Impact Objectives

- Create a method for performing gene sequencing and genotyping, particularly in genes derived from human germ cells and somatic cells, microorganisms and viruses in liquid biopsies
- Develop a new method of cost effective and high-throughput absolute quantification as a new cancer biomarker in liquid biopsy

Markers in the genome can direct medicine

*The genome provides numerous markers that can inform individual disease diagnoses and treatment options. Genome researcher **Dr Kenji Kinoshita** discusses his work aimed at improving the techniques to find and use these markers*

How have your interests in the field of genomics research changed throughout your long career?

I have several interests in this field which were developed over my career. While working at DNA Chip Laboratory Ltd and Sumitomo Bakelite Ltd I was engaged in research on DNA microarrays using highly biocompatible plastic substrates. At Mukogawa Women's University, where I currently work, I mainly conduct research on developing rapid, simple and inexpensive methods for detecting norovirus in faeces and polymorphism analysis for drug metabolising enzyme genes. I am also interested in developing a method for performing gene sequencing and genotyping quickly, simply and inexpensively, particularly in genes derived from human germ cells and somatic cells, microorganisms and viruses in liquid biopsies.

You are developing a new method of cost effective and high-throughput real time quantitative polymerase chain reaction (PCR) as a new cancer biomarker in liquid biopsy. Who will ultimately benefit from this work?

This work is aimed at benefitting any cancer patient but there are three specific types of applications. First is ultra-early detection of cancer incidence and recurrence by monitoring plasma cell free DNA levels in general and cancer patients. The second application is screening tests for primary cancer by detecting the gene sequence of circulating tumour DNA in the plasma of patients and further selecting molecular targets of a therapeutic agent. Finally, the method we are developing is superior to conventional methods in micro sampling and real time PCR measurement methods in terms of quickness, simplicity and costs, and it contributes to the development of liquid biopsy applications.

Another of your projects is looking at genetic factors for individual differences in drug efficacy and adverse drug reactions. For this you developed a dried saliva sampling kit. Why is saliva an important target?

In order to analyse and monitor gene mutations in cancer cells, it is necessary to directly collect cancer cells or collect circulating tumour DNA fragments suspended in the blood. However, genetic factors in individual differences in drug efficacy and side effects can be found by analysing germline gene mutations. Germ cells, such as leukocytes or mucosal epithelial cells, can be easily and noninvasively collected from saliva. In fact, a correlation between alcohol constitution and lifestyle-related diseases can even be predicted. We have collaborated with the National Hospital Organization Kurihama Medical Center, which conducts research on alcohol dependence and treatment effects according to alcohol constitution.

What are some of the benefits of this new sampling kit?

The kit non-invasively samples saliva from the oral cavity using a swab made of a urethane sponge. After it is dried, it can be stored for a long time at room temperature, which is the greatest advantage. In addition, sampling kits using water-soluble paper can be easily made anywhere. In developed countries, medical costs are rising due to an increase in patients with multiple diseases associated with ageing societies. Since this method is quick, simple and inexpensive for early detection of diseases, it will help prevent potential diseases by knowing their individual genotype in advance. Therefore, the prevention of recurrence after treatment and health maintenance can be made less costly. Also, indirectly, the genome information of each individual is reflected in the tendency of diseases due to lifestyle habits and the efficacy of medicines, and the spread of knowledge to be used for the prevention of diseases will reduce the rising cost of medical expenses. ●

Unlocking genetic information in blood and saliva

Research into techniques to easily sample and store blood and saliva for use in DNA sequencing are making it easier for scientists to utilise genetic information to better diagnose and treat patients suffering from diseases like cancer

Since the discovery of DNA and the wave of DNA sequencing technology that arrived in the following decades, our understanding of genetic material has skyrocketed. Now, researchers and medical doctors are starting to understand the role genetics plays in the development of diseases like cancer. Furthermore, they are beginning to tease out the role of genetics in lifestyle-associated diseases and even how alcohol consumption can impact these differently from person to person. One exciting new field is even looking at how genetics can influence the efficacy of the drugs used to treat diseases, which promises a new future of personalised medicine.

The challenges the field faces now are largely technological. The complexity of genetic information is so great that even the most powerful computers can struggle to deal with the sheer amounts of data. Even in situations where a genetic marker or mutation has been identified among the volumes of data, finding a quick an efficient way to isolate and diagnose it in patients is an obstacle. Rapid screening tests for genetic variants that are associated with disease risks or treatment success are in demand as they have the potential to improve and personalise treatment and make the overall costs of healthcare drop.

FINDING GENETIC SIGNPOSTS

Genetic researcher Dr Kenji Kinoshita, based at Mukogawa Women's University in Nishinomiya, Japan, has dedicated his career to the field of genomics and the development of diagnostic tests for diseases and genetic markers. 'Genetic markers are essentially signposts in the genome that can indicate all sorts of information; from species identity to disease risk,' he explains. 'The variation of these markers can be quite vast.' They can be genes, mutations in genes, short or long DNA sequences and even a single base pair change within a region of DNA. Depending on the application and the type of marker required there are many methods used to identify these markers as well. The one thing all methods have in common is the need for a biological sample.

