

LIVER mRNA & miRNA PROFILING OF PHENOBARBITAL-TREATED PXR/CAR DOUBLE KNOCK-OUT & HUMANISED MICE PROVIDES INSIGHT INTO MECHANISM(S) OF PB-MEDIATED MOUSE HEPATOCARCINOGENESIS.

Elcombe, Clifford R.¹; Dhritiman, Dan¹; Wolf, C Roland¹; Scheer, Nico² and Plummer, Simon M.¹.

CXR Biosciences Ltd, Dundee, Scotland¹ and TaconicArtemis GmbH, Köln, Germany².

Introduction

Previously, phenobarbital (PB)-treatment increased hepatocyte proliferation in C57BL/6J (WT) mice but not in hPXR/hCAR or PXRKO/CARKO mice (Elcombe *et al.*, 2009). To determine whether cell proliferation and potentially procarcinogenic pathways were differentially regulated by PB in the three mouse lines we performed mRNA and miRNA array analysis on total RNA extracted from livers of vehicle control or PB-treated WT, CARKOPXRKO and hCAR/ hPXR mice.

Liver mRNA and miRNA expression profiles of PB-treated WT, PXRKO/CARKO and hPXR/hCAR mice (relative to vehicle treated controls) were assessed using Agilent whole mouse genome expression microarrays and Agilent mouse miRNA arrays, respectively. Rosetta Resolver™ software was used to generate 'signature' lists of significantly ($p < 0.01$) altered mRNA and miRNA genes for each mouse line. Resolver™ ANOVA analysis was used to identify PB treatment-induced mRNA gene alterations (PB ANOVA list) that were most variable across the WT, knock out (KO) and humanised treatment groups. Ingenuity Pathways Analysis™ (IPA) software was used to identify overrepresented pathways relevant to cell proliferation, in the PB ANOVA list. Effects of the PXRKO/CARKO and humanisation in cell proliferation pathways were assessed by examining the polarity of RNA expression changes to genes contained in proliferation-relevant pathways over-represented in the ANOVA list relative to their representation in those pathways in the IPA database.

Resolver™ trends analysis was used to identify miRNAs that were altered in a reciprocal fashion relative to cell proliferation-associated mRNA genes. Homology between the miRNAs identified by this process and the 3' UTR region of the cell proliferation genes was assessed using TargetScan software and the EBI 'Emboss' alignment tool.

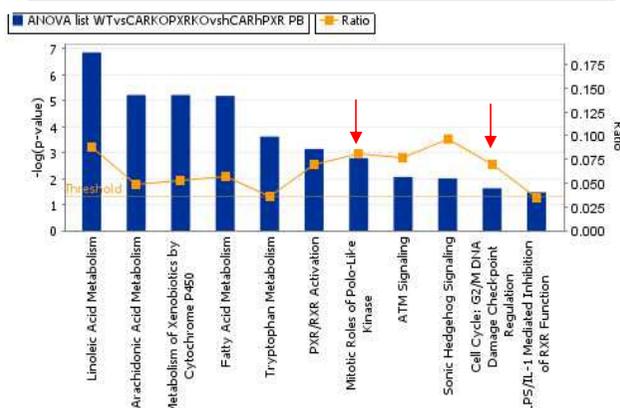
Questions

- Are there differences between PB-treated WT and PXRKO/CARKO or hPXR/hCAR mice in the content of mRNA 'signature' lists with regard to genes involved in cell proliferation?
- Do the polarities of change to mRNAs and miRNAs suggest differential activation of cell proliferation pathways in part via miRNA-mediated regulation?
- What are the mRNAs potentially targeted by miRNAs in the differentially regulated cell proliferation pathways?

