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Comparison of label-free and label-based strategies for proteome analysis of hepatoma cell lines ☆

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ABSTRACT

Within the past decade numerous methods for quantitative proteome analysis have been developed of which all exhibit particular advantages and disadvantages. Here, we present the results of a study aiming for a comprehensive comparison of ion-intensity based label-free proteomics and two label-based approaches using isobaric tags incorporated at the peptide and protein levels, respectively. As model system for our quantitative analysis we used the three hepatoma cell lines HepG2, Hep3B and SK-Hep-1. Four biological replicates of each cell line were quantitatively analyzed using an RPLC–MS/MS setup. Each quantification experiment was performed twice to determine technical variances of the different quantification techniques. We were able to show that the label-free approach by far outperforms both TMT methods regarding proteome coverage, as up to threefold more proteins were reproducibly identified in replicate measurements. Furthermore, we could demonstrate that all three methods show comparable reproducibility concerning protein quantification, but slightly differ in terms of accuracy. Here, label-free was found to be less accurate than both TMT approaches. It was also observed that the introduction of TMT labels at the protein level reduces the effect of underestimation of protein ratios, which is commonly monitored in case of TMT peptide labeling. Previously reported differences in protein expression between the particular cell lines were furthermore reproduced, which confirms the applicability of each investigated quantification method to study proteomic differences in such biological systems. This article is part of a Special Issue entitled: Biomarkers: A Proteomic Challenge.

1. Introduction

Mass spectrometry (MS)-based techniques have emerged as high-throughput bioanalytical methods within the field of proteomics. For quantitative analysis of proteomic differences, several MS-based quantification strategies have been developed over the years. Formally, these can be divided into label-free and label-based approaches. The former type implies spectral counting and ion intensity-based quantification [1–3] and the latter type metabolic, enzymatic or chemical labeling strategies. Well-established and widely used quantification methods utilizing chemical modification of proteins and peptides with stable-isotope labels are isobaric tags for relative and absolute quantification (iTRAQ) [4] and tandem mass tag (TMT) [5,6]. In both cases, the quantification relies on the measurement of intensities of so-called reporter ions. These are produced and detected at the MS^2 level, namely in course of peptide fragmentation. In contrast, label-free ion intensity-based quantification implies the determination of precursor ion abundances at MS level. The great advantage of iTRAQ and TMT as compared to other quantification methods is the ability of a multiplexed analysis of several samples within a single LC–MS/MS run (i.e. 8-plex in case of iTRAQ and 6-plex in case of TMT). Thereby, instrument time necessary for quantitative analysis is significantly decreased and variations during sample preparation, chromatography and MS acquisition can be diminished. However, commercially available labeling reagents are expensive and an additional labeling step has to be introduced in the analytical workflow. Label-free proteomics is cost-efficient and no additional sample preparation steps are needed. Furthermore, it was found to offer higher proteome coverage and a higher dynamic range [7]. However, as every sample needs to be handled separately until the final LC–MS/MS analysis, all steps ranging from sample preparation to MS acquisition can introduce variations that can bias the quantitative analysis. Furthermore, a quantitative analysis is comparatively time-consuming, as a multiplexed analysis is not possible in case of label-free proteomics. Due to these particular advantages of chemical labeling and label-free strategies, both MS-based quantification methods have become widely used in recent proteomic-based biomarker discovery studies [8–15].

In our current study a comprehensive comparison of three different approaches of quantitative proteomics was performed, namely label-free ion-intensity-based quantification and label-based techniques

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utilizing tandem mass tags incorporated at the peptide or protein level. We performed three parallel quantitative proteomic studies in which altered protein expression levels between the three well-characterized hepatocellular carcinoma (HCC) cell lines HepG2, Hep3B and SK-Hep-1 were investigated (Fig. 1). These show distinct differences regarding the expression of alpha-fetoprotein (AFP), a serological marker widely used in the clinical diagnosis of human HCC. HepG2 and Hep3B are both alpha-fetoprotein (AFP)-positive HCC cell lines, whereas SK-Hep-1 is AFP-negative [16]. Previous proteomic studies utilizing protein quantification via SILAC [17] or 2D-DIGE [16] have shown significantly different proteomic profiles of AFP-positive and AFP-negative cell lines, which makes this system an ideal model to study the performance of different quantification techniques. Proteomic data acquired in LC–MS/MS experiments were analyzed with respect to proteome coverage and reproducibility of protein identifications. Further data analyses between the different approaches were performed to assess the complementarity of the used MS-based strategies. Quantitative data were furthermore compared regarding accuracy of quantitative results and reproducibility within the same and between different quantification methods. Statistical evaluations were performed to reveal proteins with significantly different expression profiles, which were finally ranked regarding their significance and particular fold changes.

