LC-MRM, a rapid tool for high throughput quantification of protein target expression after mRNA administration

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Summary

Moderna and Biognosys have worked together on several projects with the purpose of using targeted proteomics for the high throughput quantification of protein target expression after mRNA administration. This study shows that LC-MRM is a robust and rapid tool for high throughput quantification of protein target expression after mRNA administration. Since targeted proteomics overcomes the limitations of antibody-based methods, it was used in a series of experiments focused on quantifying protein expression in a large number of samples. Here, we present examples for LC-MRM protein quantification of highly similar non-human proteins and overexpression of human proteins and demonstrate a rapid assay generation workflow for proteins where no antibody assay is available.

Introduction

Messenger RNA (mRNA) is a polyribonucleic acid used by organisms as a template for protein expression and may also serve as a tool for selected protein expression in cells. Despite decades of interest of the scientific community to engineer nucleic acids for therapeutic use, mRNA has only recently been recognized as a revolutionary human therapeutic agent (Thes et al. 2015). mRNA is a carrier of genetic information which recruits the endogenous protein translational machinery within the cell to produce active therapeutic proteins. Thus, the quantification of the target protein after the administration of mRNA therapeutic is a critical step in efficacy evaluation of any treatment. Traditionally, this has been done using antibody-based methods such as ELISA or Western blots (Matsui et al. 2015, DeRosa et al. 2016). These sensitive methods have several limitations. An antibody may not always be available for a particular mRNA target protein, especially for non-human or non-mouse species, and method development for fully validated antibody reagents often can’t be achieved in a time-frame acceptable to pre-clinical R&D. These issues are further exacerbated when it is necessary to examine multiple variants of a single protein target (point mutants, N- or C-truncated isoforms) for potential biological activity. These limitations can be addressed by applying mass spectrometry-based quantitative proteomics, a powerful high-throughput platform for monitoring protein expression that is a common technology platform for biomedical and clinical research (Pan et al. 2009). Targeted proteomics methods such as multiple reaction monitoring (MRM) are highly specific, sensitive and efficient assays for the quantification of hundreds of protein targets (Picotti et al. 2010). Protein targets from uncommon species or minor sequence differences can be resolved due to the highly specific mass spectrometer (Shi et al. 2012). Moderna and Biognosys have worked together on several projects with the purpose of using targeted proteomics for the high throughput quantification of protein target expression after mRNA administration. Since targeted proteomics overcomes the limitations of antibody-based, it was used in a series of experiments focused on quantifying protein expression in a large number of samples. Here, we present examples for LC-MRM protein quantification of highly similar non-human proteins and overexpression of human proteins and demonstrate a rapid assay generation workflow for proteins where no antibody assay is available.

Methods

Different variants of mRNA encoded for a wide range of human and non-human proteins were applied to HeLa cells. Cells were lysed in a strongly denaturing buffer and proteins were digested to peptides using trypsin. LC-MS/MS shotgun mass spectrometry was carried out for target protein detection. The three best response were selected for target protein generation. Up to three peptides for the target protein and up to five peptides representing selected housekeeping proteins were measured in LC-MRM for relative quantification of the proteins of interest. Data was analysed using SpectroDive, Biognosys’ software for the rapid analysis of multiplexed LC-MRM data.

Results and discussion

In this study an LC-MRM platform was used to evaluate the efficacy of several mRNA therapeutics by confirming the protein expression in the target cells and quantifying the relative expression across multiple mRNA variants. LC-MRM assays for 114 target proteins were generated for the quantification in 480 samples of HeLa cells in several batches. The time between sample receipt and results was only one week including the development of specific assays (Figure 1). The expression of GTP cyclohydrolase 1 (GCH1) protein in human HeLa cell lines was identified in all 5 mRNA-treated samples, but not in the control sample (Figure 2). Furthermore, sample 4 was identified as the mRNA variant yielding the highest level of GCH1 protein. The high specificity of the LC-MRM method becomes apparent especially when target proteins share large portions of their amino acid sequence and antibodies are often not able to resolve a single amino acid difference. The highly similar cat and dog erythropoietin were quantified in HeLa cells after mRNA administration using specific LC-MRM assays for either species (Figure 3a). Shared peptides between the cat and dog protein were detected and quantified in both samples (Figure 3b). This study shows that LC-MRM is a robust and rapid screening tool for protein quantification that can be applied in the development of therapeutic mRNAs. In addition, the method can be easily multiplexed with no effect on specificity or the time required to carry out the assay, and multiple peptides per protein can be analysed further enhancing the scope in results. When absolute quantification of the target protein is required, stable isotope-labelled reference peptides can be incorporated, to configure a multiplex LC-MRM assay for target proteins at attomole sensitivity.

References