfishes on the evolutionary timeline—such as the absence of a vertebral column—suggest that this species may have actually shed certain 'advanced' features found in lampreys to accommodate its distinctive lifestyle. "Hagfishes dive into rotten bodies of whales and large fish," explains Kuratani, "and make a movable knot in their long body to pull themselves out of the body they were eating." By comparison, lampreys feed on small organisms in the water or by attaching parasitically to the exterior of other fish.

Most importantly, this embryological comparative analysis has identified potential commonalities between processes observed in cyclostome development and those seen in the most primitive gnathostomes, indicating the possibility of a shared 'basic blueprint' for head formation. "This suggests a pattern that could be regarded as ancestral for all vertebrates," concludes Kuratani. In future work, his team will continue to explore the mysteries of hagfish development, but they also intend to broaden their investigation of early evolutionary history to include the amphioxus—also known as a lancelet—which is an invertebrate that scientists see as a close precursor to the emergence of vertebrate species. "We hope to reconstruct early vertebrate evolution and the establishment of the vertebrate body plan by describing it as changes in the developmental program associated with specific genomic changes," says Kuratani.

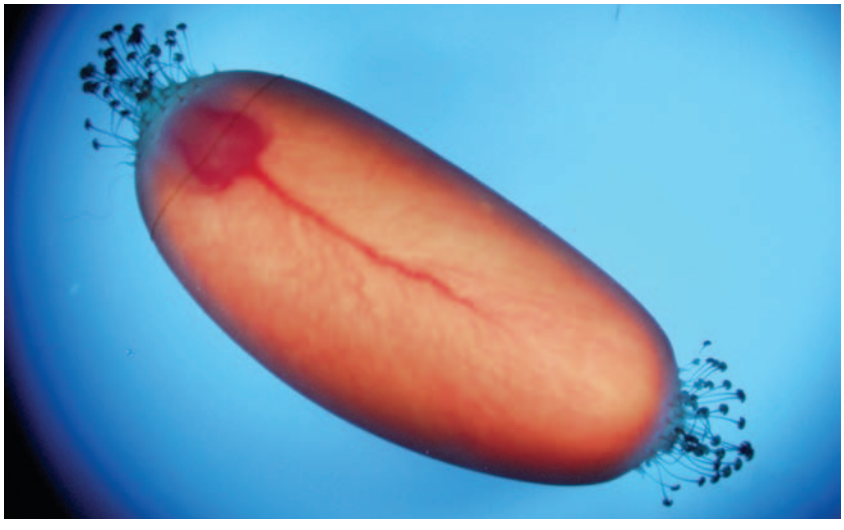

Figure 2: Growing embryo in the egg of *E. burgeri*, with the head visible at upper left.

1. Oisi, Y., Ota, K.G., Kuraku, S., Fujimoto, S. & Kuratani, S. Craniofacial development of hagfishes and the evolution of vertebrates. *Nature* **493**, 170–180 (2013).

ABOUT THE RESEARCHER

Shigeru Kuratani received his PhD from the Kyoto University Department of Zoology. From 1988 to 1991 he worked in experimental embryology in the Department of Anatomy at the Medical College of Georgia before moving to the Biochemistry Department at the Baylor College of Medicine, where he was engaged in molecular embryological research. He returned to Japan in 1994 to take on the position of associate professor at the Institute of Medical Embryology and Genetics at the Kumamoto University School of Medicine. He moved to Okayama University to assume a professorship in the Department of Biology in 1997, where he remained until he was appointed team leader at the RIKEN Center for Developmental Biology. He was appointed group director in 2005. Kuratani's research focuses on vertebrate head development and evolution, particularly the segmental organization and patterning of the embryonic head.

Melting back and forth

Experiments demonstrate unusual melting and recrystallization behavior in one-dimensional electron crystals for the first time

Unlike water ice, a one-dimensional 'Wigner' crystal on the surface of liquid helium can be made to repeatedly melt and recrystallize at the same temperature by increasing its electron density.

The melting of ice is a familiar process: the ice gradually loses its crystalline structure and becomes a featureless puddle of liquid. Normally, no amount of further heating will bring back the crystalline structure. Experiments by Hiroki Ikegami and co-workers at the Low Temperature Physics Laboratory in the RIKEN Advanced Science Institute* have now shown that a one-dimensional 'Wigner' crystal on the surface of liquid helium can be made to repeatedly melt and recrystallize with rising electron density[1].

The researchers studied a one-dimensional system of electrons floating on the surface of liquid helium using an intricate device designed to trap electrons in micrometer-sized channels. In these channels, the electrons adopt well-defined structures consisting of one or several chains with a few hundred electrons each. This arrangement provides an ideal setup for studying the behavior of such one-dimensional systems, explains Ikegami. "Generally, experiments involving regularly arranged chains are difficult because in many materials defects are inevitable. Our system is free from impurities and our sophisticated fabrication technique yields uniform channels to which the electrons are confined."

In their perfectly clean system, the researchers could control the number of electrons in each chain, and therefore the density of the one-dimensional crystals. They found that when they increased the electron density in the channel, the melting temperature at which the chains lose their well-ordered structure initially increased, as expected. At higher densities, however, they discovered that the melting temperature decreased, only to increase and then decrease again. "This sort of 're-entrant' melting behavior has been predicted in computer simulations, but it was surprising for us that we could really observe it in a real experimental system," says Ikegami.

The team came up with a detailed model that explains the periodical modulation of the melting temperature as a function of density. In essence, the model suggests that at each melting temperature change, the number of chains in the channel increases. In future research, the team plans to use their microchannel setup to perform quantum computations, in which the chains of electrons would be used to store and process information.

"Several schemes for quantum computation using electrons have been proposed," explains Ikegami. All of these schemes require single electrons to be confined to a small region—precisely the purpose for which the device developed by Ikegami and his team was made. "Our technique allows us to confine electrons in one direction, but more technical development is required before we can implement specific proposals."

1. Ikegami, H., Akimoto, H., Rees, D. G. & Kono, K. Evidence for reentrant melting in a quasi-one-dimensional Wigner crystal. *Physical Review Letters* **109**, 236802 (2012).

* Reorganized into new centers from April 2013

Silicon's double magic

The observation of a deformed atomic nucleus for a symmetric isotope of silicon suggests new forces at work

Silicon is mainly known for its use in the electronics industry, but its study may also reveal new details about the most fundamental forces of nature. Observations by Satoshi Takeuchi and fellow scientists from the RIKEN Nishina Center for Accelerator-Based Science have now shown that the silicon isotope ^{42}Si has a deformed atomic nucleus rather than the expected spherical structure, suggesting the presence of new types of forces in atomic cores[1].

The atomic nucleus contains protons and neutrons, where the number of neutrons can vary to give rise to various isotopes of a given element. The nucleus of the ^{42}Si isotope has 14 protons and 28 neutrons—'magic numbers' of each resulting in perfectly filled nuclear energy levels or 'shells'.

The nuclei of isotopes with magic numbers of protons or neutrons are usually perfectly spherical. Yet this is not always the case. "Forces may exist in the nucleus that break the shell stability caused by the magic number," says Takeuchi. The deformation caused by this breakdown of shell stability has been seen before for isotopes with a large proton–neutron imbalance. However, the observation of deformation in ^{42}Si, with magic numbers in both protons and neutrons, is particularly significant and is expected to help scientists to understand the cause of these deformations.

The experiments on ^{42}Si were only possible because of the facilities available to the RIKEN research team—the Radioactive Isotope Beam Factory (RIBF) for the production of a beam of ^{42}Si isotopes, and the DALI2 gamma-ray detector for efficient study of nuclear states. "We could not perform such experiments previously because they would have taken 100 or 1,000 times longer, and no other group in the world would conduct such a study," explains Takeuchi.

The results of the experiments suggest that ^{42}Si has a pancake-shaped nucleus (Fig. 1). This deformation differs from that for other isotopes, hinting at the involvement of a different deformation mechanism. Takuchi's team is already planning further experiments to investigate what such a mechanism might be.

"We are going to study isotopes such as nuclei around regions of ^{78}Ni and ^{132}Sn, which have magic numbers similar to ^{42}Si," says Takeuchi. "To look for isotopes with unexpected stability[2], such as ^{24}O, could also be of interest." Clarification of nuclear deformation is expected to expand our understanding of fundamental physical processes such as the evolution of stars and the formation of chemical elements in the Universe.

