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# Measurement of Cyp2b1 Protein Induction in Laser Dissected FFPE Liver Samples by Nano-LC Mass Spectrometry

Abstract# 3653

Plummer, Simon M.[1] , Rao, Frank[2], and Currie, Richard[3]

MicroMatrices Associates Ltd, Dundee, UK[1]; Dundee Cell Products Ltd, Dundee, UK[2] and Syngenta Ltd, Bracknell, UK[3];

*Correspondence: simonplummer@micromatrices.com*

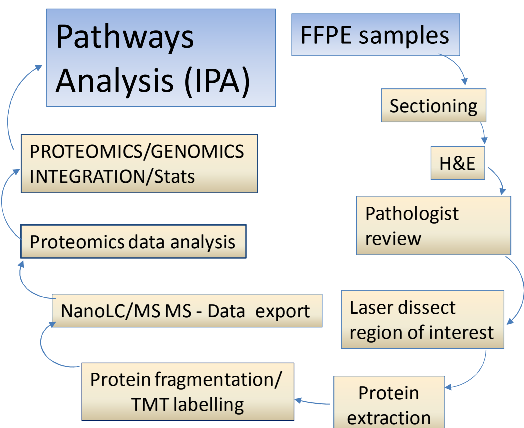
## Summary

Cytochrome p450 (CYP) gene induction is an early associative key event in the mode of action (MOA) of non-genotoxic carcinogens that act via activation of nuclear hormone receptors such as the constitutive androstane receptor (CAR), peroxisome proliferator activated receptor alpha (PPARα) and aryl hydrocarbon receptor (AHR) . Cytochrome p450 (Cyp) enzyme activity and also mRNA induction is used as a surrogate end point for assessing this MOA of putative non-genotoxic carcinogens. However both these end points are indirect indicators of changes to liver protein expression of these Cyps. Cyp protein measurement by Western blotting offers a more direct indicator of the inductive effect of compounds on these Cyps but this method is only semi-quantitative and there are difficulties applying it to formalin fixed paraffin embedded (FFPE) samples. To address this issue we have developed a method for the direct measurement of Cyp protein induction in laser dissected FFPE samples using mass spectrometry (LC MS/MS). We measured Cyp2b1 protein changes (relative to control) in laser dissected FFPE liver sections derived from Wistar rats treated with a known CAR activator – a pyrazole carboximide succinate dehydrogenase inhibitor (SDHI1).

## Methods

Wistar rats were treated with either vehicle control or SDHI1 (3000ppm) in diet for 12m under the auspices of a rat 2 year bioassay. SDHI1, a pyrazole carboximide succinate dehydrogenase inhibitor pesticide, is a Cyp2b1 inducer as reflected by PROD data (JMPR, 2011) .
Proteins were extracted from the laser dissected liver FFPE sections (4 sections) using the Qiagen Q proteome kit. 100 µg of protein was trypsin digested and labelled with Tandem Mass Tag (TMT) six plex reagents according to the manufacturers protocol (Thermo Fisher Scientific) and the samples pooled. An aliquot of the pooled sample was fractionated by high pH reversed-phase chromatography (XBridge BEH C18 Column), evaporated to dryness, resuspended in 1% formic acid and further fractionated using an in line Acclaim PepMap C18 nano-trap column using a Ultimate 3000 liquid chromatography system (Thermo Fisher Scientific) prior to performing LC MS/MS using an Orbitrap Fusion Tribrid mass spectrometer. Fold change values were calculated from raw intensity levels of unambiguously identified peptides that mapped uniquely (UniProt rat database) to Cyp2b1 using Proteome Discoverer software (Thermo Fisher Scientific). Statistical analysis was performed using Students t test.

