CHARACTERISATION OF A PHENOBARBITAL TREATMENT-INDUCED LIVER CELL PROLIFERATION RESPONSE BY INTEGRATED MRNA AND MIRNA PROFILING ANALYSIS IN WILD TYPE, CAR/PXR KNOCK-OUT AND CAR/PXR HUMANISED MICE.

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Abstract# 2919

Introduction

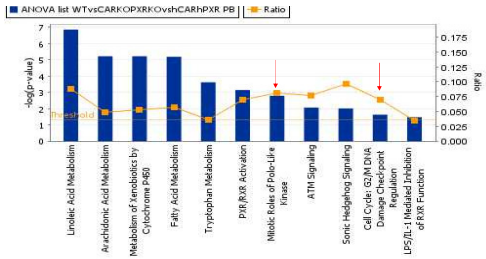
We have utilised integrated miRNA and expression microarray profiling in WT mice, CAR/PXR KO and CAR/PXR humanised TransADMET™ mice to investigate genes and miRNAs that may be involved in the proliferation response of mouse liver to phenobarbital (PB), a prototypical non-genotoxic carcinogen (NGC), in rodents. A cell proliferation response is considered pivotal for rodent liver tumour induction by NGCs, hence characterisation of this response at the molecular level will lead to a better understanding of the mechanisms involved in NGC-mediated liver carcinogenesis. Our previous work showed that only WT mice undergo cell proliferation in response to PB treatment (Ross et al., 2010) – (see treatment regimen below).

Methods

- WT (C57BL6), CAR/PXR KO and hCAR/hPXR mice reated (i.p) with PB (80mg/Kg) for 4 days.
- Livers removed, snap frozen and total RNA containing small RNA's isolated with Qiagen miRNeasy kit..
- Agilent expression/miRNA microarray analysis performed.
- ANOVA analysis performed on expression (mRNA) microarray data from control- and PB-treated WT, CAR/PXR KO and hCAR/hPXR mouse liver.
- Ingenuity Pathways Analysis (IPA) canonical pathways analysis performed on ANOVA ($p<0.01$) expression microarray gene list .
- Integration of mRNA and miRNA differentially expressed gene lists via TargetScan performed using GeneSpring software.
- Gene ontology (GO) function analysis of differentially expressed genes 'targeted' by miRNA's performed in the three different genotypes using OntoExpress and IPA.

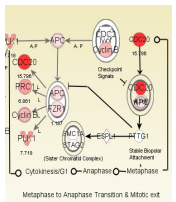
Fig 1: (A) Over-representation of cell proliferation pathways in ANOVA list; (B) Up-regulation of genes in the Polo-like kinase (PLK) pathway in WT but not CAR/PXR KO or hCAR/hPXR mouse liver.

(A)

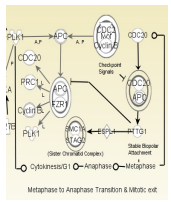


(B)

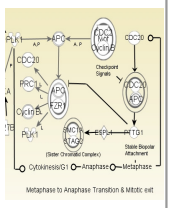
WT PB



CAR/PXR KO PB



hCAR/hPXR PB

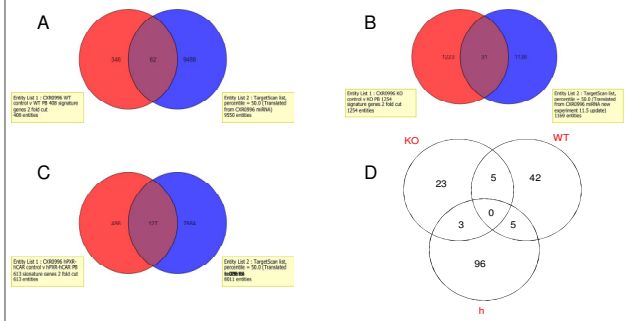


Up-regulated = red down-regulated = green Unchanged = white

Rationale

As the majority of cell proliferation-associated genes were up-regulated in the expression microarray data, we focussed on down-regulated miRNA species linked via TargetScan to significantly altered mRNA/genes.

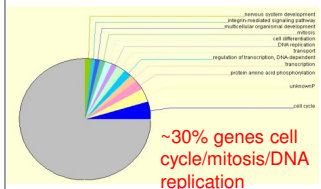
Fig 2: PB-induced differentially expressed genes TargetScan-linked with down-regulated miRNA's in (A) wild type (62); (B) CAR/PXR KO (32); (C) hCARhPXR (127) mice; (D) Venn analysis of the miRNA-targeted genes in the three mouse strains



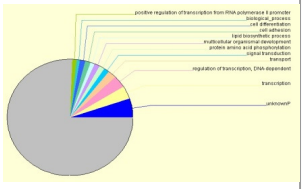
Genes 'targeted' by miRNAs were mostly different /unique in each genotype (panel D)

Fig 3: Gene Ontology Pie Charts for genes significantly altered by PB treatment that were 'targetscan linked' with significantly down-regulated miRNAs in WT; hCAR/hPXR and CAR/PXRKO mice

(WT)



(hCARhPXR)



(CAR/PXRKO)

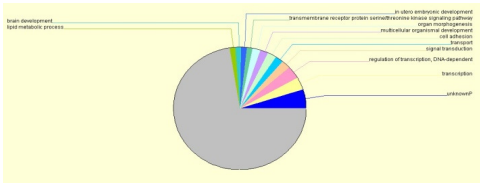


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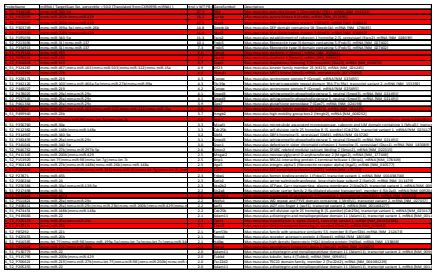
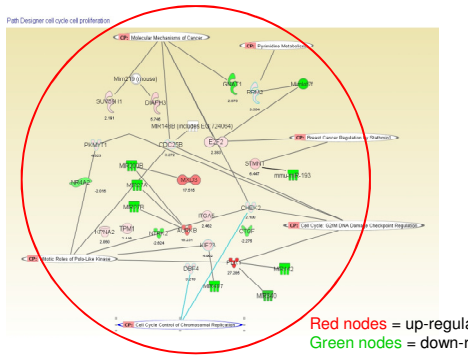
Table 1: PB-induced expression changes to genes in WT liver 'targeted' by down-regulated miRNAs
red = cell proliferation genes (~30%)



Conclusions

1. The use of transgenic mouse strains and pathways analysis tools as 'biological' filters of genomics data is useful for identification of genes potentially involved in the liver proliferation response to PB.
2. Integration of mRNA and miRNA data in the context of these TransADMET™ mouse models provides new insights into potential mechanisms of NGC - in particular indicating that miRNAs may 'orchestrate' a PB-induced cell proliferation response via CAR and PXR in WT mice.
3. These insights into mechanisms of non-genotoxic carcinogenesis have the potential to yield biomarkers useful as mode of action (MOA) 'classifiers' for non-genotoxic carcinogens (NGC's) in rodents.

Fig 4: Cell proliferation Pathways Up regulated by PB in WT and 'targetscan-linked' with down-regulated miRNAs



Red nodes = up-regulated
Green nodes = down-regulated