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Proteomics in pulmonary research: selected methodical aspects

Abstract

Recent years witness rapid expansion of applications of proteomics to clinical research including non-malignant lung disorders. These developments bring along the need for standardisation of proteomic experiments. This paper briefly reviews basic methodical aspects of applied

proteomic studies using SELDI-TOF mass spectrometry platform as example but also emphasizes general aspects of quality assurance in proteomics.

Key-words: lung proteome, quality assurance, SELDI-TOF MS.

Introduction

Proteomics, a science dealing with proteome – protein complement of the genome, has indeed reached pulmonary medicine. Though still not vast, the number of papers dealing with description and/or identification of novel proteins in lung pathobiology has been increasing (e.g. ¹⁻⁶) and currently, there has been ongoing effort to link information from gene expression and proteomics studies in order to propose candidate molecules for diagnostic and therapeutic purposes.

However, when engaged in proteomics, one has to be aware of possible pitfalls due to sophisticated methodology and a number of different formats of obtained data. Of course, there are general methodical rules applicable throughout laboratory medicine known as Good Laboratory Practice⁷. Next, specific issues appear that are pertinent only to proteomics, adherence to which is mandatory for obtaining meaningful data. These will be in brief described further and selected examples shown based on usage of SELDI-TOF MS

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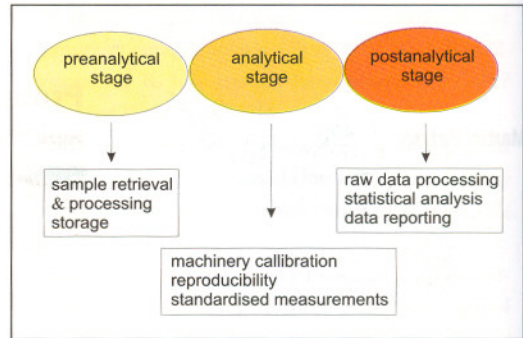
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platform (Surface Enhanced Laser Desorption Ionisation – Time of Flight Mass Spectrometry, ref. 1). The final level of complexity is influenced by the purpose of a proteomic experiment: there will be different requirements for proteomic measurements directed at discovery and validation of new biomarkers than for experiments aimed at description of proteins involved in pathological disease mechanisms. Discussing this topic is out of scope of our review, however one should be aware of existence of working groups (e.g. Proteomics Standards Initiative), which formulate set of guidelines for acquisition and namely for adequate interpretation of proteomic data (e.g. 8). Important issue for proteomic community is feasibility of re-analyses and verification of results from different laboratories. It is necessary to be able to compare proteome coverage between different laboratories or between different technical platforms and for this purpose databases have been already set⁸⁻¹⁰.

Any proteomic experiment, does not matter if directed at screen for a candidate in disease pathology, for biomarker discovery or for clinical prognostics, consists of three sequential stages (Scheme 1), methodical aspects of which will now be discussed.

Preanalytical stage

Whatever is the nature of the investigated material (serum, bronchoalveolar lavage, sputum), specimen collection must be standardised and uniform procedures must also be applied during manipulation with the biofluid and its transport to the laboratory^{11,12}: the material should be kept cool (e.g. on ice, or refrigerated to +4°C) and transported to the laboratory as soon as possible. In the labo-



Scheme 1. Sequence of stages of a proteomic experiment using example of SELDI-TOF mass spectrometry

ratory, material should be divided into portions (aliquots) and stored preferably in -80°C; shock freezing down in liquid nitrogen is recommended.

Analytical stage

Instrument set-up

Regarding the instrumentation, standard conditions are necessary: for example for SELDI-TOF MS, vacuum and other parameters such as number of laser shots should be set as required by the manual, for two dimensional gel electrophoresis appropriate temperature and constant power supply are necessary. Regular calibration (at least weekly) of an instrument is crucial^{12,13} and obtained data (for SELDI-TOF MS) should lie within the estimated range of mass/charge ratio, peak intensity and resolution and signal to noise ratio (for brief description see section “Postanalytical stage”).

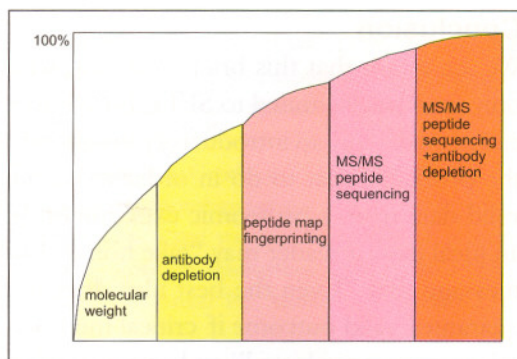
Samples

Work with samples should be organized so that minimal variations are introduced when loading the samples, e.g. in case of SELDI-TOF

MS manual pipetting has been superseded by robotic spotting. Samples should be run in at least duplicates, preferably in triplicates. Importantly, repeated freeze/thaw cycles must be avoided when processing the samples.

Postanalytical stage

After the measurements, data processing is the most important part of proteomic experiments. In most cases, analysis of the raw data from an instrument is performed by software, which requires setting of critical parameters by an operator. For SELDI-TOF MS these are: mass window, signal to noise ratio and value of normalisation factor. Because the above parameters are specific for SELDI-TOF MS, only a brief description follows. Basically, mass accuracy reflects specific aim of the experiment and is different for profiling and identification purposes. By manipulating with signal to noise (background) ratio, one can achieve more precise measurement and lower detection limits. For clinical samples, during the normalisation step, software corrects the data such that all samples have the same mean of spectral intensity and thus changes in protein content are balanced; samples with high normalisation factor are excluded from the analysis. Finally, in postanalytical stage, adequate statistics, according to aim of the experiment, should be applied and proper methods for identification of peaks or gel spots selected – this is important for confirmation of the identity of detected proteins (Scheme 2). Due to space limits we provide here only some references on statistical analyses in proteomics¹⁴⁻¹⁷ and before concluding we wish to emphasize once more general aspects of standardization process.



Scheme 2. Protein identification strategies X axis: amount of information, Y-axis: confidence level, MS: mass spectrometry

General Quality Assurance

Though it is time consuming and sometimes tricky, one should aim at achieving and running proper Quality Control (QC). This includes adopting sampling and storage protocols and performance of regular checks including personnel (operator) to eliminate subjective factors and clerical errors, performing pipette calibration, checking for uniformity and quality of used reagents and materials (chips, gels, etc.) and importantly testing for reproducibility¹⁸, e.g. employing QC samples to check for intra/inter assay variability. Usage of certified standards, which enable calibration in the required range and which are properly reconstituted, aliquoted and stored, is mandatory. Adhering to this complex QC measures, random and systematic errors should be eliminated to minimum. Finally, recording of all crucial procedures especially during preanalytical and analytical stages is critical because it allows repetition of experiments and namely analysis of all sequential steps if a problem arose. Of particular importance is recording of storage & handling conditions, instrument calibration and reproducibility records.

Conclusion

We are aware that this brief overview, with only few details limited to SELDI-TOF MS, can provide only an introductory insight into the problem what to do in order to obtain valid data from a proteomic experiment. In an ideal case, a reader may "take home" our message that "Even the best idea does not guarantee valid outcome if critical methodical points are neglected" and vice versa that "No quality control can assure that good research or clinical data are achieved if the idea & experimental design is flawed".

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