2. Materials & methods

2.1. Materials

SK-Hep-1, Hep3B and HepG2 cells were obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cell culture media, Dulbecco's phosphate saline buffer (DPBS) and cell dissociation buffer were obtained from Life Technologies (Carlsbad, CA, USA). Sixplex tandem mass tags, triethylammonium bicarbonate (TEAB), tris(2-carboxyethyl)phosphine (TCEP) and hydroxylamine were purchased from Thermo Fisher Scientific Inc. (Rockford, IL, USA). Trifluoroacetic acid (≥99.0%, TFA) and LC/MS-grade acetonitrile were from Sigma-Aldrich (St. Louis, MO, USA) and Biosolve Chemicals (Dieuze, France), respectively.

2.2. Cell culture and lysis

Human hepatoma cell lines (SK-Hep-1, Hep3B and HepG2) were grown in a humidified incubator with 5% CO_2 at 37 °C in DMEM (Hep3B, HepG2) and RPMI1640 (SK-Hep-1) supplemented with 10% fetal calf serum. Flasks with confluent monolayer of at least 5×10^6 cells were washed with DPBS, detached with enzyme-free dissociation

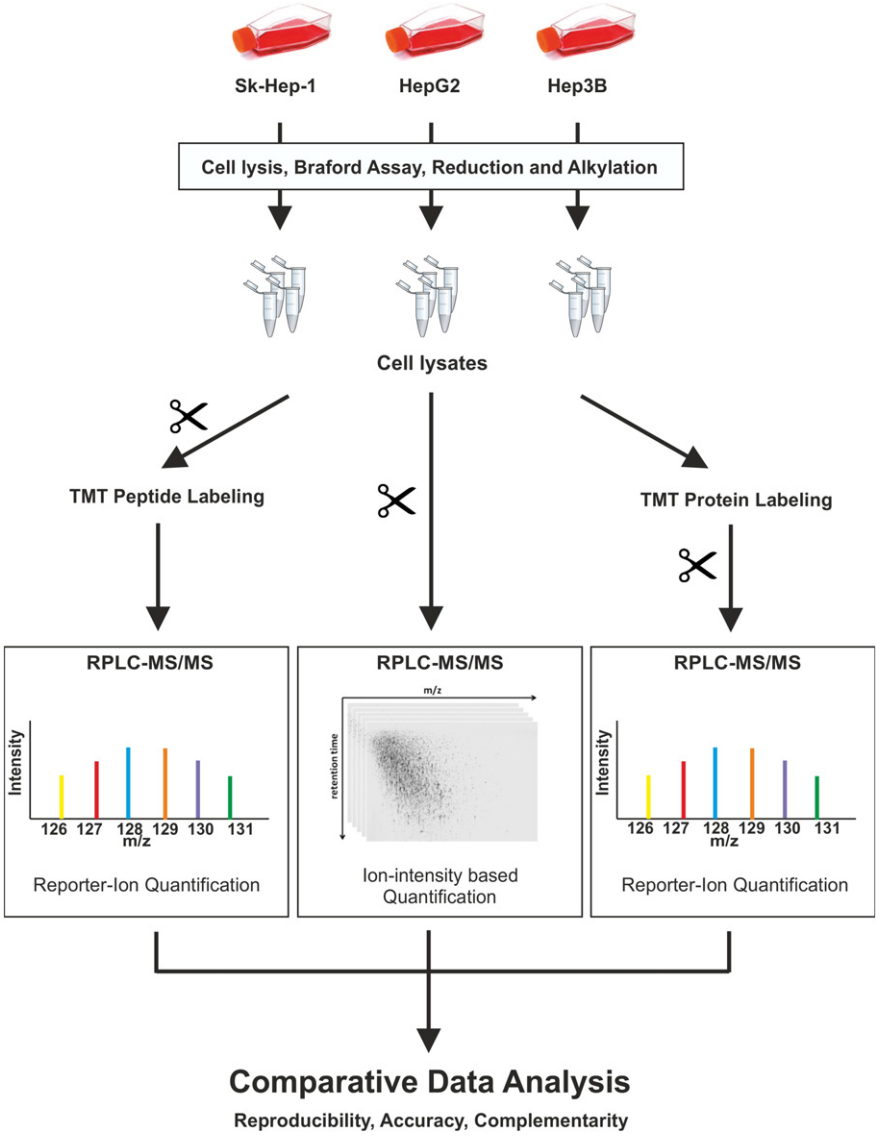

Fig. 1. Generalized experimental design of the quantitative study including three different techniques of protein quantification. Digestion steps are indicated by scissors.