1. Takeuchi, S., Matsushita, M., Aoi, N., Doornenbal, P., Li, K., Motobayashi, T., Scheit, H., Steppenbeck, D., Wang, H., Baba, H. *et al*. Well developed deformation in ^{42}Si. *Physical Review Letters* **109**, 182501 (2012).
2. Tshoo, K., Satou, Y., Bhang, H., Choi, S., Nakamura, T., Kondo, Y., Deguchi, S., Kawada, Y., Kobayashi, N., Nakayama Y. *et al*. $N = 16$ spherical shell closure in ^{24}O. *Physical Review Letters* **109**, 022501 (2012)

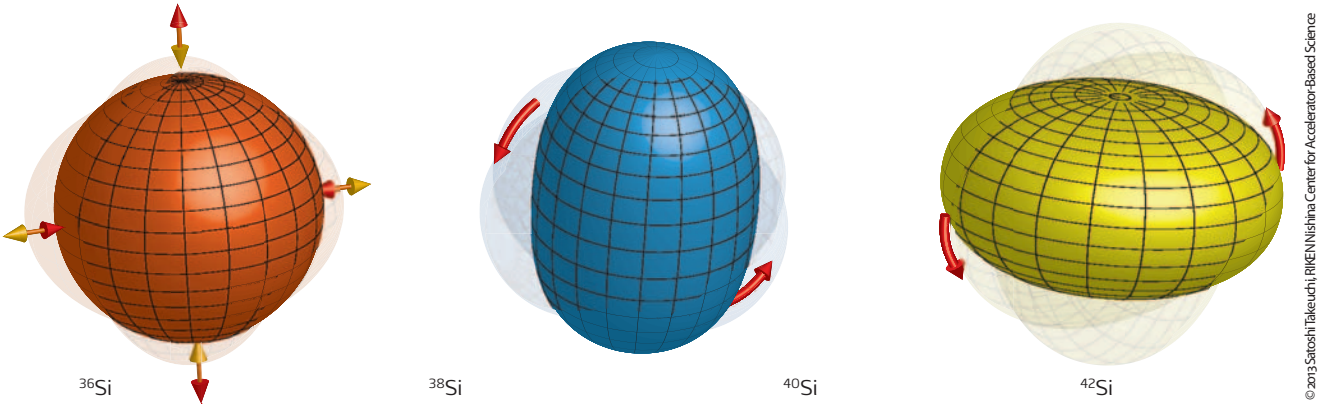

Figure 1: Nuclear shapes of silicon isotopes. The ^{42}Si nucleus should have a spherical shape similar to that for ^{36}Si, but instead has a pancake shape.

Watching bioactive molecules at work

Design rules for maximizing signal strength improve a technique that allows researchers to capture snapshots of small molecules as they move around inside live cells

Brightly fluorescing molecular tags have given scientists the unprecedented ability to see into living cells to study large molecules such as proteins. The technique is less well suited for imaging small bioactive molecules as the bulky tags required for fluorescent labeling can be larger than the molecule of interest itself. Mikiko Sodeoka and her colleagues from the RIKEN Advanced Science Institute* and the JST ERATO Live Cell Chemistry Project recently developed an alternative technique called alkyne-tagged Raman imaging (ATRI) that employs far smaller tags. The same team has now developed design rules for maximizing the signal strength of the Raman probes, making it possible to image multiple small molecules within the same cell[1].

Raman imaging identifies molecules by their characteristic interatomic vibrations. Although cells are crowded with molecules, the Raman spectrum of live cells incorporates a 'silent region' within which few molecules generate a Raman signal. Sodeoka and her colleagues previously showed that a two-carbon tag called an alkyne produced a strong signal within this region, which they exploited to image small molecules within living cells.

In their latest research, the team systematically examined various alkyne-type structures and measured the Raman signal that each tag generated. From these results, they developed design rules for creating tags with the strongest possible Raman signal. "Using this guide," says Sodeoka, "we can design potent probes more efficiently."

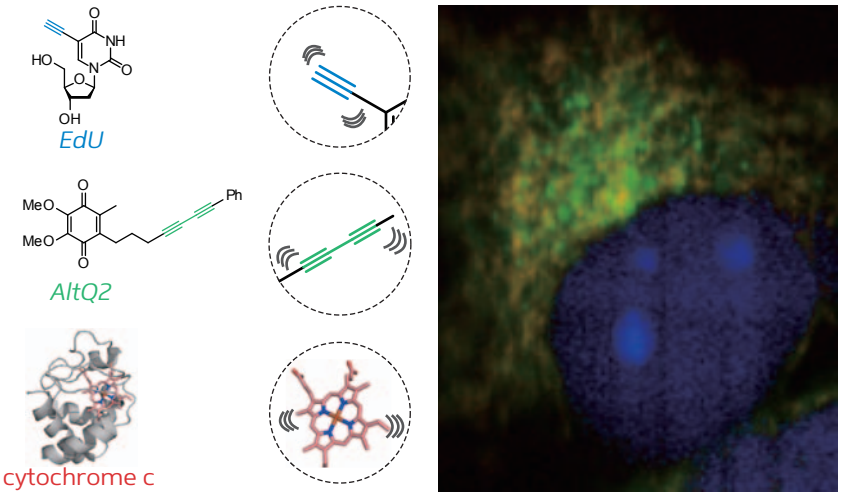

Figure 1: Simultaneous ATRI imaging of two small alkyne-tagged molecules (blue and green) and an endogenous protein (red) within a live cell.

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The researchers showed that two adjacent alkyne groups, or an alkyne attached to a benzene-like aromatic ring, made efficient tags for ATRI. Using this general design approach, they tagged and simultaneously imaged two small molecules within a live cell (Fig. 1).

"We showed two well-resolved Raman signals from small molecules, and we also obtained additional signals from endogenous molecules, such as cytochrome c," says Sodeoka. Florescence imaging, in contrast, typically generates complex overlapping signals when researchers attempt to image multiple tagged molecules simultaneously. "For a large number of target molecules, ATRI would be superior to fluorescence imaging."

Although Sodeoka's team has been able to maximize the Raman signal strength, further improvements in signal sensitivity are required to bring the sensitivity of ATRI up to a level comparable to that for fluorescent microscopy. The team plans to achieve this by improving the Raman microscope itself, as well as continuing to refine their tags. "A combination of chemical and engineering approaches could make a synergistic improvement to sensitivity," says Sodeoka. "After the improvement of sensitivity, we will apply ATRI for chemical biology research. We expect ATRI to be a key technique in this field."

1. Yamakoshi, H., Dodo, K., Palonpon, A., Ando, J., Fujita, K., Kawata, S. & Sodeoka, M. Alkyne-tag Raman imaging for visualization of mobile small molecules in live cells. *Journal of the American Chemical Society* **134**, 20681–20689 (2012).

* Reorganized into new centers from April 2013

Cancer drugs taking shape

A database of the characteristic changes to tumor cell morphology induced by anticancer therapies could help to accelerate drug discovery

In the era of molecular medicine, potentially active compounds for use in cancer therapies can be identified faster than ever before. Yet pinpointing the molecular target of an anticancer compound and deducing its mode of action remains a painstaking process. Yushi Futamura, Hiroyuki Osada and colleagues from the Chemical Biology Department of the RIKEN Advanced Science Institute* have now discovered that anticancer compounds induce a shape change in target tumor cells that is directly related to a compound's mode of action[1].

The cancer cell shape-shifting phenomenon was discovered by Futamura during a study of cells treated with the anticancer drug vinblastine, a tubulin inhibitor. He recalls being shocked by the dramatic morphological shift that the drug induced in the cells. "I remember thinking, 'Are these the same cells?'" he says.

Inspired by his initial observations of cell shape changes, Futamura and his colleagues began testing other known tubulin inhibitors and found that each had the same morphological effect—turning treated cells into flattened disks. Systematically examining other anticancer drug classes, the team discovered that each class induces its own characteristic changes in cell shape (Fig. 1). "We thus came up with the idea of making an encyclopedia of cell morphology, which we call Morphobase," says Futamura.

Morphobase now includes the results of tests on over 200 anticancer compounds with known modes of action, where shape changes in treated cells are quantified against 12 morphological parameters.

