Impact Objectives

- Identify genotoxic chemicals, evaluate the risk to humans, and remove or reduce them from the environment, thus assuring the integrity of human genome
- Developing assays and computer models for identifying genotoxic chemicals

Science for genome safety

***Dr Masamitsu Honma**, Director of the **Division of Genetics** and **Mutagenesis** at the **National Institute of Health Sciences** (NIHS) in Japan, discusses his work developing the testing system and strategy to assess the safety of new chemical compounds more effectively and accurately*

Why has genotoxic regulation been necessary and what keeps it relevant?

In the period of high economic growth in 1960s in Japan, the environment deteriorated due to rapid industrialisation. To cope with the ever-increasing population, increased production of food was essential and large amounts of pesticides and food additives were used, some of which were chemical substances with the potential to cause cancer or genetic diseases. These are called 'mutagens'. A lot of studies had been invested to detect the mutagens, understand the mechanism of mutagenicity, and control them. Currently, health hazards due to ingestion of mutagenic substances through the environment and food are strictly regulated to be almost zero. This is exactly the result of the victory of regulatory science.

What should we do at the next era? This is a major issue common to all scientific societies, not limited to our research field. I said that we conquered the serious mutagenic substance in our environment. However, many new mutagens may exist in our surroundings, but it may just be impossible to see in the past method. Stick to the traditional ways and do not overlook new threats. A more sophisticated strategy is necessary.

What is it you hope to learn?

Originally, we assessed the safety of drug substance and drug products in the development of pharmaceuticals. However, as there is no threshold for mutagenicity, it has become necessary to pay attention to a trace amount of impurities contained in pharmaceuticals. The *in silico* method involving quantitative structure activity relationship (QSAR) is used for assessment of the impurities. In addition, the threshold of toxicological concern (TTC) was introduced for the management. Learning new technologies and new concepts in regulatory science is my new challenge.

How will society benefit from this work?

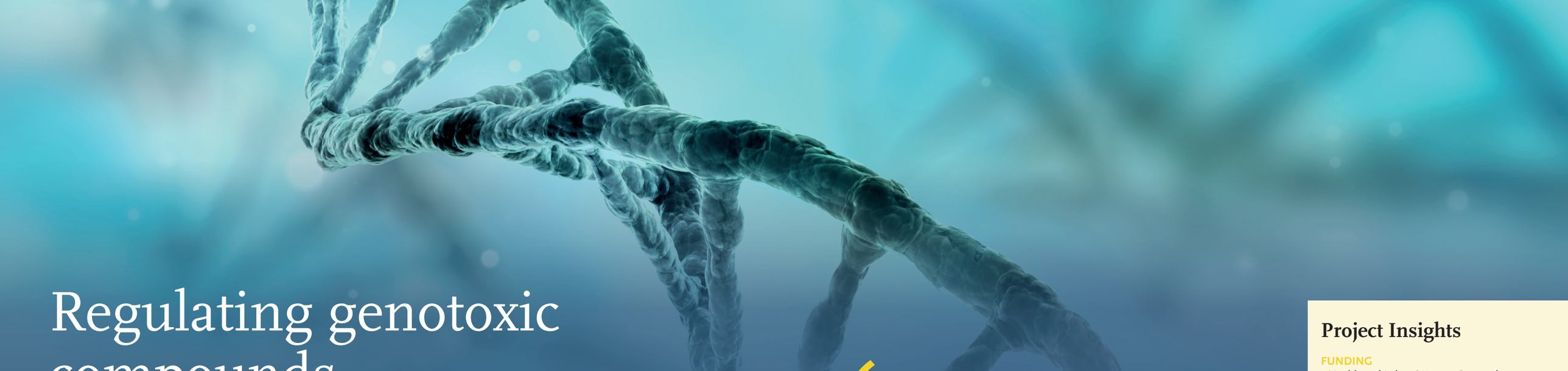
People are pursuing further safety for pharmaceuticals. Therefore, pharmaceutical companies need to manufacture high-quality pharmaceutical products through technological innovation. However, we cannot reduce the risk to zero. Regulatory science aims to achieve both innovation and risk thinking. We believe that the regulatory science will ultimately benefit companies, patients and society.

