BIOMARKER-PROFILER : a dedicated solution for simultaneously discover & quantify candidate biomarkers.

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INTRODUCTION

Discovery of candidate biomarker is an important step to evaluate drug efficacy in treatment or to detect diseases at early-stage. This kind of studies are largely conducted with high-throughput MS-based techniques, and consists in samples comparison through differential analysis approaches, based on iBAQ, LFQ or spectral counting signals. However, these comparisons rely on relative quantification and don't allow obtaining accurate proteins quantity assessment, which however can appeared a important information. Based on this statement, we developed a BIOMARKER-PROFILER integrated workflow, a high-resolution MS-based workflow for biomarkers discovery and quantification, occurring in a single analysis. Validation step is then done with targeted MS analysis, or biochemical approach such as ELISA.

CONTEXT & STRATEGY

The objective of the study was to discover & quantify potential biomarkers, consequently to gene silencing in keratinocyte cells culture. BIOMARKER-PROFILER was developed to answer this technical challenge (fig 1).

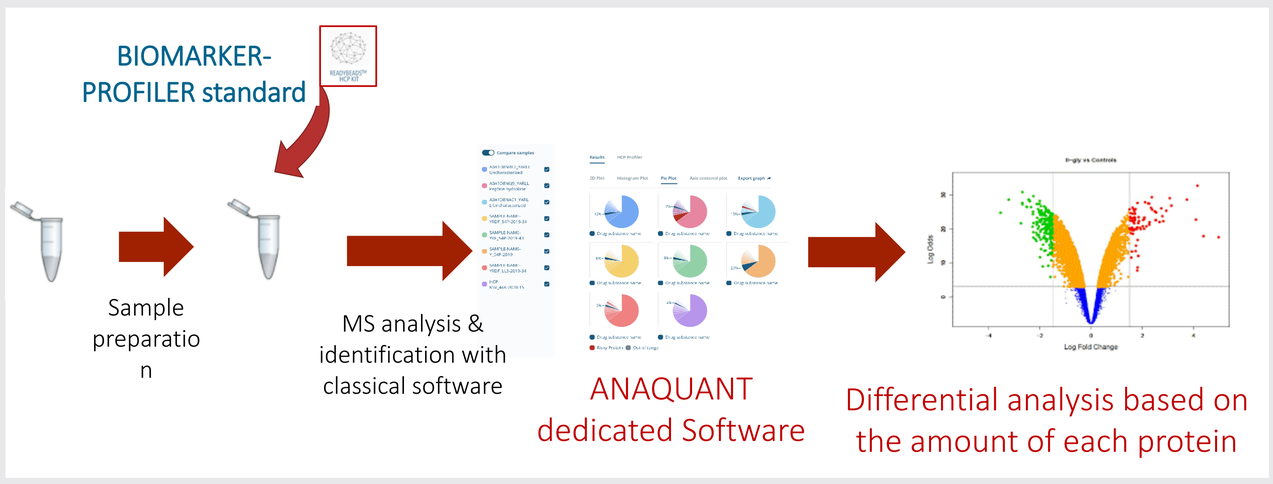

Figure 1: BIOMARKER-PROFILER integrated workflow.

This solution is based on an innovative standard relying on a hydro-soluble bead containing an internal calibration curve including several peptides at well-defined concentration levels; and an integrated software for user-friendly data management and viewing. The standard is added into the sample before MS analysis, and calibration curve acts as a internal normalizer. Consequently, differential analysis can be done, based on concentrations, and candidate biomarkers can be discovered.

EXPERIMENTAL DESIGN

2 keratinocyte strains were compared, the wild one and a genetically modified one. In each group, 6 independent cultures were done, and analyzed according the following workflow.

