Genome engineering of quantifiable protein tags

June 6 2016

Cell biologists' most notorious approach to detect and semi-quantify proteins, western blotting, could well be on its way down. Professor Sven Eyckerman (VIB/UGent) and colleagues developed a set of universal protein tags that warrant protein quantification via targeted proteomics techniques. The development and applications of these new tags - named Proteotypic peptides for Quantification by SRM (PQS) - are described in the online, open access journal *Scientific Reports*.

Getting an accurate view on the cellular concentration of a protein remains a challenging task. Antibody-based approaches like western blotting and ELISA are sensitive and convenient, but detection can be hampered by inadequate antibodies or epitope masking. Alternative quantification strategies like selective reaction monitoring (SRM) do not require any immunoaffinity reagent, but instead rely on the use of targeted mass spectrometry to monitor the expression level of a protein. Despite the clear use for targeted proteomics, assay development remains challenging as multiple, proteotypic reporter peptides need to be selected for each studied protein.

The team of Eyckerman envisioned a universal SRM assay based on protein tags, which takes away the need to meticulously optimize targeted proteomics assays for each protein of interest. "Ideally, we would tag proteins with a small peptide flanked by optimal tryptic contexts and characterized by optimal MS properties for SRM-based detection and quantification", says PhD student Giel Vandemoortele (VIB/UGent).

To bring this idea into reality, the lab teamed up with the bioinformatics group of Lennart Martens (VIB/UGent) and the proteomics lab of Kris Gevaert (VIB/UGent). The scientists mined the proteome of the hyperthermophile Pyrococcus furiosus for unique sequences with optimal mass spectrometry characteristics. "The rather extreme habitat of this archaebacterium results in a truly unique proteome, which provided us with a great source for unique peptides", says Martens. For the peptides to be universally applicable in a wide range of model organisms, the team specifically looked for peptides proteotypic for all eukaryotes and all E. coli strains. Two ideal peptides were retained and found suitable for direct sensitive detection and quantification in complex lysates. "Before, I needed to setup an SRM assay for each protein of interest, which was labor intensive and often had a low success rate", SRM expert An Staes (VIB/UGent) explains. "This painful assay development is now obsolete."

To introduce the quantifiable tags into the genome of human cells, the scientists put new recruits of the genome engineering toolbox into action. "With the CRISPR/Cas9 system we could efficiently tag endogenous proteins in mammalian cell lines cultures, providing a universal quantitative read-out system for the tagged proteins", Eyckerman comments. "This adds a powerful novel aspect to genome editing. An accurate and sensitive quantification of an endogenous protein is now possible, either directly or after purification of the protein within a protein complex."

More information: Giel Vandemoortele et al. An extra dimension in protein tagging by quantifying universal proteotypic peptides using targeted proteomics, *Scientific Reports* (2016). DOI: 10.1038/srep27220

Provided by VIB (the Flanders Institute for Biotechnology)