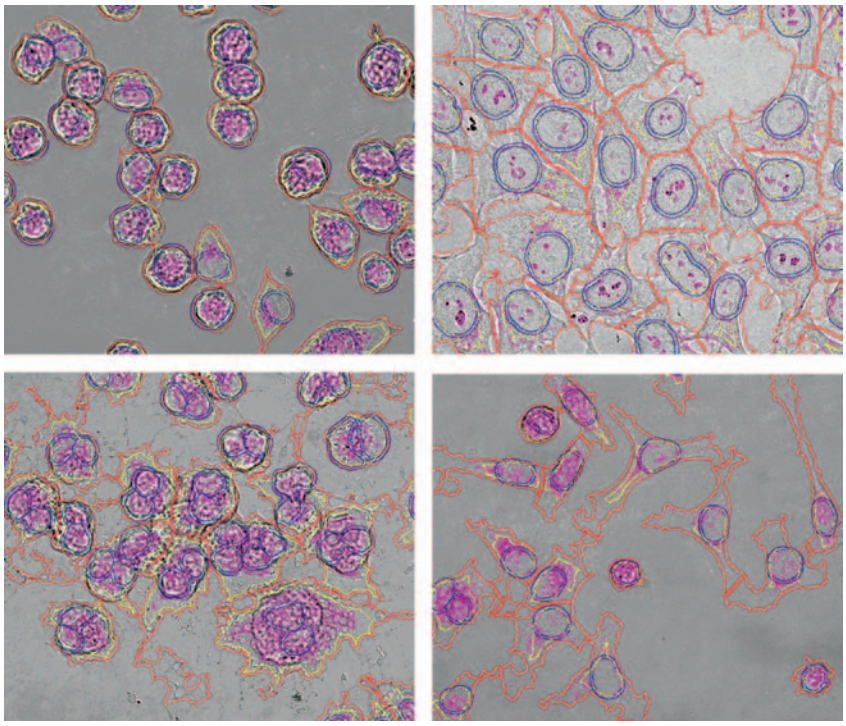

Figure 1: Microscopy images of stained tumor cells revealing some of the characteristic cell morphologies induced by anticancer drugs with different modes of action.

The researchers evaluated the potential of Morphobase as a predictive tool for rapid determination of anticancer compound modality by testing three anticancer compounds with unknown modes of action. By comparing the observed changes in treated cells with results in the database, they quickly established that all three were tubulin inhibitors. Further tests using conventional techniques confirmed the result.

Osada, Futamura and their colleagues are continuing to use Morphobase to establish the mode of action of novel anticancer compounds with promise as potential drug leads. The researchers are also refining and expanding the database by broadening the drug reference set and the range of drug doses for which morphology data is maintained. "These improvements and extensions to the system could improve its capability to identify the molecular mechanisms of drug activity, and in discovering novel drug candidates as well," says Futamura. The database has the potential to significantly cut the time it takes to establish the mode of action of novel anticancer compounds, removing a significant bottleneck in drug discovery research.

1. Futamura, Y., Kawatani, M., Kazami, S., Tanaka, K., Muroi, M., Shimizu, T., Tomita, K., Watanabe, N. & Osada, H. Morphobase, an encyclopedic cell morphology database, and its use for drug target identification. *Chemistry & Biology* **19**, 1620–1630 (2012).

* Reorganized into new centers from April 2013

Stopping the rot

Genomic analysis reveals the genetic basis of pathogenicity in crop-blighting fungi

The fungi *Colletotrichum orbiculare* and *C. gloeosporioides* represent major threats to crops around the world, responsible for blight and post-harvest rot in plants as diverse as melons, strawberries, mangoes, coffee and leaf crops. Both of these fungi are 'hemibiotrophs'—first growing parasitically on living plant tissue then killing the host and feeding on the remains. Ken Shirasu, Pamela Gan and colleagues of the Plant Immunity Research Group at the RIKEN Plant Science Center* have now sequenced the entire genomes of these two fungi and predicted their gene functions to identify the genetic basis for their hemibiotrophic pathogenicity[1].

Fungal pathogenicity in plants is known to occur through biochemical interaction between the fungus and host involving genetically encoded secreted proteins. The researchers found through their genomic analysis of *C. orbiculare* and *C. gloeosporioides* that both species, while having quite different genomes, exhibit similar 'expanded' gene classes that are not augmented in non-hemibiotrophic fungi. These included genes for digestive enzymes that degrade plant cell walls, for chitin-binding proteins that may help protect the fungal cell wall from plant defenses, and for the synthesis of secondary metabolites likely to be related to pathogenicity. Genes for small secreted proteins (SSPs), which are thought to facilitate fungal colonization, were also similarly expanded.

Shirasu's team also studied gene expression in *C. orbiculare* during infection of the model plant *Nicotiana benthamiana* (Fig. 1). They found distinct patterns in the timing of expression over the life-cycle of the fungus: SSPs, secondary metabolite synthesis enzymes and some chitin-binding proteins were found to be active early in host colonization, whereas digestive enzymes were most concentrated during plant death. The timing of secondary metabolite synthesis indicates that fungal secondary metabolites are not predominantly toxins as previously assumed, but instead may contribute to the manipulation of host growth during colonization. The presence of high levels of SSPs at an early stage suggests these are also important for colonizing the host.

The findings highlight the importance of different gene classes for all stages of hemibiotrophic fungus development, and the set of genes identified provides a crucial base for future investigation into pathogen activity. Ultimately, this research could lead to control strategies for these and other fungi, such as the development of crops with genes that provide resistance to secreted fungal proteins. "Knowing which plant resistance genes are effective against which pathogens aids in informing the deployment of rapid and targeted crop management strategies," says Gan.

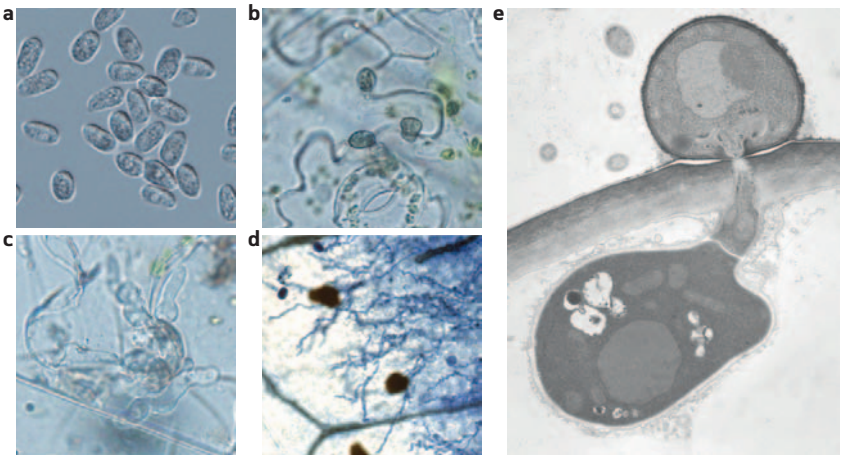

Figure 1: Life-cycle stages of *Colletotrichum orbiculare*. (a) Germination, (b) penetration of plant cells, (c) fungal proliferation, (d) development of fungal filaments associated with plant death. (e) Fungal infection of plant cell, showing fungal penetration into the cell and the formation of an infection vesicle.

Reproduced from Ref. 1 © 2012 P. Gan *et al.*

1. Gan, P., Ikeda, K., Irieda, H., Narusaka, M., O'Connell, R.J., Narusaka, Y., Takano, Y., Kubo, Y. & Shirasu, K. Comparative genomic and transcriptomic analyses reveal the hemibiotrophic stage shift of *Colletotrichum* fungi. *New Phytologist* **197**, 1236–1249 (2013).

* Reorganized into a new center from April 2013

Understanding metabolism through computer predictions

Combining computer-based statistical analysis with mathematical modeling can improve our understanding of metabolic pathways

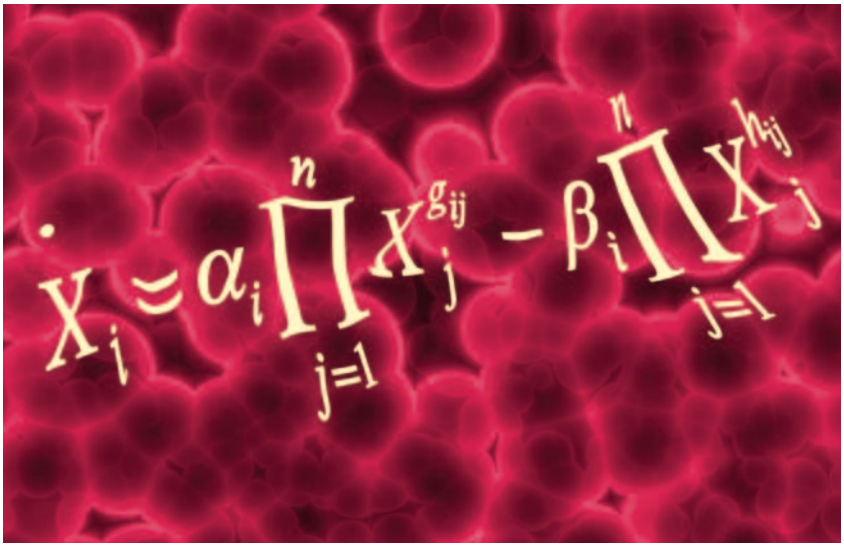

Figure 1: Starting with time-series data of metabolite concentrations, Hirai's team used a combination of statistical analysis and computer modeling to predict reaction networks for different metabolites within a metabolic system.