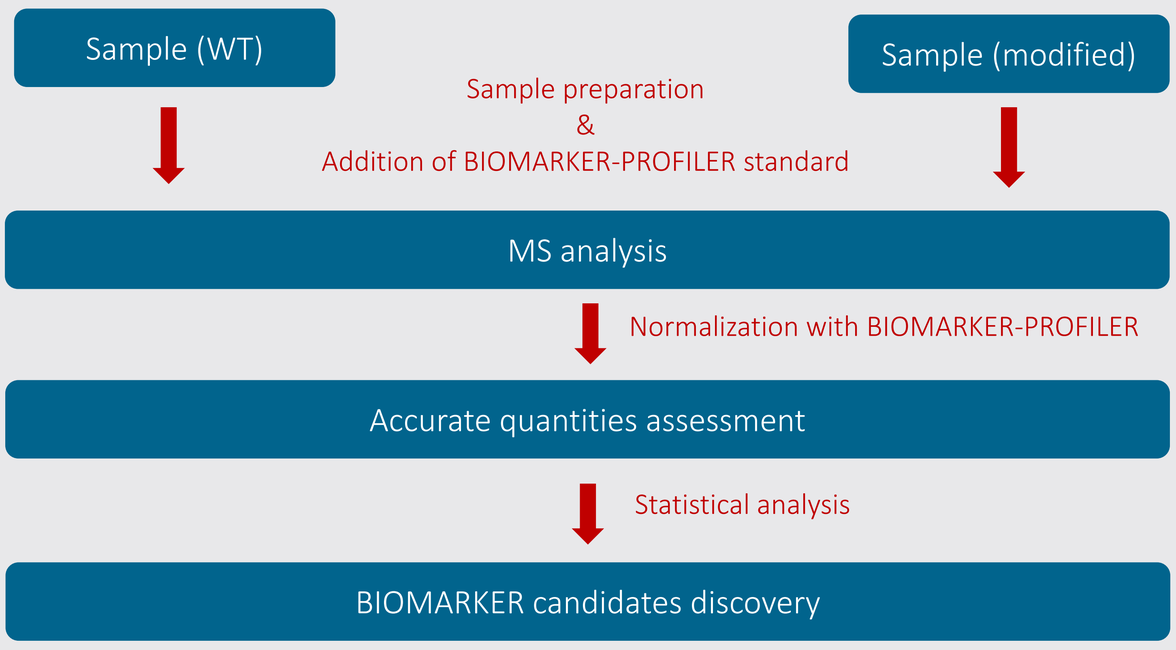

Figure 2: Experimental design done for discovering biomarkers in genetically modified keratinocyte.

EXPERIMENT SUITABILITY

Unlike to classical approaches, BIOMARKER-PROFILER relies on internal calibration curve, which normalize samples in regard of preparation & analytical induced variations; and reflects only biological variations. After this normalization, correlation plot between the 2 strains confirms a similar proteome expression, which ensure that differential analysis makes sense at biological level. Quantification accuracy was tested, CQ was added in each sample at 50 fmol. Over the 12 replicates, the average estimated quantity was found at 43 fmol, with CV calculated at 11%.

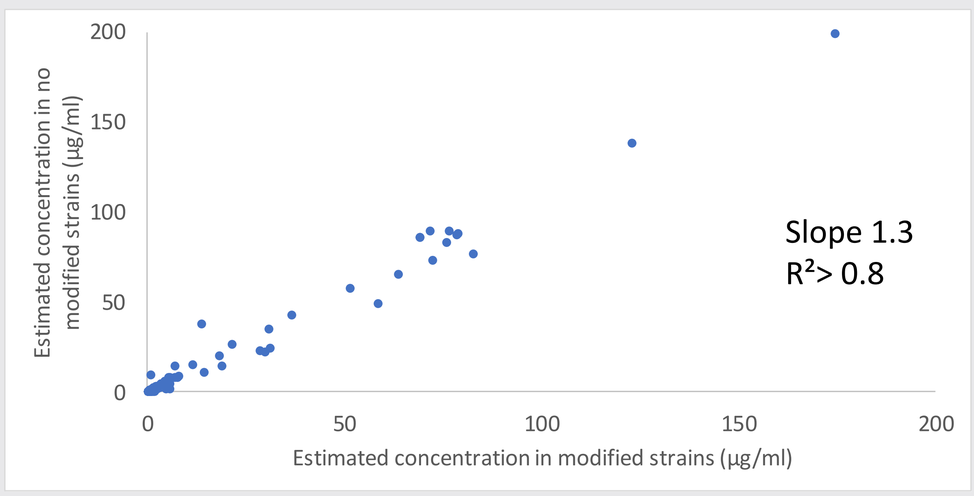

Figure 3: Correlation-plot of 2 keratinocyte strains based on estimated concentrations.

DIFFERENTIAL ANALYSIS

Differential analysis was done based on the estimated quantities.

538 & 483 proteins were quantified in wild and modified strains respectively. 5 proteins were significantly differentially expressed (fold change > 2 and pvalue < 0.05.

CONCLUSIONS

To our knowledge, BIOMARKER-PROFILER is the first analytical approach allowing both, identification & quantities assessment of biomarker candidates. In this study we identify 5 candidates. The knowledge of concentration allows a really faster & easier development of validation assay.