Being that these markers are found in the DNA of an individual, isolating the DNA from a biological sample is the first step to any method. Obviously, some sample types are easier to get than others. Blood or saliva, for example, is easier to obtain than tissues found deeper in the body. 'This allowed us to sample non-invasively by developing several new DNA preparation methods that require only water-soluble paper for dried saliva and PVA sponge for dried blood,' clarifies Kinoshita.

PRESERVING BLOOD AND SALIVA

One of the most common genetic markers used in research is a single nucleotide polymorphism (SNP). These are single base pair changes in a stretch of DNA that is associated with a condition or trait. To find these DNA must sequenced, a process that starts with a sample preparation process where DNA is isolated and purified. 'General sample-preparation processes are labour-intensive and time-consuming, and it is costly to use simplified kits for large-scale studies,' outlines Kinoshita. 'Recently, we developed simple SNP genotyping methods in which sample-preparation processes were eliminated.' This new process involves taking swabs of saliva, where DNA is available from leukocytes and mucosal epithelial cells, and stores them on a water-soluble paper. 'The unique sponge used in the swab penetrates the saliva into the paper where it can be stored after it dries for long periods of time at room temperature,' he clarifies. These samples can then be used directly in the sequencing process. This method is reducing costs and allows samples to be collected much more easily in the field. 'Along with my collaborators, we are able to perform large-scale epidemiological investigations into SNPs associated with drug metabolism that are aimed at improving individual treatments for women with breast cancer,' says ▶

> *The PVA material allows efficient sample collection and preservation, which sufficiently prepares the sample for DNA sequencing, thereby simplifying the sampling process*

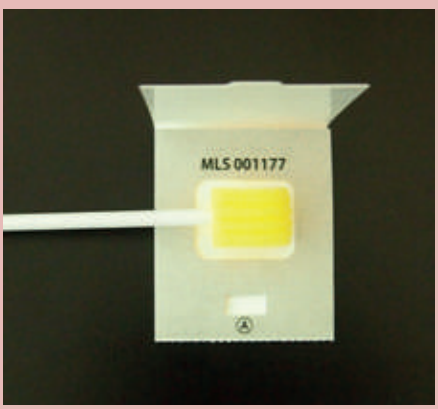
Kinoshita. Breast cancer is the most common type of cancer affecting Japanese women and variations in a specific gene, detectable using the saliva test, indicate how well a patient can metabolise anti-cancer drugs, improving dosing calculations and the estimations of risks from side-effects.

Kinoshita has also been at work developing similar tests to be used on dried blood. 'We have created a new sampling method to collect dried whole blood or plasma samples using a Poly Vinyl Alcohol (PVA) sponge,' he confirms. This PVA sponge can be used as a liquid biopsy because within the blood it captures resides in cell free or circulating tumour DNA. 'Essentially this is circulating tumour DNA associated with a cancer cell that circulates in the blood and can be used to diagnose cancers and, furthermore, when it is sequenced it can provide information as to the type of cancer and potential weaknesses that can be exploited during treatment.' According to Kinoshita this method, like the saliva protocol, greatly reduces costs. 'The PVA material allows efficient sample collection and preservation, which sufficiently prepares the sample for DNA sequencing, thereby simplifying the sampling process,' he points out.

BETTER SAMPLES, BETTER HEALTHCARE

The usefulness of a rapid, efficient, easy-to-use and cheap method for sampling and storing DNA cannot be understated. In terms of costs, reducing sampling and processing time and the need for cold-storage of samples, DNA is usually stored at -80°C long term, these new methods will save the health care system financial costs. The ease of use and portability also means that hard to reach populations can benefit from these tests as well, which will again save money by providing efficient personalised care and early diagnoses for diseases.

Kinoshita and his colleague Dr Ichikawa work at the Bio Education Laboratory where they are training pharmacists with the information gleaned from these techniques. In particular, using the methods described here they have gathered data on how a SNP in genes associated with alcohol metabolism can contribute to lifestyle-related diseases. This data is important for pharmacists who are not only required to contribute to medical treatment but also health maintenance. 'Studies on the correlation between alcohol consumption and frequency of alcohol consumption, and the incidence of lifestyle-related diseases, such as breast cancer are required,' says Kinoshita. 'Training on the alcohol polymorphism test and promoting an understanding of the association between the gene polymorphism and the illnesses caused by alcohol drinking needs to be offered to pharmacists.' The pharmacists in turn can help the researchers recruit patients for trials and provide valuable feedback for their studies on drug metabolism. The wealth of genetic information these tests make available has only begun to be implemented and could vastly improve health and health care systems globally. ●



Saliva sampling by a homemade DnaCapter kit


Central Campus of Mukogawa Women's University

Project Insights

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BIOS

Dr Kenji Kinoshita is a Professor in the School of Pharmaceutical Sciences at the Mukogawa Women's University, where he has been a faculty member since 2006. Previously, he worked at Brown University as a research professor from 1997 to 2002. His research interests lie in the genome area, especially development of cost effective and high-throughput genotyping methods as a new cancer biomarker in liquid biopsy. In the near future, his research base will be the Bio Education Laboratory.

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