High-resolution nano LC-MS/MS proteomics and CORAVALID™ data processing: For a new approach of substantiation tests.

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Introduction

With more than 5500 full text articles published about it, **skin proteomics** is taking increasing importance for *in vitro* testing, using usually primary fibroblasts or keratinocytes cultures, skin patches, or reconstituted epidermis. **Proteins carry more mechanistic significance with them than genes as they represent a snapshot of the functional state of the cell, and of specific mechanisms or pathways, and integration of various levels of regulation.**

Currently the best tool to perform proteomics is Nano Liquid Chromatography coupled to Tandem Mass Spectrometry (Nano LC-MS/MS). Nano LC allows to use very small sample amounts and to obtain better separation and sensitivity. **Tandem Mass Spectrometry enables non targeted protein identification** through the use of related peptides. Despite its advantages, as an –omics paradigm, MS generates big data, which can usually only be handled by specialists, at the price of important time and resources, potential data losses and results hard to readily use. On the other hand, **good data processing can make the best of MS data**, by showing us what is statistically relevant without biases, revealing additional information that can be directly understood by human minds.

In this study, we used **primary fibroblasts treated by TGF beta** as a key player in proliferation, differentiation and apoptosis.

Material & Methods

Samples from primary cultured fibroblasts exposed to TGF-beta and unexposed control were processed for whole proteome extraction, then passed on a **reverse phase Nano LC system** (nano LC Eksigent) coupled with an **high resolution MS/MS system** (TripleTof 5600 ABSciex). A Mass/charge and retention time library was generated for human peptides based on public data, these peptides were then correlated by our processing algorithm with the experimental peaks generated by label-free quantitative analysis. Sample data were then compared and protein fold changes and t-test values assessed (**Figure N°1**).

Fold changes > 2 or < 0.5 were further analyzed; by using **CORAVALID™** (**Figure N°2**), our expert workflow for transcriptomics and proteomics data processing. It is a complementary analysis of identification and relative quantization data, using specific data from specialized, quality databases (metabolic and signalling pathways, biological or molecular functions, cell components, interactors, orthologs, and so on). It yields condensed and meaningful information.

Results

Results are summarized in **Figure 3, below**.

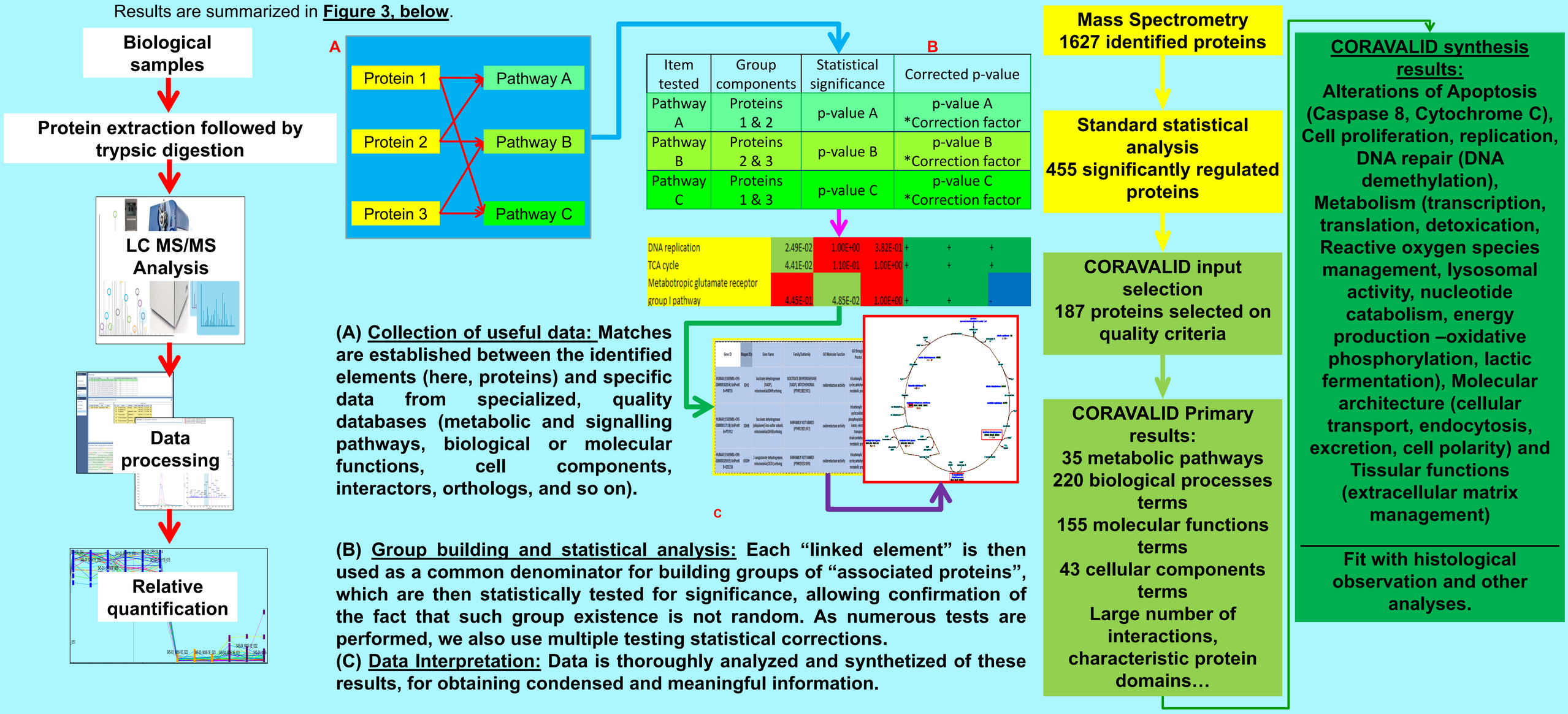

Figure N°1: Nano LC MS/MS Workflow

Figure N°2: CORAVALID™ data processing Workflow

Figure N°3: Results summary

Conclusion

While there were evident discrepancies between some of these observations and literature and between observations themselves, data depth and knowledge of methods allowed us to understand these, showing potential differential cell behavior depending on their environment in the culture, just as it exists physiologically. Our global results were correlated with the histological observations of the cultures at a macroscopic level (cell count, proliferation over time). We were not able to check our hypothesis about the discrepancies with what is usually described as fibroblast reaction to TGF-beta, as we would have needed to follow the culture at a finer scale and over time. However, we were able to **identify a lot of regulators; effectors; signalling and metabolic pathways and define their biological context and repartition more accurately and easily thanks to the method**, while adding clarity and statistical significance to this information, allowing quick integrations of these into current researches.

Proteomics Nano LC- MS/MS coupled with CORAVALID™, our expert data processing workflow, allows for a complete view on the effects on the cell and is a very efficient tool for substantiation tests of active ingredients.

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