**Fig 1:** Laser Dissection Targeted Proteomic Profiling Workflow




## Results

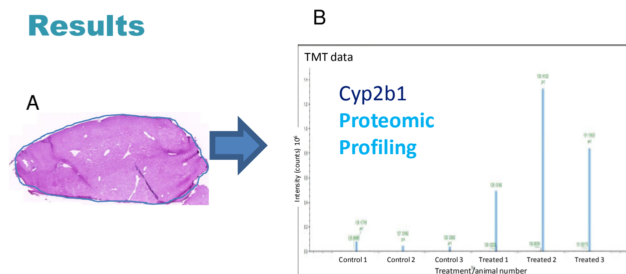
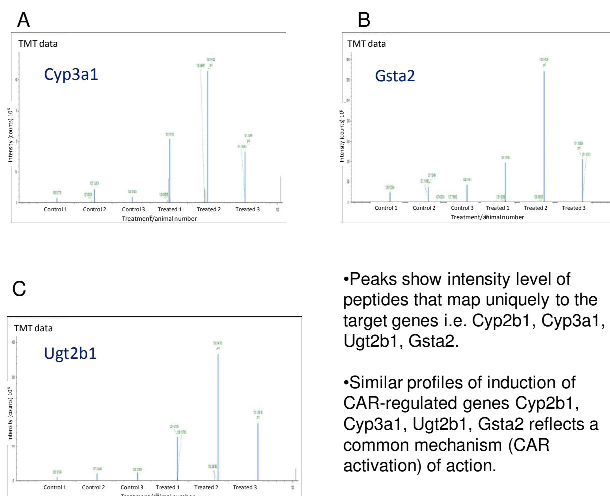



**Fig 2. (A) Wistar rat liver laser dissection. (B) Cyp2b1 intensity level of 3 control and 3 SDHI1-treated Wistar rats**




•Peaks show intensity level of peptides that map uniquely to the target genes i.e. Cyp2b1, Cyp3a1, Ugt2b1, Gsta2.

•Similar profiles of induction of CAR-regulated genes Cyp2b1, Cyp3a1, Ugt2b1, Gsta2 reflects a common mechanism (CAR activation) of action.

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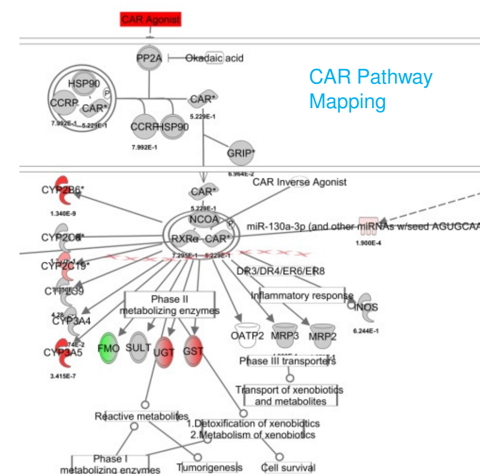
| Gene_Name | UniProt Protein.Group.Accession | Fold.change | P.value |
|---|---|---|---|
| Cyp2b2 | P04167-2;P00176 | 8.556 | 0.02 |
| Cyp2b1 | P00176;P13107;G3V9D2 | 21.235 | 0.02 |
| Cyp3a1 | P04800 | 9.991 | 0.043 |
| Ugt2b1 | P09875 | 11.951 | 0.054 |
| Gsta2 | F7F2H5;P04903 | 5.653 | 0.076 |
| Gstm1 | P04905;P08009;Q5BK56 | 2.754 | 0.087 |

**Fig 3. Intensity levels of (A) Cyp3a1; (B) Gsta2; (C) Ugt2b1 in 3 control and 3 12m SDHI1-teated Wistar rats. (D) Table showing fold change and p value (T test) data for CAR pathway genes.**

**Figure 4. Integrated transcription profiling and proteomic profiling data overlayed on the CAR signalling pathway.**




**Key:** Red nodes = up-regulated genes; green nodes = down-regulated genes

## Conclusions

•The profiles of induction of CAR target genes Cyp2b1, Cyp3a1, Ugt2b1 and Gsta2 are consistent with a common mechanism of induction (CAR activation).

•Integration of mRNA profiling (microarray) and proteomic profiling data gives more accurate indication of the biological response (pathway biomarker) to SDHI1 treatment.

•The effect of SDHI1 on Cyp2b1 protein levels is concordant with biochemical data showing a ~200 fold induction (relative to control) of PROD (7-pentoxyresorufin *O*-dealkylase*)* activity in rat liver microsomes from SDHI1-treated (2000ppm) rat liver (JMPR 2011)

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