Clinical Chemistry

Protein-Specific Glycoprofiling for Patient Diagnostics

Article:
D.J. Lefeber.
Protein-Specific Glycoprofiling for Patient Diagnostics.
Clin Chem 2015;62:9-11.
http://www.clinchem.org/content/62/1/9.extract

Guest:
Dr. Dirk Lefeber is an Associate Professor and Clinical Laboratory Geneticist with the Radboud University Hospital in Nijmegen, The Netherlands.

Bob Barrett: This is a podcast from *Clinical Chemistry*, sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Protein glycosylation is increasingly recognized as a crucial moderator of protein function, offering an additional layer of biological information over genomics and proteomics.

Modern tools for analyzing the carbohydrate portion of proteins have shown abnormal protein glycosylation in numerous human diseases. Unlike the genetic template for protein synthesis, the process of protein glycosylation is not directly encoded by the genome.

The January 2016 issue of *Clinical Chemistry*, a special issue devoted to mass spectrometry and the clinical laboratory published a paper describing congenital disorders of glycosylation in patients with certain enzyme deficiencies.

That article was accompanied by an editorial on protein specific glycoprofiling by Dr. Dirk Lefeber. He is Associate Professor and Clinical Laboratory Geneticist with the Radboud University Hospital in Nijmegen, The Netherlands. Dr. Lefeber is our guest in this podcast.

Doctor, what exactly is protein glycosylation?

Dr. Dirk Lefeber: Yeah, so protein glycosylation is a rather new field of research, and we all know about genes and proteins and the codes are quite fixed and dictate the synthesis of proteins. But there is another layer of biological information that we call protein glycosylation. So this enforced the addition of sugars to a protein, and this can be a wide range of sugars that can be added to a protein.

So the remarkable thing is that each individual protein can be glycosylated in a different way. So in that way you get a lot of different glycosylated proteins or different glycosylated protein forms.

So maybe to quote a few examples, the best known example I think is the blood group system that we all know, the ABO blood group system that discriminates individuals, and the difference between the blood group systems is really dependent on differences in glycosylation on blood cells.

Bob Barrett: Are most plasmas in urine proteins glycosylated?

Dr. Dirk Lefeber: Yes, indeed! So the vast majority of proteins in plasma and maybe also in urine, in urine we usually do not see a lot of proteins, but in plasma the majority of proteins is glycosylated.

You could say that of all the proteins that we have in the human body about 50% is glycosylated, but if you look really in body fluids, so the secreted proteins that occur in plasma, but also in cerebrospinal fluid, for example, we estimate in general that this is even higher, so like 90% or more of the proteins is glycosylated.

Bob Barrett: Your editorial in *Clinical Chemistry* refers to a paper published in the same issue regarding congenital disorders of glycosylation. Please tell us about the importance of that work.

Dr. Dirk Lefeber: Congenital disorders of glycosylation are genetic defects that result in abnormal protein glycosylation. So the process that we discussed of protein glycosylation is defective in these patients. The defective glycosylation leads to abnormal function of a lot of proteins in the body and thereby these patients suffer from a wide variety of severe clinical symptoms.

So the importance of this paper is as follows. There is a subgroup of genetic defects that account for about more than 20 different genetic defects, and it has always been thought that the affect on glycosylation was more or less the same, and the authors have now shown that for some subtypes they see very specific glycosylation abnormalities that can distinguish these subtypes from other subtypes.

So for a couple of reasons this work is important, that is for patient care and for research, equally alike. So for patient care it’s important that we are now able to identify very specific glycosylation abnormalities and thereby significantly shorten the time to diagnosis for those patients. And in addition, the work also illustrates that it's important to analyze glycosylation differences on individual proteins.

Bob Barrett: Well, how will analysis of protein glycosylation aid individualized diagnostics?

Dr. Dirk Lefeber: Well, that's because you combine the information of the protein level and on the glycan structure. So until now the majority of biomarker research has focused on the study of protein levels alone. The process of protein glycosylation and the factors that control it are only partially known.

What we are starting to learn is that an increasing number of factors influence the process of protein glycosylation. These could be genetic factors or environmental factors.

So thereby the glycosylation profile of a certain protein provides a kind of image of the disease state or health state of a cell. And this state is different from person to person and therefore provides a much more precise biomarker of disease, if you compare that by measuring protein levels alone.

So the future will be to measure protein levels and its glycosylation status and thereby this gives more information on the patient and thereby leads to better individualized biomarkers of disease.

Bob Barrett: Doctor, what new technologies have aided in making this work potentially useful in diagnostics, and do we still need key improvements in instrumentation?

Dr. Dirk Lefeber: Yeah, that's a good question. So new developments in mass spectrometry are now allowing to analyze the glycosylation of individual proteins, but still we need some improvements on two major aspects I would say.

So there's a discovery phase of glycoprotein biomarkers and for that phase we still need to improve on the ability to simultaneously profile glycans of many different proteins in complex mixtures. And if you have to apply this new technology in patient care, we have to zoom in on very specific glycoproteins, and for that we need to be able to quantify exact changes in the glycosylation of individual proteins.

Bob Barrett: Well finally, let's look ahead, how far are we from applications that will impact diagnosis and therapy in a clinical setting?

Dr. Dirk Lefeber: The technology for analyzing glycomes is called glycomics, and this field has very well developed in the last decades, and usually people analyze global glycome profiles of complete protein mixtures. So the information of individual proteins is lost.

In more recent years there is also new technology that is able to analyze glycosylation profiles of individual proteins

within a complex mixture, like, for example, the plasma glycoprotein.

For genetic disorders the first examples have already appeared in patient diagnostics, but for more common disorders several steps still have to be taken, but it's becoming a real option now that within, I think, five years several glycoprotein-based biomarkers are widely used in individualized patient care.

Bob Barrett: That was Dr. Dirk Lefeber, an Associate Professor and Clinical Laboratory Geneticist with the Radboud University Hospital in Nijmegen, The Netherlands. He has been our guest in this podcast from *Clinical Chemistry* on protein glycosylation and human disease. His editorial appeared in the January 2016 issue of *Clinical Chemistry*, a special issue devoted to mass spectrometry and the clinical laboratory.

I am Bob Barrett. Thanks for listening.