The chemical interactions among molecules and enzymes in an organism's metabolic system drive growth, reproduction and even evolution. Understanding the pathways that link enzymes and the molecular products of metabolism, called metabolites, can help scientists control metabolic reactions and therefore enhance organism productivity. This can be particularly useful in agriculture and industry.

It is already possible to show whether enzymes are activated or inhibited by certain metabolite interactions through intensive laboratory experiments, and correlations among metabolites can be deduced through statistical analysis of metabolite concentrations in a living organism. However, complete reaction networks of correlated metabolites remain difficult to identify.

Masami Yokota Hirai and Kansuporn Sriyudthsak at the RIKEN Plant Science Center*, in collaboration with Fumihide Shiraishi from Kyushu University in Japan, have now developed a method involving a combination of mathematical modeling and statistical techniques that allows probable metabolic reaction networks to be predicted and identified[1].

"In order to gain insight into the molecular mechanisms and biological functions of metabolism," says Hirai, "we have combined two important approaches to comprehensively understand metabolic reaction networks."

The team used time-series data of metabolite concentrations to predict the metabolic reaction network for the bacteria *Lactococcus lactis*. A disturbance in the concentration of one type of metabolite has a knock-on effect on the concentrations of other metabolites in the system. Consequently, analyzing the changes in metabolite concentrations over time can help to produce a picture of the metabolic reaction network.

Firstly, Hirai and her team carried out data smoothing through regression analysis. Next they used a test known as Granger causality to determine if one metabolite's set of time-series data could be used to forecast that of another metabolite. In this way, the team was able to eliminate metabolites that were unrelated.

The researchers then constructed a mathematical model based on the remaining metabolites to create a probable reaction network. The resulting predictions correlated closely with pathways already established for the bacteria.

"Our novel approach not only predicts metabolic pathways, but the mathematical model can also simultaneously analyze metabolism to determine its characteristics," explains Hirai. "For example, the model can find metabolic bottlenecks—where a healthy metabolic system is limited in some way—and can also predict the genes or enzymes responsible for causing them."

The team believes that their combined modeling approach could prove useful in large-scale metabolism studies in the future, and could also help in measuring individual enzyme activities and identifying entirely new metabolic pathways.

1. Sriyudthsak, K., Shiraishi, F. & Hirai, M.Y. Identification of a metabolic reaction network from time-series data of metabolite concentrations. *PLoS ONE* **8**, e51212 (2013).

* Reorganized into a new center from April 2013

Cellular networks up close

High-powered microscopic techniques give scientists a detailed view of a critical component of the cellular infrastructure

The cellular interior is criss-crossed by protein-based cables known as microtubules, each formed from 13 'protofilaments' composed of the protein tubulin. Microtubules are also associated with a host of other specialized proteins that help coordinate the transport of molecular cargoes and link microtubules to intracellular structures.

A research team led by Yuko Mimori-Kiyosue from the Optical Image Analysis Unit of the RIKEN Center for Developmental Biology is involved in the study of a subset of proteins that preferentially localize near the microtubule ends and regulate their assembly and disassembly. By performing an up-close examination of two such proteins, end-binding 1 (EB1) and colonic–hepatic tumor-overexpressed gene (ch-TOG), the team have now revealed surprising new details about the organization of microtubule ends[1].

Scientists have long believed that EB1 specifically accumulates at the microtubule tip, although the detailed structure of this region has proved difficult to observe. "The dynamic configuration of microtubule ends has never been studied in living organisms, mainly due to limited resolution of microscopy techniques," explains Mimori-Kiyosue. Her team overcame these limitations through the use of an ultra-high resolution imaging strategy, and was surprised to discover that EB1 is not actually the endmost microtubule protein. EB1 typically accumulates in comet-shaped structures, and for over 90% of the EB1 comets examined, ch-TOG was situated even further along the microtubule, indicating that it instead is the endmost protein (Fig. 1).

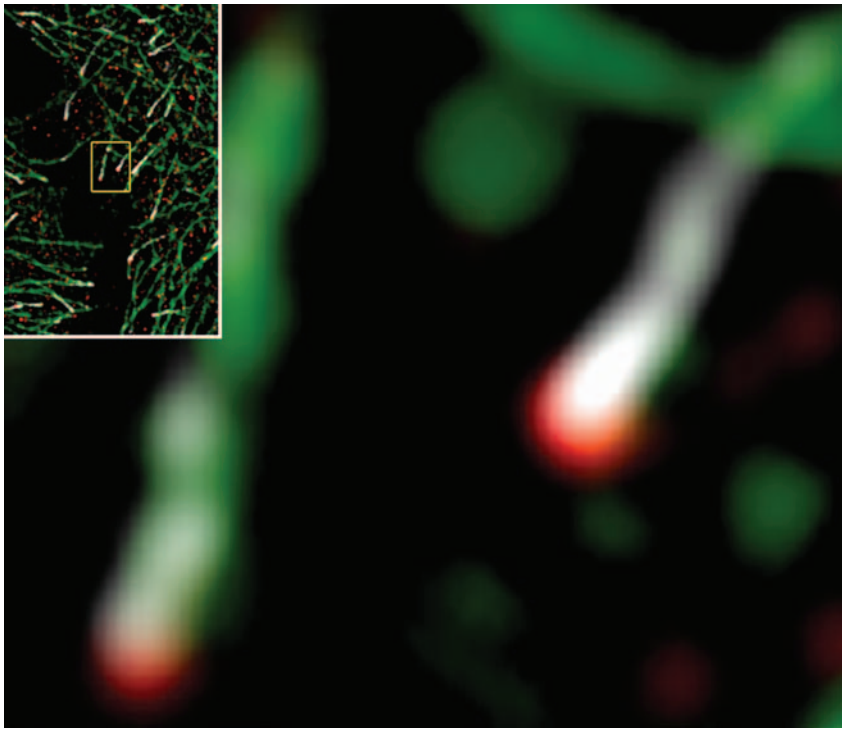

Figure 1: High-resolution microscopy of fluorescently labeled microtubules (green) in cultured cells reveals that ch-TOG (red) is consistently positioned much closer to microtubule ends than EB1 (white), shown inset and in close-up, right.

Microtubule ends are constantly growing and shrinking as tubulin subunits are added or removed, and depletion experiments demonstrated that EB1 and ch-TOG both contribute to the maintenance of this dynamic state in an independent and complementary fashion. However, Mimori-Kiyosue's team also identified a distinct role for EB1 in attaching microtubule ends to the inner surface of the cell membrane, which it accomplishes through interaction with other specialized membrane-anchoring proteins. Having these anchor points slightly removed from the end likely prevents such interactions from interfering with tubulin addition or removal at the microtubule tip. Mimori-Kiyosue finds this novel function of EB1 particularly interesting. "Appropriate organization of the microtubule network is very important," she says, "since microtubules serve as rails for cellular trafficking, which need to be placed correctly to deliver important materials to the correct destination."

Future studies by Mimori-Kiyosue's team should further clarify the role of these proteins in microtubule maintenance. As microtubules are core components of the cell division machinery and therefore primary targets for cancer drugs, these findings could in turn facilitate the discovery of new therapeutic agents.

1. Nakamura, S., Grigoriev, I., Nogi, T., Hamaji, T., Cassimeris, L. & Mimori-Kiyosue, Y. Dissecting the nanoscale distributions and functions of microtubule-end-binding proteins EB1 and ch-TOG in interphase HeLa cells. *PLoS ONE* **7**, e51442 (2012).

Stem cell therapy for Parkinson's disease

Dopamine-producing neurons derived from bone marrow stem cells yield improvements in monkeys with Parkinson's disease

Parkinson's disease is a neurodegenerative disorder characterized by the death of dopamine-producing neurons in the midbrain, resulting in motor symptoms such as tremors and stiffness. The cause of cell death remains unknown and researchers have long sought a way to replace the lost dopamine-producing cells. A study led by Takuya Hayashi from the RIKEN Center for Molecular Imaging Science* now suggests that in monkeys such neurons can be derived from bone marrow stem cells and then transplanted back into the brain to reverse the symptoms of this devastating disease[1].