Can you talk generally about some of the work underway at the Division of Genetics and Mutagenesis (DGM)?

The mission of DGM at NIHS is to identify genotoxic chemicals, evaluate the risk to humans, and remove or reduce them from our environment through administrative regulations, if necessary, thereby assuring the integrity of the human genome. To accomplish the mission, we are engaged in the development of various genotoxicity assays, as well as research on mechanisms of mutagenesis and DNA repair and application of this knowledge to the risk assessment of chemicals. We are also working on the regulation of chemicals through participation in administrative committees in regulatory agencies. As to specific research projects, we are developing a gene mutation assay system using bacteria, mammalian cells and animals, and studying about the mechanism of DNA repair and chemicals mutagenesis using these assay systems. Recently, we have been conducting studies such as detection of epigenetic mutagens and monitoring of mutations in blood cells of human populations that may have been exposed to mutagenic substances in their environments.

What kinds of toxicological research are you planning?

The pharmacological action of pharmaceuticals is 100 per cent dependent on their chemical structure. Likewise, its toxicity should be determined by chemical structure. In the current toxicology, the results of biological testing are regarded as absolute truth. However, biological tests always have uncertainties. Toxicological tests on a chemical do not always give the same results. We may abandon biological test supremacy and should change toxicology from biology to chemistry. The progress of QSAR and AI technology could make this possible. In particular, considering animal welfare, the abolition of toxicity tests using animals is important for the formation of a wholesome society. ▶



Regulating genotoxic compounds

*The **National Institute of Health Science** in Japan is tackling the assessment of chemical compounds for genotoxicity through basic research, assay development and the use of Artificial Intelligence*

The last 200 years of extraordinary technological development has benefitted people across the globe. Advances in medicine and food technology have had a particularly large impact. Medical technologies have allowed for the curing of multiple diseases previously endemic to humanity. At the same time, the burden of aging has lessened and previously dangerous conditions have been rendered extremely manageable. Where food is concerned, advances in fertilisation, pest management and preservation have vastly increased the quality and quantity of food available. This has led to the massive increases in global population and wealth seen across that time period. However, in pursuit of these noble aims, chemicals have been developed and applied on a large scale and have had a significant negative impact on human health. These compounds typically have some sort of mutagenic effect on DNA which can either be toxic or lead to the development of cancer.

Instances where novel compounds were later proven to be harmful became more prevalent in the middle of the 20th century. Many countries around this time began the process of instituting tighter regulation on new chemicals that may come in contact with humans directly or indirectly through the environment. Regulations of these types attempt to test novel chemicals for their safety and assess the risk of their use. The aim, of course, is to balance innovation with a strong consideration of human and environmental health. In order to maintain this balance effectively, robust methods of testing toxicity need to be developed, maintained and improved upon. Doing so requires a strong basis in foundational research science. This is the work of Dr Masamitsu Honma, Director of the National Institute of Health Sciences (NIHS), Japan. Honma and his team are researchers who work on uncovering DNA repair mechanism and partake in consultations on national regulations for new chemicals.

ASSAYING TOXICITY

Toxicity at a genomic level can cause a variety of problems for those affected. The two of greatest concern are passing on dangerous mutations from a parent to a child and the second is cancer. Genotoxic mutations leading to dangerous mutations can take various forms. Double-stranded DNA breaks and mutation of bases from one type to another are just two of the most serious of these. Both require immediate corrective action from the cell and both can lead to potentially life-threatening changes. The most genotoxic compounds tend to introduce these sorts of problems. 'Most cells, most of the time, are able to correctly identify and repair DNA damage due to an array of DNA repair pathways available to them,' explains Honma. 'However, with bad breaks, there is no guarantee and given that a single mutation can be sufficient to cause cancer, there is no tolerated level of exposure to a genotoxic compound.'
In order to sufficiently assess and understand the genotoxicity of a compound, it is necessary to develop robust testing systems. Mammalian cell lines are the go-to living system in which to test genotoxicity. 'However, it is not sufficient to merely expose the cells to a compound, extract the genome and search for mutations,' says Honma. 'There must be reliable ways in which to measure the rate and type of mutations.' Equally, it is also essential to understand the mechanisms of repair that these cells employ and be able to decide if they are employed or not. This work is the focus of Honma and the NIHS. They have developed various assays to test genotoxicity. One such assay revolves around the introduction of a unique restriction cut-site in the genome of a cell line. 'The TSCE / TSCER system allows us to qualitatively and quantitatively trace the fate of a single double-stranded break in the human genome,' Honma outlines. 'This system is also the ultimate low dose radiation model and can be applied to safety assessments of genome editing technology such as CRISPR /Cas9.'

