Proteome Sciences

Investigation into biomarkers of antidepressant response in a mouse model of depression using isobaric labels (tandem mass tags) and 2DE

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GENDEP

Overview

- GENDEP is an international pharmacogenetic study focussed on the identification of markers of antidepressant response.
- Escitalopram and nortriptyline (study drugs), represent the main classes of antidepressants shown to be effective; although adequate response to a single drug is observed in only 40-70% of patients. Reliable prediction of the clinical response of a patient to a specific antidepressant is therefore vital to the successful treatment of depression.
- Using a mouse model of depression and quantitative proteomics, namely 2DE and Tandem Mass Tags[1] (TMT), we are aiming to gain insight into the molecular mechanisms underlying depression and identify biomarkers of therapeutic response.

Introduction

GENome-based therapeutic drugs for DEPression (GENDEP), a European Framework six funded programme, has three major interconnected themes aimed to identify markers of antidepressant response. The first is a human pharmacogenomic study, involving 19 European centres, which is focussed on the prediction of therapeutic response to antidepressants and adverse effects. The second is a set of basic science studies using animal models and *in vitro* experiments using cell lines. These studies are to investigate drug effect, to gain further insight into the mechanism of action of the study antidepressants and identify biomarkers of response.

Here we present a proteomic study on hippocampi from a mouse model of depression to assess antidepressant (escitalopram and nortriptyline) effect. Complementary approaches were used namely 2DE (protein level) and isobaric mass tagging using TMT (peptide level).

Methods

The study animals comprised 144 groups of mice that covered all combinations of the following factors:

Strains: C57, FVB, 129, DVB

Depression model: Chronic mild stress (CMS), maternal deprivation (MD), environmental control (ENV)

Drug treatment: Escitalopram, nortriptyline, saline

Drug administration: Chronic or acute

Sex: Male (2DE), female (TMT)

Behavioural testing and transcriptomic data were available for all 144 groups of mice and used to select a subset of the groups (to focus on the identification of drug response specific markers) for proteomic analyses.

Proteomic approach

Specifically, proteomic analyses were conducted on 18 distinct groups of mice out of the total of 144 groups. The groups selected comprised combinations of those factors highlighted in red in the previous schematic. Further, male hippocampi were analysed using 2DE (four hippocampi per group; biological replicates) and female hippocampi using TMT (three hippocampi per group).

2DE Analysis A total of 144 2DE gels were run; 18 groups, 4 biological replicates**,** and each sample was analysed in duplicate (technical replicates). 2DE was performed on pH 4-7 (IPG strips) in the first dimension and 24 cm, 10 % acrylamide gels in the second dimension. Proteins were visualised using mass spectrometry-compatible silver stain.

TMT analysis Hippocampal proteins were digested and labeled with TMTsixplex reagents (Figure 1). A total of 54 samples were available for TMT analyses (18 groups, 3 biological replicates). Fourteen sixplex experiments were undertaken, which comprised four experimental samples and two reference samples (pool of all 54 samples) per sixplex (Figure 2). Samples for each sixplex experiment were mixed prior to reversed-phase and SCX purification, lyophilised and analysed by MS (dda, QToF2, Waters) over an 90 min ACN gradient (5-30%).

Data analysis 2DE images were analysed using Progenesis Samespots, reporter ion peak areas (centroid values) were analysed using in-house software, statistical data analysis was performed in SIMCA-P and R.

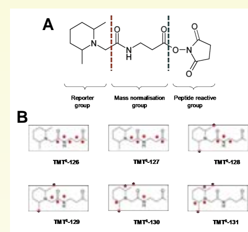

Figure 1 Structure of TMTsixplex reagents. **A** The common structure of TMT reagents showing the reporter group, the mass normalisation group and the protein reactive group. The orange dotted line demarcates the cleavable linker, to generate the reporter ion. The green dotted line demarcates the point of attachment to the protein after the labeling process. **B** The isotopic substitutions (^{13}C, ^{15}N) in each TMTsixplex reagent are indicated by red asterisks.

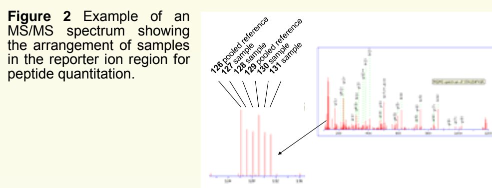

Figure 2 Example of an MS/MS spectrum showing the arrangement of samples in the reporter ion region for peptide quantitation.