Hayashi, Mari Dezawa from Tohoku University and their colleagues injected ten adult male cynomolgus monkeys (crab-eating macaques) with a neurotoxin that induces a Parkinson's-like condition. They then obtained bone marrow samples from the monkeys, isolated the marrow's mesenchymal stem cells (MSCs), and treated the cells with growth factors to direct them to differentiate into A9 dopaminergic neurons—the neuronal subtype that is most severely damaged in Parkinson's patients. The researchers subsequently transplanted the differentiated cells back into the forebrain of five of the donor monkeys, while the other five animals received a sham operation.

The procedure is an example of an 'autologous' transplantation, involving cells derived from and transferred to the same individual. Autologous transplantation eliminates the possibility of immune rejection, making this approach attractive for eventual use in the clinic.

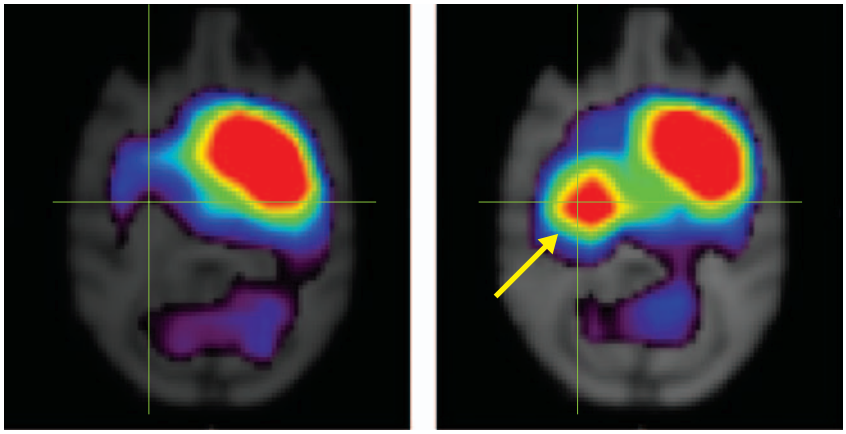

Figure 1: Brain scans showing the distribution of dopamine transporter (DAT) in the brain of a monkey before (left) and after (right) implantation of dopamine-producing neurons derived from the monkey's own mesenchymal stem cells.

Several months later, the treated monkeys, but none of the untreated subjects, exhibited improvements in motor behaviors, such as performance in a hand-reach task. Positron emission tomography scans of the cell-engrafted monkeys' brains revealed a dramatic increase in the expression of dopamine transporter (DAT), a membrane-spanning protein that helps clear dopamine from the synapse (Fig. 1).

DAT expression in monkeys that received the transplanted neurons remained above pre-treatment baseline levels for more than seven months after the operation. Further analyses at nine months demonstrated the existence of cells positive for DAT and other markers indicative of dopaminergic neuron function in the engrafted striatum—the forebrain region in which the MSC-derived neurons were implanted. The findings are consistent with functional integration and survival of the transplanted tissue.

Hayashi next plans to compare the efficacy of transplanting differentiated versus native MSCs in this monkey model. He also hopes to start translating his system for human applications. "Our newly developed system of cell-based therapy restored motor function of animal models with Parkinson's disease," says Hayashi. "We should now test whether we can derive functional and viable dopaminergic cells from human MSCs."

1. Hayashi, T., Wakao, S., Kitada, M., Ose, T., Watabe, H., Kuroda, Y., Mitsunaga, K., Matsuse, D., Shigemoto, T., Ito, A. *et al.* Autologous mesenchymal stem cell-derived dopaminergic neurons function in parkinsonian macaques. *The Journal of Clinical Investigation* **123**, 272–284 (2012).

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Generating cancer blueprints for future therapies

HIDEWAKI NAKAGAWA

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"Cancer treatment will soon change dramatically," says Hidewaki Nakagawa, team leader of the Laboratory for Biomarker Development at the RIKEN Center for Genomic Medicine (CGM). Cancer develops as a result of an accumulation of mutations in the genome. In response, Nakagawa is conducting analytical studies to decode and compare the genomes of cancerous and normal cells from liver cancer patients to identify these mutations and their locations in the genome.

Cancer is a disease of the genome

Cancer, medically known as a malignant neoplasm, is the leading cause of death among Japanese people, and the number of cancer-related deaths has been increasing year on year. In 2010, they accounted for 29.5% of all mortalities; one in three deaths was attributable to cancer. Including those who have been treated and cured, one in two Japanese people will contract cancer. Increases in the number of cancer patients and cancer mortalities pose a serious problem not only in Japan, but also throughout the world. Reducing the cancer mortality rate by preventing the development of cancer and establishing new therapies is therefore an issue of great urgency for humankind.

"Until 1999, I was engaged in clinical practice as a surgeon, providing medical treatment for many cancer patients. Although I am now not involved in clinical practice, I still have the wish to contribute to cancer treatment. Hence, I am planning to generate and catalogue cancer blueprints," says Nakagawa.

There are two types of cancer: hereditary and non-hereditary. Since the 1980s, many causal genes for hereditary cancers have been discovered and it is increasingly evident that cancer occurs due to gene mutations. In hereditary cancers, a mutation exists in the causal gene, not only in the cancer cells, but also in all normal cells that constitute the patient's body. This mutation will also be transmitted from parent to offspring.

However, in non-hereditary cancers, mutations—due to various causes—occur in the genes of normal cells, which in turn accumulate and disrupt cell function, producing cancer cells. The cancer cells repeatedly divide in a disorderly manner, leading to tumors. Any genetic mutation occurs only in the cancer cells; the mutation is never transmitted from parent to offspring. In this way, most cancers lack a hereditary nature.

"Cancer is a disease of the genome," says Nakagawa. The genome refers to the genetic information retained by each organism. It is composed of DNA, a sequence of four types of bases: adenine (A), cytosine (C), guanine (G), and thymine (T). As such, DNA is present in the nuclei of all cells and the regions of DNA that carry protein synthesis signals are called genes.

Nakagawa continues, "In a patient with non-hereditary cancer, all the base sequences of the genomes in normal

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Figure 1: Next-generation sequencer
This is a system for decoding the base sequences in a genome, capable of reading about 600 billion base sequences in 12 days, equivalent to the minimum number of base sequences required for the extensive analysis of 6 donor human genomes.

cells and cancer cells are decoded and compared to determine the locations of the mutations on the genome and how they were induced. This is a cancer blueprint. It is anticipated that if the site of mutation is located, and its nature is known, it will be possible to achieve early detection using them as indexes, and to develop new drugs that target them."

Generating a cancer blueprint

The project to generate cancer blueprints is being conducted within the framework of the International Cancer Genome Consortium (ICGC). Founded in 2008, the ICGC currently consists of members from 14 countries in addition to the European Union (EU). "The ICGC aims to decode and compare all genomes of normal cells and cancer cells in important cancers, and identify and catalogue genome mutations in the cancer cells. Currently, 47 projects covering 20 types of cancers are underway with the participation of 2 Japanese organizations, RIKEN's Center for Genomic Medicine (CGM) and the National Cancer Center, which are analyzing liver cancers," explains Nakagawa.

Liver cancer is relatively common in Asia and Africa, and ranks third in terms of worldwide mortality rates, following lung cancer and stomach cancer. In Japan, around 40,000 individuals are newly diagnosed with liver cancer every year, and more than 30,000 die from it; often they are infected with the hepatitis B or C viruses and develop liver cancer via chronic hepatitis and liver cirrhosis. Many other patients contract liver cancer as a result of hepatitis infection after receiving a blood transfusion or blood products. Although therapies for liver cancer are available, at present none are sufficiently effective. For these reasons, Nakagawa and his colleagues chose liver cancer as their target.

Nakagawa's team collected and analyzed 27 samples of both cancerous and normal cells from 25 patients with liver cancer. The human genome comprises about 3 billion base pairs and to 'read' these sequences, the latest technology, known as a next-generation sequencer (Fig. 1), was used. The analysis was then repeated 30 times to prevent misreadings and omissions. Accordingly, the number of base sequences read in the project totals about 7 trillion. This is equivalent to the number of characters in a 40,000-year newspaper subscription, or 470,000 copies of the *Kojien*, one of the most authoritative Japanese dictionaries.

