Taiwan Develops New Biochip Technology

Scientists at Taiwan’s Academia Sinica have developed a new biochip technology, which could have important implications in cancer diagnostics. This technology was recently described in the *PNAS Early Edition* in the week of February 5, 2007, by the well-known chemist and President of Academia Sinica Dr Chi-Huey Wong, postdoctoral research fellow Dr Tsui-Ling Hsu, and collaborators from The Scripps Research Institute in California.

Using a “glycan tagging system”, the method allows for the monitoring of cell surfaces and the intracellular trafficking of glycoproteins. It is known that more than 80% of human proteins have sugars attached, called glycoproteins. Protein glycosylation plays a central role in mediating protein function in living organisms, and altered glycosylation is often associated with inflammation and cancer metastasis.

Therefore, the detailed correlation between glycosylation and biological or pathological status is of great interest, and may provide information for disease diagnosis and treatment. According to the researchers, their new biochip technology can monitor this correlation, and will thus be useful for isolating and identifying the glycoproteins that are differentially expressed under various physiological or pathological conditions.

Together with another newly developed set of “sugar chips”, also reported by Dr Wong’s group and published online on January 31, 2007, in *Nature Protocols*, this new method has great potential for industrial use in high-throughput diagnostic tools that analyze posttranslational protein glycosylation in disease states.

Scientists Develop Cost-Effective Nuclear Medicine Kit

Scientists in India have developed a new cost-effective nuclear medicine kit to tackle the growing number of infectious diseases in the country. The kit — which gives specific detection, location, and treatment response — is superior to radiological techniques, and is being sought after by foreign countries and organizations including the International Atomic Energy Agency.

“We have developed a single-vial cold kit of ciprofloxacin for bacterial infection imaging called Diagnobact™,” said W. Selvamurthy, Chief Controller of Research and Development in the Life Sciences and Human Resources, Defence Research and Development Organization (DRDO).
The cold kit contains a cocktail of various reactants, including the antibiotic ciprofloxacin. When the radioisotope Technetium-99m ($^{99m}$Tc; a gamma emitter) is added to the cold kit, it binds to ciprofloxacin, which can be injected into the patient. According to Selvamurthy, the radiation dose is less than or equal to that used in a CT scan and is quite safe. The kit can detect and locate bacterial infections from any part of the body, including bones and joints.

After the technique was developed, a 2-year multicentric human trial was conducted in 20 hospitals in India, including the PGI, Chandigarh, Jaslok, and Hinduja hospitals in Mumbai as well as the Sir Ganga Ram Hospital in Delhi. “The multicentric trial confirmed and validated the techniques and the clinical utility of Diagnobact™ in diagnosis of infectious lesions,” Selvamurthy said. He added that this technique is more effective than other radiological techniques because it detects metabolic changes, which are the first to occur at the site of infection, instead of anatomical changes.

The kit was invented by A. K. Singh, who owns the patent and has worked on the project since 1997. Each vial costs Rs800 in the Indian market.

**Protein Found Key to Human Flu Transmission**

Researchers have discovered that influenza hemagglutinin — a type of protein found on the surface of influenza viruses that is used to bind to host cells — appears to play an important role in the virus’s ability to transmit efficiently among humans. These findings were published in the online early edition of the journal *Science*.

Terrence M. Tumpey from the US Centers for Disease Control and Prevention and coauthors studied the hemagglutinin protein covering the surface of the 1918 influenza virus, which they recreated in 2005. This virus caused a pandemic that claimed the lives of at least 50 million people between 1918 and 1920, and shows genetic sequence similarities to avian influenza viruses.

Researchers changed two amino acids in the 1918 virus hemagglutinin from a mammalian configuration to an avian configuration, and inoculated ferrets with it. Ferrets are expected to be good predictors of influenza virus transmissibility among humans. The inoculation of ferrets with the 1918 “avian” hemagglutinin virus caused severe disease, but healthy ferrets placed close enough to the sick ferrets to catch it remained healthy.

The findings suggest that, for an influenza virus to spread efficiently, its hemagglutinin must prefer to attach to host cells found in the human upper airway instead of host cells found predominantly in birds. The transmission ability among humans is an essential property of a pandemic virus. Understanding flu transmission will assist researchers in their challenge to stop the spread of influenza, especially as concerns mount with the current avian flu epidemic in chickens and the possibility that it will spread to humans.
IMCB Partners with Hitachi for Better Diagnostics

Cancer diagnosis may soon be made earlier, resulting in better treatment options and more lives being saved, due to research collaboration between the Institute of Molecular and Cell Biology (IMCB) at Singapore’s Agency for Science, Technology and Research (A*STAR) and Hitachi Asia. The project, which started in 2004, has resulted in a diagnostic assay for the early detection and diagnosis of cancer, based on the detection of DNA methylation.

“Collaborative interest for this project started after I did a TV broadcast interview that was aired in Japan, where I spoke on bioscience developments happening in IMCB and Singapore. A representative from Hitachi saw this show and was very excited to work with us,” explained Masafumi Inoue, Principal Coordinator of the Translational Research Facility at IMCB who led this project.

This assay detects DNA methylation, which is a type of chemical modification to DNA that involves the addition of a methyl group at the carbon 5 position of the cytosine ring. DNA methylation is an epigenetic modification that alters gene expression without any change in gene sequence. It is an inheritable change that regulates gene transcription. Recent studies have shown that alterations in DNA methylation are common in a variety of tumors.