STANDARDISATION

Developing robust *in silico* assays for new compounds has been one of Honma's main aims. He and his team are now taking advantage of advancements in artificial intelligence (AI) and Deep Learning in order to teach software to recognise patterns in genotoxic chemicals. The aim is to educate the software in chemical structure, DNA structure and types of DNA mutations and the interlink between the three. With enough information fed into the system, the programme should be able to accurately predict the effects an unknown compound may have. Honma is using the quantitative structure activity relationship (QSAR) model which focuses on chemical structure to predict toxicity and is aiming to improve its predictive powers. 'The International Conference on Harmonization guideline allows the use of *in silico* approaches for predicting mutagenicity,' says Honma. 'This is the first international guideline that addresses the use of QSAR models in lieu of biological toxicological studies for human health assessment. Therefore, QSAR models of mutagenicity now require higher predictive power.' There are several reasons to do this. A computer-based model has the potential to help screen a large variety of compounds for safety and thereby highlight the best candidates to be selected for tests on living cells. This would both aid the discovery of new compounds and prevent mistakes that would be costly both in money and potentially in health of any test subjects.

> *"The TSCE / TSCER system allows us to qualitatively and quantitatively trace the fate of a single double-stranded break in the human genome"*

In addition to their *in silico* work, Honma and the Institute are attempting to better understand the different DNA repair systems within cells. Different repair systems lead to different methods of repair and, in cases where the system fails, different types of genetic damage. Understanding these processes is essential to being able to predict and test for genotoxicity. In the DNA repair field, one issue is that many researchers are conducting their work in different cell types with different backgrounds. Honma has developed one parental cell line and created a huge variety of repair-defective lines from the parental line. 'In regards to the advantages of this cell line if the cells are different, their phenotypes will also differ,' explains Honma. 'This makes it difficult to compare results. We use human lymphoblastoid cell line TK6 as parental cell line. We have made over 120 kinds of DNA repair deficient TK6 cells and offer them to researchers worldwide. These researchers will verify the results of their research by confirming the function of the DNA repair that they study in the TK6 background.'

THE FUTURE OF REGULATION

There are many dimensions to investigating genotoxicity. Honma and the NIHS are covering them all. This is essential to understanding how genotoxicity occurs. The knowledge and expertise acquired from the basic research feed into the Institute's ability to predict and test for genotoxicity. *In silico* prediction is becoming a cornerstone of regulatory science and having accurate data with which to feed it is vital. However, *in silico* predictions can only go so far and robust, repeatable and accurate *in vivo* testing are still required to uncover the genotoxicity of a chemical. Honma has been conducting all three key areas and in doing so has positioned the NIHS at the forefront of regulatory science.

Project Insights

FUNDING
- Health and Labor Sciences Research Grants in Japan
- Japan Agency for Medical Research and Development (AMED) Grant
- Japan Society for the Promotion of Science (JSPS)

CONTACT
Dr Masamitsu Honma

T: +81 44 270 6672
E: honma@nihs.go.jp
W: http://www.nihs.go.jp/dgm/index-e.html

BIO

Dr Masamitsu Honma is the Director of the Division of Genetics and Mutagenesis in the National Institute of Health Sciences (NIHS), and the Professor of Graduate School and School of Pharmaceutical Sciences, Osaka University, in Japan. He received a PhD degree from The University of Tokyo in 1989. After studying as a postdoctoral fellow at Harvard School of Public Health, he started research works in the field of genetic toxicology in NIHS. Honma has studied DNA repair, mutagenesis, genotoxicity QSAR models, etc., and published more than 200 papers. He has been actively involved in Environmental Mutagen Society (EMS), and is now a president of Japanese and Asian EMS. Honma is responsible for a lot of regulatory works in Japanese and international bodies.

NIHS

科研費 KAKENHI