Experimental Outline

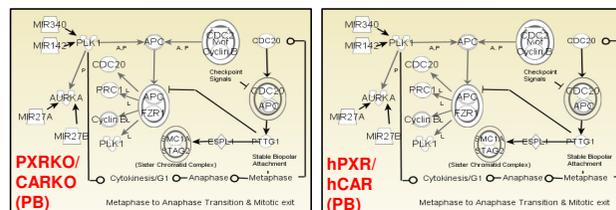
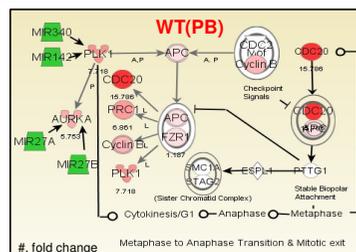
- WT, PXRKO/CARKO or hPXR/hCAR mice treated with four daily doses of PB (80 mg/kg, i.p.) or vehicle control (saline).
- Total RNA isolated from vehicle- or PB-treated WT, PXRKO/CARKO, hPXR/hCAR mouse liver and (a) mRNA labelled with cyanine 3 (Cy3) nucleotides using an Agilent low input RNA labeling kit or (b) miRNA end labelled using the Agilent miRNA complete labeling and hybridisation kit.
- Labelled mRNA and miRNA samples hybridised on Agilent mouse whole genome or Agilent mouse miRNA oligo microarrays in one colour experiments.

Fig. 1: Over-representation of cell proliferation-relevant pathways



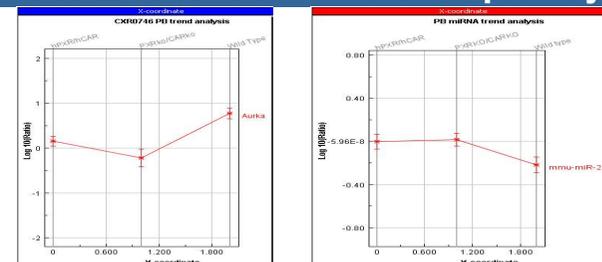
IPA canonical pathways analysis identified two pathways relevant to cell proliferation, namely mitotic roles of polo-like kinase (PLK1) and cell cycle G2/M DNA damage check point regulation, that were over-represented in the PB ANOVA list.

Fig. 2: Polo-like kinase (PLK1) pathway only upregulated in PB-treated WT mice



Genes involved in regulation of mitosis by PLK1 that were over-represented in the PB ANOVA list. Examination of the polarity of gene expression changes caused by PB treatment relative to the PLK1 pathway indicated that this pathway was selectively up-regulated in WT but not in PXRKO/CARKO or hPXR/hCAR mice. Red nodes, up-regulated; green nodes, down-regulated.

Fig. 3: Four miRNA species were reciprocally altered relative to PLK1 /AURKA pathway



Resolver™ trends analysis identified four miRNA species that were altered in a reciprocal fashion relative to the PLK1/AURKA pathway, namely mmu-miR-340-5p and mmu-miR142-5p (PLK1) and mmu-miR27A and mmu-miR27B (AURKA).

Fig. 4: miRNA homology to the 3' UTR region of the PLK1 gene

	predicted consequential pairing of target region (top) and miRNA (bottom)
Position 255-261 of Plk1 3' UTR	5' ... CUGUCUUCUUCUUAAGCUUUUAU ...
mmu-miR-340-5p	3' ... UUAAGUCAGAGUAAAGAAUAUU ...
Position 255-261 of Plk1 3' UTR	5' ... CUGUCUUCUUCUUAAGCUUUUAU ...
mmu-miR-142-5p	3' ... UCACUACGAAAGAU---GAAUAUC ...

Targetscan software analysis indicated that these miRNA species had significant homology to the 3' UTR regions of the PLK1 gene.

Conclusions

- ANOVA analysis of PB-induced mRNA gene expression identified a list of genes that was most variable across the treatment groups (WT vs. PXRKO/CARKO vs. hPXR/hCAR mice).
- IPA representation analysis of the PB ANOVA list indicated that cell proliferation pathways regulated by polo-like kinase (PLK1) and G2/M DNA damage checkpoint genes were over-represented in the list.
- The polarity of mRNA gene expression changes in the PLK1 signalling pathway indicated that this pathway was induced (activated) by PB treatment in WT but not PXRKO/CARKO or hPXR/hCAR mouse liver.
- Resolver™ trends analysis of PB-induced miRNA expression changes indicated that mmu-miR-340, mmu-miR-142-5p, mmu-miR27b and mmu-miR27c could be involved in mediating the PB-induced changes to mRNAs in the PLK1 signalling pathway.
- The data indicate that PB-treatment induces PXR/CAR-mediated induction of the PLK1 pathway in liver which is associated with alterations to miRNAs targeting this pathway and suggests that mouse but not human isoforms of these receptors are involved in this effect.

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