In the IMCB–Hitachi project, the assay detects the methylation of a specific gene: the tumor suppressor gene. While IMCB’s team selected special genes and developed the primer, partners at Hitachi developed the probe. “If you imagine DNA transcription being like a train moving along a rail track, DNA methylation basically puts rocks onto the track, which can derail the train. In the same way, the methyl groups are like the rocks which can interfere with transcription and suppress the expression of the tumor suppressor gene. In humans, this may contribute to the formation of cancer,” said Inoue.
Although the majority of the research work was done at IMCB, Hitachi provided funding and technology for the detection machines installed at IMCB as well as a Hitachi Asia-based researcher who regularly went to IMCB.

The success of this research project led to a licensing agreement, which was announced in November 2006, between Hitachi Asia and Exploit Technologies (the commercialization arm of A*STAR) to further develop the diagnostic assay into a viable commercial test kit. Before these kits can be used in research or hospital settings, Hitachi will have to take them through clinical trials, obtain proper approvals from bodies such as the FDA, and manufacture the kits. “Hitachi has expressed interest in manufacturing the kits here in Singapore, as well as to do some of the trials here. Of course, they also have good linkages with hospitals in Japan and internationally, so will be able to develop the right channels for distributing the kits globally for the trials,” said Inoue.

With this diagnostic assay moving towards commercialization, Inoue is now concentrating on other research projects. One of them is looking at detecting avian flu in both humans and birds. His team at the Translational Research Facility and Tan Tock Seng Hospital’s team aim to create a more sensitive assay that can detect influenza A, influenza B, and the H5N1 strain. “The main objective of bioscience research work is to help people, and this is what I am trying to do — to develop early diagnostics that can eventually help to save lives,” added Inoue.

Creating Safer and Cheaper Therapeutic Options for Stem Cells

As stem cell research continues to advance, the therapeutic potential of these precursor cells will increase and new applications will arise to tackle a growing range of diseases. A team from the Genome Institute of Singapore (GIS) has developed a novel method for generating mesenchymal stem cells (MSCs) from human embryonic stem cells (hESCs) that is set to further expedite new clinical applications for stem cells.

“Mesenchymal stem cells are a specialized type of adult stem cells which, apart from hematopoietic stem cells, are the only type of stem cells that are currently being used in the clinic. This is why they are so important,” said Dr Sai-Kiang Lim from GIS. “The derivation of MSCs from hESCs is not new. But the problem remains of how to get a sufficient number of pure MSCs, which can be used for therapeutic purposes.”

Traditional protocols of deriving MSCs from hESCs use methods such as exposing the hESCs to another species’ cells or introducing potentially cancer-causing genes to immortalize the cells; this limits their applicability. There are also the problem of teratomas and difficulties in getting the hESCs to differentiate into a particular cell type. GIS’ method sought to overcome these problems by establishing cell lines from the hESCs, thus theoretically allowing the cells to be propagated indefinitely. GIS has already demonstrated that their MSCs can be propagated more than twice as many times as that previously done in adult bone marrow MSCs. GIS also identified markers to generate highly purified MSC cultures, which are more suited to clinical stem cell applications.
“We took hESCs and trypsinized them, breaking them down into single cells. Usually, when you do this, the hESCs will differentiate into many cell types. We then put the cells into a medium, which contains two growth factors that promote the growth of MSCs. So, we select for the MSCs and let the rest of the cells die. Although our protocol is simple, it is robust and we have already tested it out on two different hESC lines,” said Dr Lim.

She went on to explain how markers have made GIS’ protocol more sophisticated, “In order to get a homogeneous highly purified MSC culture, we would need to do many rounds of trypsinization, so we used markers to shorten the process. We looked at genes which were expressed in both the hESC and MSC cultures. We then pulled out a list of candidate genes, looking specifically for genes that encode for surface antigens. From here, we were able to identify genes whose expression is high in hESC cultures but low in MSC cultures and vice versa.”

While MSCs have been used to treat a number of diseases, their actual mechanism is not known. Recent developments have suggested that MSCs secrete certain factors that promote tissue repair. This leads to the possibility of using the secretions instead of transplanting the MSCs, thereby avoiding the problems of immunocompatibility. To find out more on these secreted factors, GIS grew MSCs in a chemically defined medium. After 3 days of culture, GIS analyzed the medium and identified over 200 unique gene products. “A literature search of the past 10 years has identified only 29 proteins secreted by the MSCs. So our discovery of 201 gene products is significant,” said Dr Lim.

Dr Lim added, “We then wanted to know whether this medium, with the secreted factors, will have the same therapeutic effects as the MSCs.” To answer this question, GIS worked with the Utrecht Medical Centre in the Netherlands. Early data from mouse model studies showed that GIS’ medium results in significant improvements in mortality rate and heart function after acute myocardial infarction (heart attack). GIS has also worked with the National University of Singapore and the Harvard Medical School to compare their derived MSCs with industry standards.

“The next step is to move our work into pig models; and if this gives us promising indications, we will progress to clinical trials. In preparation for this, top cardiac clinicians, such as Professor CN Lee and his team at the National University of Singapore and National University Hospital, are collaborating with us on the pig models. We are also working with Steve Oh and Andre Choo at the Bioprocessing Technology Institute to develop processes to scale up production of clinical-grade media,” said Dr Lim.