"It was impossible to read such a large number of base sequences several years ago," says Nakagawa. The Human Genome Project—an international project aiming to decode the human genome—commenced in 1990 and finished decoding all the base sequences in 2003; in total, it took 13 years to read the 3 billion base pairs. "As the performance of sequencers has been improving remarkably, the speed of analysis has been increasing about 10-fold every year. Thanks to these technical innovations, it has become possible to generate cancer blueprints."

The vast amount of genome data obtained in the CGM study is analyzed by the center's Laboratory for Medical Informatics (headed by team leader, Tatsuhiko Tsunoda) using a supercomputer located at the Human Genome Center, Institute of Medical Science, University of Tokyo. Designed and constructed for dedicated use in genome analysis, the supercomputer is capable of high-throughput analyses, making it possible to determine the locations and modes of cancer cell genome mutations and thus generate cancer blueprints.

Cancer genome mutations

A point mutation is a type of mutation in which a single base within the genomic sequence is substituted, deleted, or inserted. The team's analysis showed that, on average, each cancer cell contained around 11,000 point mutations. Genome structure abnormalities include mutations such as copy number alterations (where the number of chromosomes—normally two—has increased or decreased), translocations (where a portion or all of a chromosome attaches to another chromosome), and inversions (where base sequences are inverted). In the analysis, each cancer cell was shown to contain 21 such genome structure abnormalities, on average.

"This is nothing more than an average number. Some cancer cells have a large number of point mutations, and others have few. Genome structure abnormalities are also diverse, and copy numbers as well as the sizes and locations of base sequences with translocations or inversions are also variable (Fig. 2, parts A and B)," comments Nakagawa. "Although it is readily understandable that genome mutations vary depending on the organ where cancer develops, I was astonished by these remarkable differences, despite the fact that all cell samples analyzed were from patients with liver cancer."

In liver cancer, multiple tumors, known as multicentric tumors—and distinct from metastases—can develop at

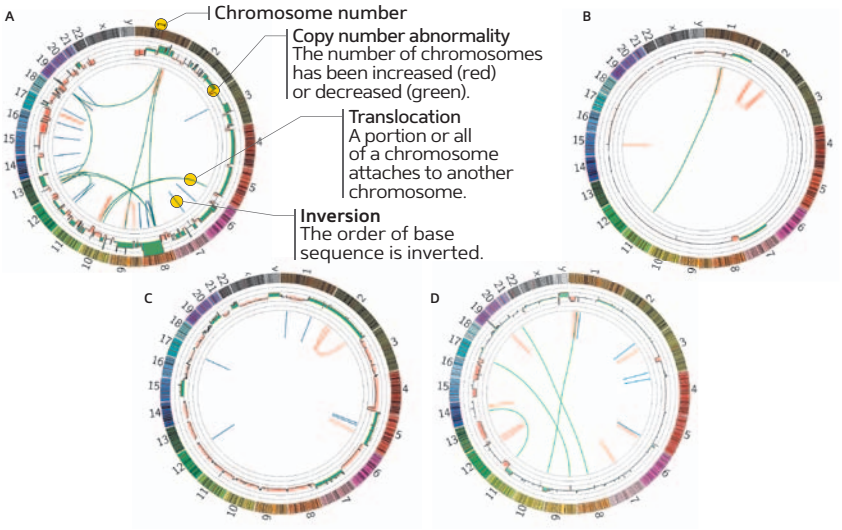


Figure 2: Genome structural abnormalities in liver cancer

These diagrams compare cancer cell genomes and normal cell genomes in liver cancer, indicating the sites and natures of the mutations found. Although all the data was obtained from liver cancer, the genome mutations observed are diverse. Diagrams C and D show multicentric tumors that developed in the same patient's liver, but where genome mutations are not identical. Reproduced from *Nature Genetics* **44**, 760–764 (2012) © 2012 A. Fujimoto, *et al.*

various sites in the liver. Nakagawa was particularly surprised at the analytical results for a pair of cancer cell samples isolated from such tumors in two different patients. "Each set of cell samples was obtained from the same liver cancer in the same patient. I predicted that the genome mutations would have some commonalities. However, the locations and natures of the genome mutations differed completely between the two samples despite their derivation from the same patient (Fig. 2, parts C and D). I was astonished. Genome mutations in cancer cells seem to be extremely diverse."

Using base substitution patterns

Nakagawa analyzed the samples with a focus on the pattern in which the base sequence is substituted in point mutations. A base substitution pattern refers to the manner of substitution of the four kinds of bases; for example, A is substituted by T, and T is substituted by C. "The two cancer cell samples from multicentric tumors were found to have completely different mutation sites despite the fact that the tumors developed in the liver of the same patient, the genome mutations probably have the same cause. I suspect that base substitution patterns are influenced by the cause of the liver cancer," says Nakagawa.

A wide variety of factors are known to cause liver cancer, including the hepatitis viruses, alcohol, obesity, diabetes, and a broad range of poisons. Analysis with a focus on the drinking habits of liver cancer patients revealed similar base substitution patterns in a group of patients who were habitual drinkers. Additionally, an association between the type of hepatitis virus that led to infection and the base substitution pattern was also observed. "Analyzing base substitution patterns may make it possible to estimate the causes of liver cancer."

Furthermore, Nakagawa thinks that it may be possible to predict the occurrence of multicentric tumors by studying the base substitution pattern within them. If no metastasis occurs for several years after a tumor is surgically removed, the patient will feel assured that they have been cured of their cancer. In the case of liver cancer, however, multicentric tumors often develop about 10 years after removal of the cancer. "If the genome of the resected tumor is found to have a base substitution pattern that is characteristic of multicentric tumors, the patient is judged to be likely to develop multicentric tumors. Early detection and prophylaxis of multicentric tumors may be facilitated by shortening the interval of periodical health checkups and paying attention to lifestyles." However, further extensive studies will need to be conducted to determine whether there is an actual association between base substitution patterns and liver cancer causalities.

Mutations in chromatin regulation genes

Genome mutations in liver cancer, like its causes, seem to be highly diverse. Interested to see if there was any commonality among such mutations, Nakagawa

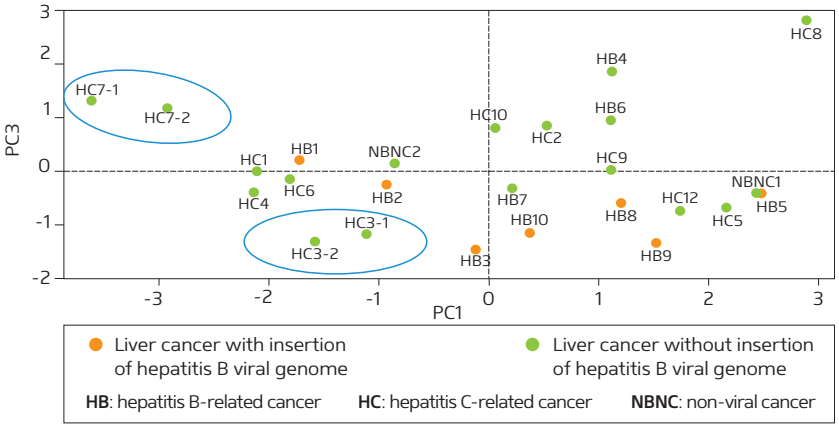


Figure 3: Various genome base substitution patterns in liver cancer

The genomes in 25 cancer cell and normal cell samples from 23 liver cancer patients were compared and analyzed for base substitution patterns. Numerical figures indicate sample numbers. The multicentric tumors in the same patient had very similar base substitution patterns (blue circles). Originally, a total of 27 samples were collected from 25 patients, out of which 2 samples from 2 patients were excluded from the analysis because their genome abnormalities were markedly different from those in the other samples. Reproduced from *Nature Genetics* **44**, 760–764 (2012) © 2012 A. Fujimoto, *et al.*

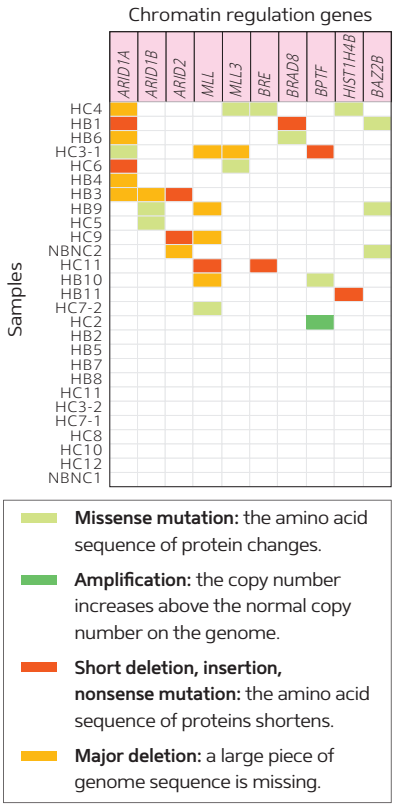


Figure 4: Mutation status of chromatin regulation genes in 27 samples from liver cancer patients

At least 1 chromatin regulation gene has mutated in about 60% (16 out of 27) of samples from liver cancer patients. Reproduced from *Nature Genetics* **44**, 760–764 (2012) © 2012 A. Fujimoto, *et al.*

detected genes with mutations in 27 liver cancer cell samples. He detected mutations in chromatic regulation genes across 16 of the 27 samples (Fig. 4).

