Measuring modified protein structures

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Swiss researchers have developed a new approach to measure proteins with structures that change. This could enable new diagnostic tools for the early recognition of neurodegenerative diseases to be developed.

Cells regulate protein functions in a wide variety of ways, including by modifying the protein structure. In an instant, a protein can take on another form and perform no or even the "wrong" function: in humans, proteins that fold wrongly can cause serious diseases such as Alzheimer's, Parkinson's or cystic fibrosis. Some of these proteins also have a tendency to "infect" other molecules of the same type and congregate into insoluble so-called amyloid fibrils or plaques. These amyloids can damage cells and tissues and make people ill.

Method breaks the shackles

Until now, there has been a lack of methods that enable structurally modified proteins to be recorded quantitatively in complex biological samples. Although there is a series of techniques to study structurally modified proteins, such as x-ray crystallography, nuclear magnetic resonance spectroscopy and other spectroscopic techniques, they cannot be used to analyse complex biological samples. Other procedures that researchers have used to study structural changes ofproteins in cells also have their limits: prior to the analysis, the proteins of interest have to be specifically marked to enable the scientists to observe them in samples. However, this approach is only possible for a few proteins in a sample.

The team headed by Paola Picotti, a professor of protein network

biology, has now found a way to measure the majority of structurally modified proteins in any biological sample, which can contain thousands of different proteins. Picotti and her team have succeeded in measuring quantities of structurally modified proteins directly from a complex protein mixture as it occurs in cells, without cleaning or enriching the samples.

Combination of several methods

For their new method, the researchers combined an "old" technique and a modern approach from proteome research. First of all, familiar old digestive enzymes such as proteinase K are added to the sample, which cut the proteins depending on their structure into smaller pieces known as peptides. The fragments can then be measured using a technique which Picotti played a key role in co-developing during her time as a postdoc at ETH Zurich (as ETH Life reported). Known as Selected Reaction Monitoring (SRM), this method enables many different peptides to be sought specifically and their quantities measured. Based on the peptides found, proteins that were originally present in the sample can be determined and quantified.

What makes it so special: the digestive enzymes cut the same kind of proteins that have different structures in different places, resulting in diverse fragments. Like a fingerprint, these fragments can be clearly assigned to the individual structures of the protein.

"This means we can use the method to analyse structural changes of specific proteins or entire protein networks in a targeted fashion and measure numerous proteins at the same time," says Picotti.

Works for protein responsible for Parkinson's

Based on their new method, the researchers devised a test to specifically measure the "healthy" and "sick" versions of the protein alpha-synuclein in complex, unpurified samples such as blood or cerebrospinal fluid. Alpha-synuclein is thought to cause Parkinson's when its structure is modified. The pathological structural variety congregates with its own kind to form amyloid fibrils, which harm neuronal cells.

With the aid of the test, the scientists managed to measure the exact amount of pathogenic and non-pathogenic alpha-synuclein directly in a complex sample. The test also yielded information on the structure of the protein. "It shows us which parts of the protein change and turn into the new pathological structure," says Picotti.

Increasing number of amyloidoses

For the time being, the concentration of alpha-synuclein cannot be used as a biomarker as the levels of the protein are too similar in the blood or cerebrospinal fluid of Parkinson's sufferers and healthy people. "Nevertheless, it is possible that the ratio of pathological versus nonpathogenic alpha-synuclein structure changes with time, along the progression of the disease" suspects the ETH-Zurich professor. "As the new method enables us to measure both structures of the alpha-synuclein protein in a large variety of samples, it might be possible to use this to develop new biomarkers for this disease in the future," she hopes. Using the method, it might also be conceivable to discover other, as yet unknown amyloid-forming proteins that are connected to diseases without prior knowledge.

Both applications – the quantification of a specific known protein with a modified structure and the discovery of new proteins with variant structures – are highly relevant from a medicinal perspective, Picotti explains. "The number of amyloidoses, i.e. diseases that develop due to changes in protein structures, increases every year."

More information: Feng Y, De Franceschi G, Kahraman A, Soste M, Melnik A, Boersema P, Polverino de Laureto P, Nikolaev Y, Oliveira AP, Picotti P. Global analysis of protein structural changes in complex proteomes. *Nature Biotechnology*, published online 14th Sept 2014, DOI: 10.1038/nbt.2999

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