Results

__2DE Analysis__ Following image analysis, the 2DE data set contained volume data for 717 spots. Spot volumes were corrected for background and normalised. Pearson correlations for the $\log_{10}$ transformed data from technical replicate gels were computed to assess reproducibility. All replicates had correlation coefficients of ≥ 0.87 and so the replicate values were averaged prior to subsequent analysis.

Principal component analysis (PCA) was used to summarise variance in the data set. This is useful for the detection of technical artefacts (e.g. batch effects) and outlying observations. PCA indicated that there were no obvious issues to be addressed in this data set.

Partial least squares (PLS) modelling was used to relate variation in the 2DE spot volume data set to the experimental treatment (both drug and depression model) and strain data. The one component PLS model was able to explain 23% of variance in the experimental treatment groups (Figure 3) mainly accounting for strain differences.

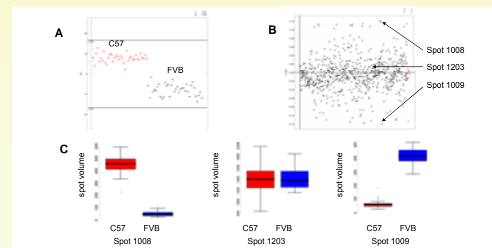

Figure 3 PLS scores (**A**) and weights (**B**) plots for the PLS model of the 2DE data. The scores plot shows clear separation of the C57 and FVB mice. The weights plot shows those spots that most discriminate between the two strains (top – increasing in C57, bottom – increasing in FVB). **C**) box plots summarising the distribution of three selected spots in the two strains C57 (red) and FVB (blue), highlighting the regulation of the spots seen in the weights plot.

__TMT Analysis__ The reporter ions measured in each sample were normalised to constant sum area. Precursors ions were excluded from analysis if the ratios of the two reference samples fell outside the range 0.83 – 1.2. This filter removed many of the precursors with low intensity reporter ions (Figure 4; blue regions). Additionally, reporter ions were excluded if their intensity fell below a minimum threshold of 10 counts (Figure 4; pink region). Peptides were identified using SEQUEST and peptide/protein prophets. The reporter ion ratios (relative to the TMT^6-126 reference) of all precursors assigned to a given protein were averaged. Following these filters, 10,013 protein abundance measurements, describing 381 unique proteins, were taken forward for analysis.

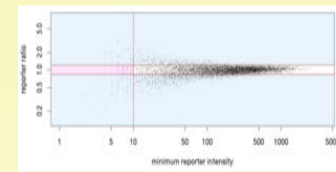

Figure 4 scatter plot showing the relationship between reporter ratio and minimum reporter intensity for the pooled reference samples. Precursors in the blue shaded regions were excluded based on reference ratio. Precursors in the pink shaded region were excluded based on intensity.

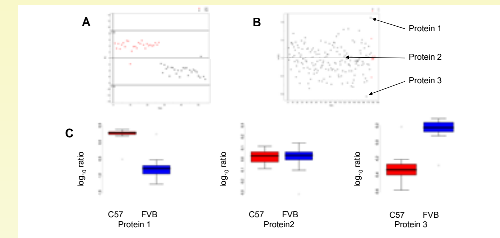

Figure 5 PLS scores (**A**) and weights (**B**) plots for the PLS model of the TMT data. The scores plot shows clear separation of the C57 and FVB mice. The weights plot shows those proteins that most discriminate between the two strains (top – increasing in C57, bottom – increasing in FVB). **C**) box plots summarising the distribution of three selected protein in the two strains C57 (red) and FVB (blue), highlighting the regulation of the proteins seen in the weights plot.

The data set was imported into SIMCA-P and analysed using PCA. All data was suitable for further analysis. PLS was used to relate variation in relative protein abundance to the experimental treatment and strain data. A one component model was able to explain 24% of the variance in the experimental treatments (Figure 5), similar to that found in the 2DE, the variance explained mainly accounted for strain differences.

Conclusions

- Using complementary proteomic approaches both models clearly high-lighted the many strain differences in the data sets.
- More subtle differences pertaining to treatment are being further explored in these proteomic data sets along with protein identification of 2DE spots that best explain the experimental factors.
- The biological significance of these data, in-line with the aims of GENDEP, will be further examined through the integration between these and other proteomic and transcriptomic data sets available across GENDEP.

References

1 Dayon L. *et al.* (2008) Relative quantification of proteins in human cerebrospinal fluids by MS/MS using 6-plex isobaric tags. *Anal Chem.*, 80(8):2921-31.