To achieve a higher level of organization in the cell nucleus, DNA winds around histone proteins, forming a structure known as chromatin. When the chromatin structure is loose, RNA can be transcribed from the DNA, but when the structure is condensed, transcription does not occur. Any mutation in a chromatin regulation gene means that the transcription of RNA from DNA may not occur normally. "Mutations of chromatin regulation genes were detected in 60% of the liver cancer cell samples, so I think there is a potential for developing a new therapeutic drug for liver cancer that targets chromatin regulation genes," predicts Nakagawa.

In May 2012, Nakagawa and his colleagues published the results of their study of whole genome decoding in the 27 liver cancer cell samples in *Nature Genetics* and also released a briefing document to the media. "Our achievement was also featured in newspaper articles. In addition, the relevant news page on RIKEN's website was visited many times. Those visiting the site probably included liver cancer patients and their families, with expectations for advances in the treatment of the disease. I want to live up to their expectations. The cancer research I am conducting cannot be meaningful unless I meet such expectations."

The ICGC project is currently analyzing 500 cell samples for each of 20 important cancers; in Japan, the CGM and the National Cancer Center are both studying 250 samples and will proceed to catalogue liver cancer blueprints.

Drug development

The researchers involved in the ICGC project are obliged to immediately post their analytical results on the ICGC's website, www.icgc.org. The Japanese teams have already published information on the liver cancer cell genomes of 77 samples. "All the published data is freely available at no cost, not only for scientific research, but also for new drug development at pharmaceutical companies. Cancer blueprints provide fundamental data for new modalities of cancer treatment, representing a common heritage of humankind. We, ourselves, can only produce the cancer blueprints, and I really hope that pharmaceutical companies and the like will develop new drugs based on our work," explains Nakagawa.

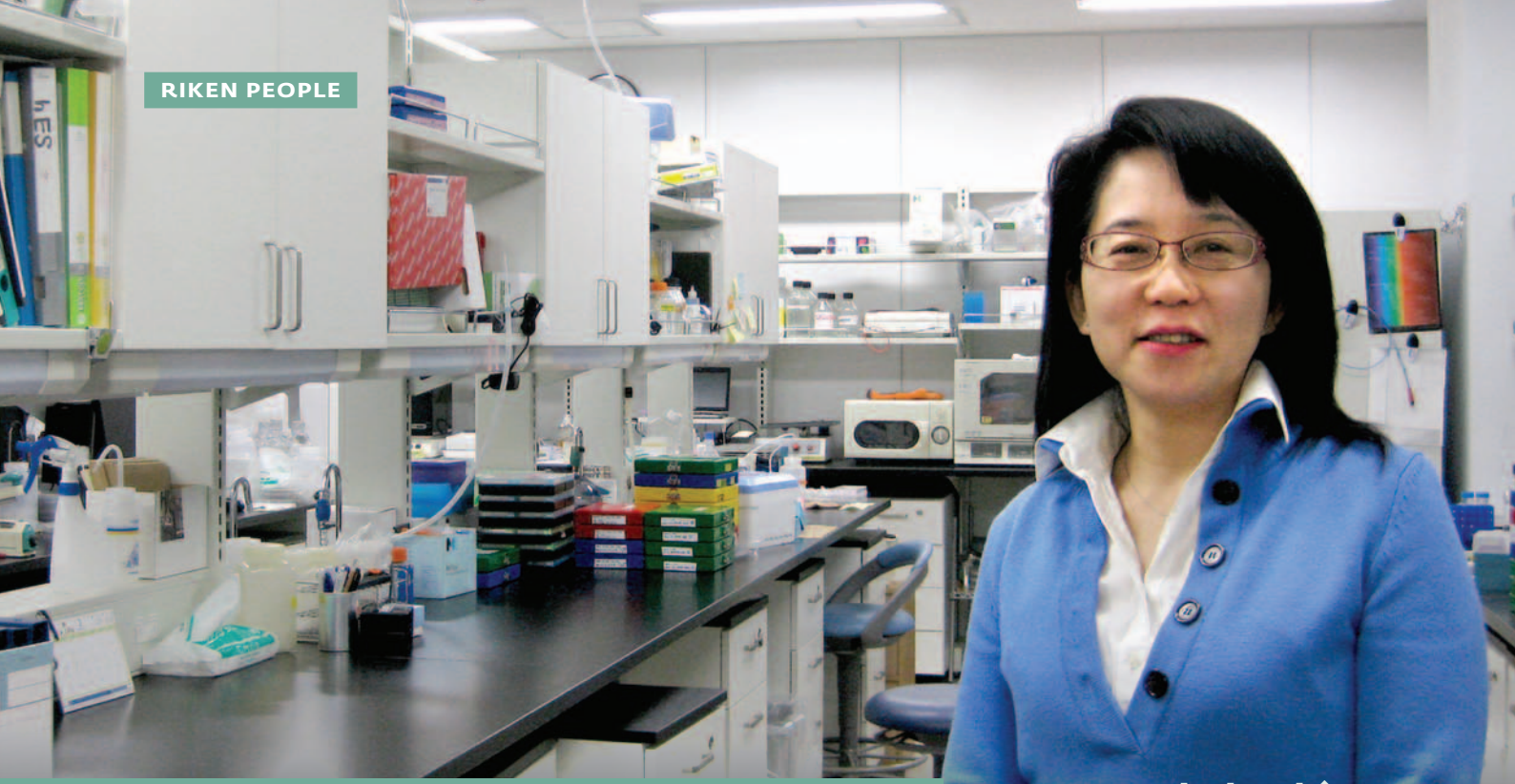
During the days when he was a practicing surgeon, Nakagawa viewed surgical removal as the first choice of treatment for cancer, believing that chemotherapy was auxiliary and not so effective. However, an event occurred that forced him to change his notions. "My father contracted lung cancer. In addition, the cancer was surgically inoperable. Reportedly effective chemotherapy was performed, and surprisingly the tumor shrank! Although he died two years later, I realized that surgical resection was not the only option for treatment. If developed, an excellent cancer therapeutic drug would improve the prognosis for survival of a greater number of patients. Wanting to undertake such work, I decided to go in that direction for my basic research."

Now, a new type of medicine—known as 'molecular targeted drugs'—is attracting attention. Conventional chemotherapeutics attack and kill cancer cells; however, because they also kill normal cells, the problem of adverse reaction arises. Contrastingly, molecular targeted drugs act selectively on molecules that are specific to cancer cells only, and do not attack normal cells.

Nakagawa reflects on the potential of these novel therapies: "Molecular targeted drugs cannot serve as a panacea that will cure all patients. However, they are expected to be highly effective in certain groups of patients. Although many molecular targeted drugs have been developed one after another and are currently the mainstream of cancer therapeutics, their availability is still insufficient to cope with the vast diversity of cancer cell genome mutations. We are hoping for the earliest advent of the day when cancer cells are examined for genome mutations, and a molecular targeted drug fitting each mutation can be chosen for the treatment. To help realize this, I am now engaged in continuing work to generate cancer blueprints."

ABOUT THE RESEARCHER

Hidewaki Nakagawa graduated from Osaka University School of Medicine in 1991. He obtained his PhD in hereditary colorectal cancer from Osaka University in 2000 and was later appointed assistant professor at the Institute of Medical Science, University of Tokyo. In 2008, he moved to the RIKEN Center for Genomic Medicine, as team leader of the Laboratory for Biomarker Development. His research focuses on whole genome sequencing and biomarker development for prostate and GI cancers through genetics and proteomics approaches. He also contributes to the International Cancer Genome Consortium (ICGC).



