Interview with Professor Dennis Lo

Prof. Dennis Lo runs a research group at the Chinese University of Hong Kong and is a leading researcher in the field of circulating nucleic acids in plasma and serum. Analysis of circulating nucleic acids is used in diverse applications, including cancer detection, prenatal diagnosis, and rapid assessment of trauma victims. He spoke to QIAGEN about the significance of this field, and about the work in his lab.

When were circulating nucleic acids in plasma first discovered?

Plasma and serum contain low levels of freely circulating nucleic acids, and this was first reported in 1947 by two French scientists. In the 1960s and 1970s, circulating DNA was found in patients with systemic lupus erythematosus and cancer, but the technology for studying this further at that time was limited, and the field did not progress significantly until the 1990s. In the mid-1990s, studies showed that some of the circulating DNA in the plasma of cancer patients contained carcinogenic mutations, suggesting that it had been released from tumor cells. A year or two later, our group started to develop the idea that if a tumor can give rise to circulating DNA, an unborn baby might produce the same effect. Indeed, in 1997, we found DNA originating from the Y chromosome in the plasma of women expecting baby boys. Furthermore, we found donor-derived male DNA in the plasma of women who had received liver or kidney transplants from male donors.

What are the main applications of studying circulating DNA in plasma?

There are several important applications. A basal level of circulating DNA is found in healthy individuals, but in cancer and during pregnancy, amounts increase by approximately tenfold. Circulating DNA can be used for detection and follow-up of a wide range of cancers. It can also potentially be used for prenatal diagnosis of a number of genetic diseases [see accompanying article on page 4], including Down Syndrome, achon-droplasia, and myotonic dystrophy. Levels of fetal DNA in serum can also be used to detect problems such as pre-eclampsia, pre-term labor, or the possibility of rhesus incompatibility between mother and fetus. For example, the International Blood Group Reference Laboratory in Bristol in the UK has recently launched a diagnostic service using fetal DNA in maternal plasma to assess the blood group of unborn babies. Data from several laboratories show that this method has an accuracy of over 95%.

Another very significant area is rapid prediction of life-threatening complications in trauma victims. As circulating DNA is produced when cells die, we expected that patients hospitalized following incidents of trauma, such as traffic accidents, would have elevated levels of circulating nucleic acids, and this has been shown to be the case. Healthy individuals have about 500–1000 genome equivalents per milliliter, and this is increased in accident victims. In fact, it has been shown that nearly 100% of trauma victims with complications such as multiple organ failure or acute respiratory distress syndrome have levels of plasma DNA above

100,000 genome equivalents per milliliter. Measurements of plasma DNA levels can therefore be used to predict the likelihood of these serious complications, and if necessary, aggressive therapy can be given at an early stage in an attempt to reduce the chance that they occur.

Can you detect elevated levels of these nucleic acids in trauma victims rapidly enough to be useful in emergencies?

Speed is very important here, of course. Using QIAamp® Kits, we can isolate DNA from plasma in 45 minutes. Then we quantify the DNA using quantitative, real-time PCR, and we can predict the likelihood of serious complications within 2 hours. This is a new direction for DNA analysis, moving it away from the clinic and into the emergency room setting.

Which areas are your group currently working on?

We are interested in studying circulating nucleic acids in patients with liver cancer or nasopharyngeal cancer, both of which are common cancers in Hong Kong. To diagnose liver cancer, we look for aberrant methylation patterns of multiple genes, including the tumor suppressor p16 gene, in circulating DNA. To detect nasopharyngeal cancer, which is closely associated with Epstein Barr virus (EBV) infection, EBV DNA is used as a plasma-based tumor marker, which has greater than 95% accuracy.

In our work on fetal DNA, an important focus is developing standardized protocols for the isolation of circulating nucleic acids in plasma, because we have shown that processing methods used can have a marked effect on the amounts of DNA measured [see accompanying article on page 4]. So far, publications on prenatal diagnosis of fetal disorders have all examined autosomal dominant disorders. We are working on extending the technology to autosomal recessive disorders, such as congenital adrenal hyperplasia and β-thalassemia.

Finally, a new and developing interest is in the study not only of circulating DNA, but also RNA. This is potentially very exciting, because it could provide a method of non-invasive gene expression profiling, which would yield insights into dynamic processes during pregnancy. For example, this approach may yield information on the cell types responsible for the release of fetal nucleic acids into maternal plasma.

What methods do you use to purify circulating nucleic acids in plasma?

Isolation of circulating RNA is quite difficult, as it is not very stable. We are currently trying out a range of methods, including RNeasy® Kits. We have also tested a number of different methods to purify circulating DNA, including home-made methods such as phenol/chloroform extraction or boiling, and various commercial kits. We found that QIAamp Kits are highly robust, and we now use them routinely for purification of circulating DNA. ■