Protein quantification - key technology for future research

In this exclusive interview, Professor Tetsuya Terasaki, Ph.D, Distinguished Professor at the Tohoku University in Japan talks about his most recent research concerning drug transporters, the role of PBPK modeling and protein quantification as the key technology to shorten the gap between the in-vitro and clinical phase regarding DDI. He also talks about his work as a senior scientific advisor with Bertin Pharma, explaining the nature of their collaboration.

IQPC: In terms of research tools, where do you see the biggest chance for a breakthrough?

T.T.: In terms of a breakthrough, physiologically based pharmacokinetic (PBPK) modelling will be much more important in the future, because the most critical information for predicting drug absorption, distribution, metabolism, excretion (ADME) in the body is the functional protein concentration in the organ, not only in animals but also humans and the disease condition. We have established a comprehensive method to qualify the functionary important proteins, that is of course, a transporter protein and also enzyme proteins and receptors and channels which are very important proteins not only for the drug's ADME, but also drug efficacy and/or toxic effect. In order to predict those protein functions in vivo, PBPK models are a very useful tool to integrate ADME, efficacy and toxic effect of the drug after the administration. PBPK models were first developed approximately 50 years ago as BJKK models and then simplified and used for lots of ADME studies. Although blood flow rate and organ volume were measured and have been used for the analysis, biochemical parameters, i.e., intrinsic activity of drug metabolizing enzyme, the permeability clearance across the plasma membrane is not so easy to determine in humans, especially in the disease condition. In vitro systems such as human liver slices, liver microsome or cDNA transfected cell lines have been used to determine these parameters, while concentration of responsible functional proteins both of these in vitro system and in vivo organs were not previously known. Now, it is possible to measure these functionally responsible proteins by LC-MS/MS combined with in silico peptide selection methods. Therefore, in the very near future, we believe the reliability of predictions based on PBPK models should increase significantly by using protein concentration determined for both in vitro and in vivo together with the intrinsic activity per functionally responsible protein amount. The progress of this research will solve the critical question of understanding the large gaps between animals and humans including the disease condition. As a result, the success rate of clinical trials could be increased significantly in the future. This is the most important challenge to be faced by the drug discovery and development community.

IQPC: What in your opinion will be the key to shortening the gap between in-vitro and the clinical phase with regards to transport-mediated DDI?

T.T.: Again, protein quantification is the key technology to shorten the gap between in vitro and the clinical phase because it is possible to measure the activity if you have a cDNA transfected cultured cell line of responsible transporters as an in vitro system. Based on the uptake or efflux studies using the in vitro cultured cell line, it is possible to compare the activities among different compounds, which would be useful to select an appropriate candidate compound as a substrate or inhibitor. However, one of the most difficult questions is how we could extrapolate the in vitro activity to in vivo activity of the transporter in a rational way. Information concerning the protein concentration of responsible transport activity was missing previously, and as a result nobody knew how much a transporter protein expresses in the organ of both animal and human. Therefore, in order to have a more precise prediction based on an in vitro system by means of PBPK model or if any, a pharmacodynamic (PD) model, the key parameters can now be determined by the quantitative targeted absolute proteomics technology (QTAP).

IQPC: Could you tell us a little more about this protein quantification method, QTAP?

T.T.: It means that target peptides for quantification are selected only from amino acid sequence information, so time-consuming procedures such as the selection of peptide fragments for the quantification based on the wet laboratory work by using mass spectrometry equipment are unnecessary. This in silico method of how to select an appropriate peptide fragment has been approved by the EU patent office and the patent has been issued in North America, China and Japan.

With the amino acid sequence of the protein, we can almost instantaneously select the appropriate peptide fragment which will be produced by tryptic digestion of the target protein to be quantified. That means for instance, if you want to set up 100 different transporter protein quantification using a traditional proteomics approach you have to inject the samples expressing the target protein a certain number of times after tryptic digestion to LC-MS/MS, this is not so straight forward and is very time-consuming. Our method however is very easy. If you want to quantify ABC transporter such as MDR1 or BCRP or SLC transporters such OCT1 or OATP1B3, all you need is the amino acid sequence of the transporter protein then almost immediately you can select the peptide fragment. It is also very important to pre-treat the sample to be quantified in an appropriate manner such as solbilization of the membrane proteins and tryptic digestion. We have spent a lot of time and effort to solve many practical problems in order to establish a reliable, robust and reproducible method.

The technology is based on LC-MS/MS

combined with the in silico peptide selection method but we also use the term quantitative targeted absolute proteomics - QTAP. The reason we use this term QTAP is to distinguish from the traditional global proteomics approach, which has been used for the protein discovery studies.