Delving into stem cell research

Masayo Takahashi

Project Leader
Laboratory for Retinal Regeneration
RIKEN Center for Developmental Biology

What do you do at RIKEN?

As the project leader at the RIKEN Laboratory for Retinal Regeneration, I coordinate research and clinical projects with a specific focus on induced pluripotent stem (iPS) cell research.

What made you decide to become a doctor and researcher?

I remember reading a biography of Marie Curie when I was a child and I was greatly inspired by it. I set myself the goal of becoming a physician-scientist to fulfill my dream of working in both a clinical and research environment.

What is a physician-scientist?

It is someone who is both a clinical doctor and a researcher, and today this role is a popular concept in the US. This role gives many opportunities for clinicians to develop research skills and it is a trend I hope will gain momentum here in Japan.

The reading of the characters in your name means "govern the generation"—it's a very special name with a sense of power attached to its meaning. Can you tell us more about it?

The Chinese character for 'masa' in my name comes from my grandfather. Typically in girls' names, *masa* is written with a different Chinese character which means "Japanese elegance", while mine means "to govern". My name may sound more powerful in its meaning, but I must say I rather like it.

Why did you join RIKEN?

Upon completion of my appointment at Kyoto University Hospital, I was looking for a position that would give me opportunities to develop professionally and personally. RIKEN Kobe Institute ticked all the boxes, so it was a natural choice for me.

What do you like about working at RIKEN?

Working at RIKEN gives me the flexibility to undertake both research and clinical work. Two years after I started my research at RIKEN, President Noyori introduced the idea of a 'baton zone'—a concept in which basic research meets applied research. The 'baton zone' is unique to RIKEN and allows researchers to explore the possibilities of real-life applications as well as providing opportunities for science and business to work in partnership on efficient technology transfer. The baton zone created a unique environment in which I was able to realize my dream to carry out translational research.

Please tell us about your research and other work at RIKEN.

My main research focuses on iPS cells. Our lab aims to perform clinical trials, with our first trial using iPS cells on six patients to commence soon. If the results are positive, we hope to go on to larger scale clinical trials.

I have also set up a company as part of the RIKEN Venture Systems—an initiative of the RIKEN Collaborations Division and a part of RIKEN's 'baton zone' concept. I look forward to the results I may achieve from this.

Why were you drawn to iPS research?

I was first attracted to stem cell research early in my career, when I was at the Salk Institute in San Diego 15 years ago, where I learned about neural stem cells—a form of somatic stem cell. After that, I carried out retinal cell transplantation therapy with a certain type of stem cell. However, I soon discovered that somatic cells are not suitable to be donor cells as they do not proliferate very well. Subsequently, I moved on to embryonic stem (ES) cells and, while working at the Kyoto University Hospital, I collaborated with Yoshiki Sasai, who is also based at the RIKEN Center for Developmental Biology, Kobe Institute. While we achieved success

some three years later by demonstrating the use of primate ES for treatment of retinal cell conditions, we were hesitant to apply its use on humans as it requires immunosuppression. However, shortly after, to my greatest excitement, the technology of iPS emerged. I jumped at the opportunity and approached Shinya Yamanaka's lab immediately to obtain samples of these cells.

Does the high cost of producing iPS pose a potential barrier?

Yes, I must admit the high production cost is indeed an issue we have to deal with. At present, the actual cost of creating cells that are suitable for treatment is less than 1 million yen, but the cost due to regulations amounts to about seven times that. However, if we can automate the process, we would be able to reduce the cost significantly.

What is your approach to research at RIKEN?

I'm a person who is always thinking about five or ten years into the future. Whenever I have a discussion with others in the field, I often make suggestions for new ideas—to which many will tell me they are impossible. But I think it is of the utmost importance to look beyond our current technology and achievements and to strive for something even bigger and more ambitious.

I'd like to ask you about the so-called Moriguchi incident. The day after the announcement of Shinya Yamanaka's Nobel Prize, there was a Japanese researcher who claimed he had treated patients with iPS cells, though it was eventually proven to be untrue. What is your take on this?

I first heard about it when a reporter approached me to make a comment. I took it at face value initially, as I certainly did think Harvard is very capable of doing it first. However, in my opinion, there is too much focus on who's first and who's not. I would rather we all work towards the common goal of achieving a breakthrough in human stem cell research and application.

Why do you think the reporter believed the story?

Perhaps he didn't fully understand the topic of regenerative medicine. Fortunately, he is a rare example. Every three months, I give a briefing to the mass media, and I am pleased to say that all the journalists who have attended the events so far are very well-informed and educated in this area of research.

As a female principal investigator at RIKEN, have you encountered any difficulties?

Not at all—the working environment here is very conducive to women's participation and career advancement. Unlike working at a hospital, I have very regular hours. This allows me to go home at a reasonable time. RIKEN also provides on-site childcare facilities for its researchers and office staff. In addition, RIKEN promotes many programs on gender equality.

All the success you have had must come with much hard work. What do you do to unwind?

I love movies and music. I go to temples very often too, a habit I cultivated back when I was living in Kyoto. However, in all honesty, I admit I do have retinal regeneration racing through my mind 24 hours a day.

What is your hope and vision for the future in terms of retinal regeneration?

I hope to develop a comprehensive research center for retinal degenerative disease. Currently, we already have a platform in place that focuses on a variety of areas; from genetic diagnosis to counseling and treatment. We could definitely work towards a seamless incorporation of research with clinical work. A new center such as this would bring us a step closer to making regenerative medicine a reality and would offer an accessible service not only to people in Kobe, but also to people all over Japan and around the world.

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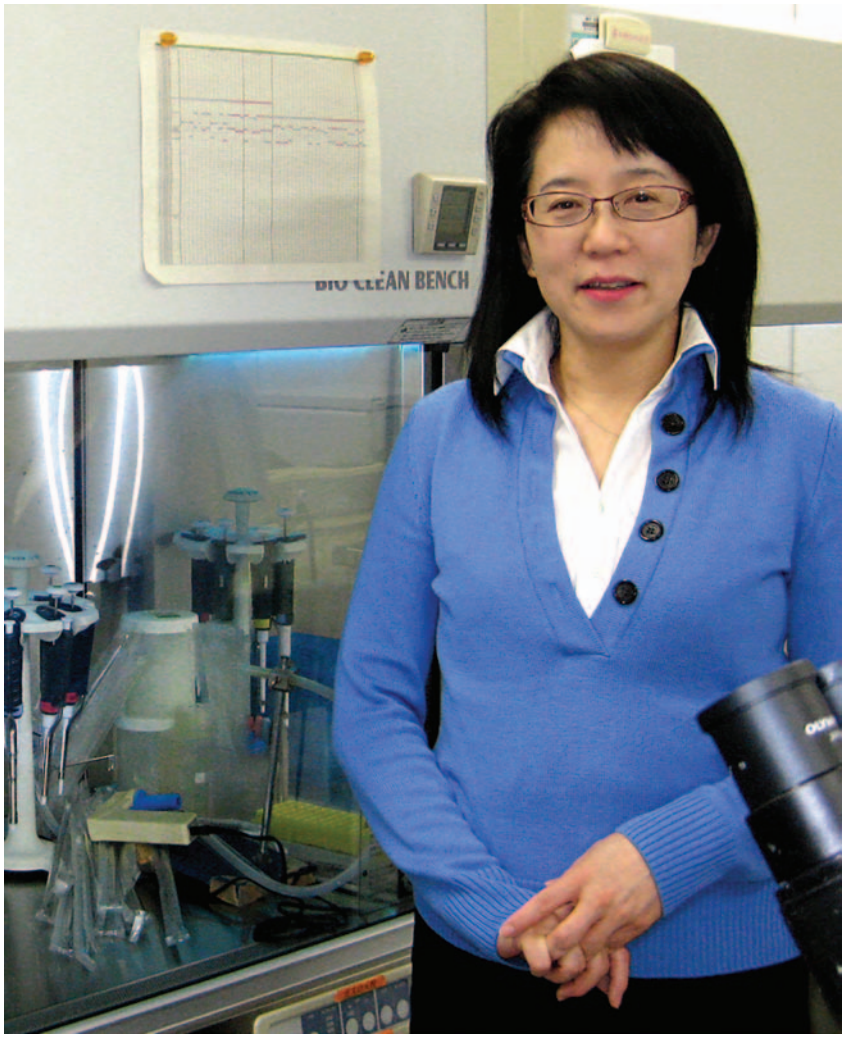

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