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Targeted MRM Proteomics is a Better Protein Quantification Method Over Western-Blotting

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Sensitivity and selectivity of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for quantification of small molecules encouraged biologists to utilize this platform for protein quantification using peptide as a surrogate. MS based protein quantification, commonly referred as multiple or selective reaction monitoring (MRM or SRM) proteomics, relies on selective quantification of surrogate peptide(s) in a digested protein sample. The selectivity is achieved by the MRM characteristic of triple quandrupole MS instrument in which unique daughter ion generated from fragmentation of a parent ion (i.e, ionized analyte peptide) is monitored. This application of MS based targeted proteomics become wider with the availability of protein databases and *in silico* methods for surrogate peptide selection in the last five years. For instance, successful completion of human genome project and the ongoing human proteome project enabled us to select surrogate peptides for MRM quantification of nearly every human protein [1,2]. Because of its increasing popularity, Nature Methods declared targeted proteomics by mass spectrometry (MS) as Method of the Year for 2012 [3].

On the other hand, western-blotting (or protein immunoblotting) is a conventional protein quantification method which is a standard technique in almost every biology laboratory. Therefore, it is a challenge for biologists to see beyond western-blotting, and accept and adopt MRM proteomics in routine protein quantification work. Considering the latter, it is important to compare the advantages and disadvantages of both these methods. Aebersold et al. [4] recently recently touched this topic in their editorial in Molecular & Cellular Proteomics and advocated the use of MRM proteomics. As a user, I present here my perspective on this topic focusing on the differences between the two methods on various aspects, i.e., application and speed, ease of use, quality of data and ability to confirm results. i) MRM proteomics can be used to quantify multiple proteins in a shorter time (approximately 10 proteins in 20 min) [5]. As the latter does not rely on antibodies, it can quantify every protein for which the sequence is known. On the other hand, western-blotting is mostly applicable to one protein at a time and requires antibodies. We even use MRM to quantify complex membrane bound proteins for which developing good antibodies is a huge challenge [5-9]. ii) Western-blotting is recognized as a routine method and can be applied to any laboratory; however, MRM needs sophisticated MS instrument with expertise to run the same. Despite that, any laboratory that is using MRM for quantification of small molecules, can adopt this technology in protein quantification. Further, with the availability of *in silico* tools, it takes approximately 1 hour to select a surrogate peptide and confirm the signal in a digested biological sample using MRM method. iii) As synthetic peptide is used as calibrator, MRM proteomics can deliver absolute protein levels when the protein digestion efficiency is established. Sensitivity of MRM method depends on peptide characteristics and may vary widely. In general, if the best peptide is used for MRM quantification, it is comparable or better in sensitivity than western-blotting. This is because signal to noise ratio is significantly high and a minimal sample volume is required in MRM. The MRM sensitivity can be further increased substantially by using stable isotope standard capture with anti-peptide antibodies (SISCAPA) [10,11]. As protein digestion is reproducible and many sample preparation steps can be automated, MRM offers better precision. iv) The quality of data is also better in the latter because it rely on multiple signals, i.e., multiple peptides and multiple MRM transitions. The results can be easily confirmed by using another independent surrogate peptide.

So, LC-MRM outperforms western-blotting in many aspects except that a dedicated facility with skilled personnel are required to run these sophisticated instruments and therefore the initial costs are higher. However, with the pace of technology, it is not difficult to imagine that the small, economical and user-friendly bench-top LC-MRM instruments will be available soon. The main advantage of LC-MRM is its ability to quantify various proteins in multiple samples with great high-throughput. This is particularly applicable in protein quantification in biofluids, tissues, cells and cell lines for applications like, biomarker discovery and validation, patient stratification, clinical trial monitoring, development of companion diagnostics, drug toxicity assessments in serum or urine samples, genomics-proteomics correlations, systems biology/pharmacology (network analysis), systems based pharmacokinetic (SBPK) modeling, pharmacokinetics-pharmacodynamic (PKPD) modeling, pharmacokinetic (PK) studies of bio-therapeutics, population variability in protein expression, quality control studies of biologics.

References

1. Legrain P, Aebersold R, Archakov A, Bairoch A, Bala K, et al. (2011) The human proteome project: current state and future direction. Mol Cell Proteomics 10: M111 009993.
2. Picotti P, Aebersold R (2012) Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions. Nat Methods 9: 555-566.
3. (2013) Method of the Year 2012. Nat Methods 10: 1.
4. Aebersold R, Burlingame AL, Bradshaw RA (2013) Western blots versus selected reaction monitoring assays: time to turn the tables? Mol Cell Proteomics 12: 2381-2382.
5. Prasad B, Evers R, Gupta A, Hop CE, Salphati L, et al. (2013) Interindividual Variability in Hepatic Oatps and P-Glycoprotein (ABCB1) Protein Expression: Quantification by LC-MS/MS and Influence of Genotype, Age and Sex. Drug Metab Dispos.
6. Prasad B, Lai Y, Lin Y, Unadkat JD (2013) Interindividual variability in the

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hepatic expression of the human breast cancer resistance protein (BCRP/ABCG2): effect of age, sex, and genotype. J Pharm Sci 102: 787-793.

7. Lee N, Hebert MF, Prasad B, Easterling TR, Kelly EJ, et al. (2013) Effect of Gestational Age on mRNA and Protein Expression of Polyspecific Organic Cation Transporters during Pregnancy. Drug Metab Dispos .
8. Edson KZ, Prasad B, Unadkat JD, Suhara Y, Okano T, et al. (2013) Cytochrome P450-Dependent Catabolism of Vitamin K: Ï‰-Hydroxylation Catalyzed by Human CYP4F2 and CYP4F11. Biochemistry .
9. Deo AK, Prasad B, Balogh L, Lai Y, Unadkat JD (2012) Interindividual variability in hepatic expression of the multidrug resistance-associated protein 2 (MRP2/ABCC2): quantification by liquid chromatography/tandem mass spectrometry. Drug Metab Dispos 40: 852-855.
10. Razavi M, Frick LE, LaMarr WA, Pope ME, Miller CA, et al. (2012) High-throughput SISCAPA quantitation of peptides from human plasma digests by ultrafast, liquid chromatography-free mass spectrometry. J Proteome Res 11: 5642-5649.
11. Anderson NL, Jackson A, Smith D, Hardie D, Borchers C, et al. (2009) SISCAPA peptide enrichment on magnetic beads using an in-line bead trap device. Mol Cell Proteomics 8: 995-1005.