IQPC: What could be a desirable scenario for drug transporters' role in drug development in the next 10 years?

T.T.: The most important but fundamental question that we should answer, is "are there any unknown transporters responsible for drug absorption or hepatic transport or renal elimination such as MDR1, BCRP, OAT1, OAT3, OCT2, OATP1B1 and OATP1B3 ?" I believe that currently no one can conclude that there is no other functionally important transporter for the ADME of drug and candidate compounds for a new drug. In order to answer this question, we have to search through all the putative transporter proteins highly expressing in the plasma membrane of small intestine, liver, kidney, brain capillaries and diseased organ such as cancer cells in human. According to our preliminary study, there are certain numbers of unknown or unnamed candidate transporter proteins expressing abundantly in the human plasma membrane. The second step is to clarify the transporter function. We expect part of this step will present some challenges. Setting up a cDNA transfected cell line for each potential candidate transporter protein is the easy part. The difficulty lies in finding a substrate of the transporter. This is the critical part together with clarifying the driving force of the transporter function. The third step is, again a PBPK model. So that is our ultimate goal and I think this study could be completed within ten years, of course funding support from EU, USA and Japan will be a key requirement for such a big research project.

IQPC: Can you tell us more about your work as a senior scientific advisor with Bertin Pharma and the MS2plex technology?

T.T.: Three years ago, we set up a company on campus called Proteomedix Frontiers and the logo is on the box of the MS2plex kit (the MS2plex kit enables membrane protein quantification such as ABC transporter, SLC transporter, CYP and UGT enzyme and is based on the validation work and methodology developed by Professor Terasaki and his colleagues). Bertin Pharma has the right to use the patent covering this technology based on the contract with our company (Proteomedix Frontiers). Should a customer request any specific target protein quantification which is not in the MS2Plex catalogue, we could select the peptide for the protein quantification and create an "On-Demand Kit".

The reason we collaborated with Bertin Pharma is that they have a very strong background in producing protein qualification kits such as ELISA assays. They have a lot of expertise in handling these kits. Protein is not a simple material to handle, for instance, it must be kept at a very low temperature for shipping and specific knowledge is required to ensure a high level of quality is maintained with the kits. Our company is very small and we do not have that particular expertise or experience, therefore we collaborated with Bertin Pharma. Of course, Bertin Pharma has a very strong background and focus in ADME and they have a large distribution network in the world. They also have a lot of experience in customer support, which I believe, is a very important requirement for productization.

IQPC: What would you like to achieve with this technology?

It is my goal and my partner's goal to make our methodology the de facto standard for protein quantification including membrane transporters. By that I mean a global standard, the benchmark protocol around the world. There are slightly different methods already published by different scientists, but we believe that both our protocol and the result are very reliable. It is my personal mission to make a contribution to the international scientific society. That is my dream and the driving force behind my collaboration with Bertin Pharma.

IQPC: Thank you.

About Prof. Dr. Terasaki:

Dr. Tetsuya Terasaki is currently the Distinguished Professor of Tohoku University. He received B.S. degree in Pharmacy from Kanazawa University in 1977 and Ph.D. degree in Biopharmacy from University of Tokyo in 1982. He was appointed Assistant Professor of Kanazawa University in 1982, Associate Professor of University of Tokyo in 1992, Professor of Tohoku University in 1996 and Distinguished Professor of Tohoku University in 2008. He completed a postdoctoral training in blood-brain barrier research and was a Visiting Assistant Fellow at UCLA School of Medicine from 1985 to 1987.

Dr. Terasaki has received four international awards: the Ebert Prize from American Pharmaceutical Association (APhA) in 1985, the Meritorious Manuscript Awards from American Association of Pharmaceutical Scientists (AAPS) in 1996 and 2010 and AAPS Fellow in 2004. He also received two research achievement awards: the Academy of Pharmaceutical Science and Technology, Japan (APSTJ) Award in 2007 and the Japanese Society of Study for Xenobiotics (JSSX) Award in 2007. He served as the special advisor to the President of Tohoku University (Nov. 2006- March 2009) and Vice-Dean of Graduate School of Pharmaceutical Sciences (April 2008-March 2010).

His major research interests are pharmacoproteomics, quantitative targeted absolute proteomics-based brain barrier research, drug delivery to the brain and cancer tissue, and the physiology and molecular biology of blood-brain barrier function. He has published extensively in journals, with 226 original research articles and 96 review articles and contributed chapters to books.