

Transcript Profiling of PXR and CAR Knock-out and Humanized Mouse Liver Provides Insight into Endogenous Roles of the Human PXR/CAR Isoforms.

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Pathways Analysis PXRKO and hPXR

Introduction

To investigate PXR- and CAR-regulated endogenous and species specific functions we performed transcript profiling and pathways analysis on RNA extracted from the livers of mice in which the murine pregnane X receptor (PXR) and constitutive androstane receptor (CAR) genes were removed (PXR KO, CAR KO mice) and/or replaced with human PXR or CAR (hPXR, hCAR mice). Liver RNA expression profiles of these mouse lines, relative to C57BL/6J wild type (WT) mouse liver, were assessed using Agilent whole mouse genome expression microarrays. Rosetta Resolver™ software was used to generate a 'signature' list of significantly ($p < 0.01$) altered genes for each mouse line. Ingenuity Pathways Analysis™ (IPA) software, was used to examine differences between the knock out (KO) and humanised lines in pathways regulated by these receptors. Effects of the PXR/CAR KO's and humanisations in classical and functional pathways were assessed by examining the polarity of RNA expression changes to genes contained in pathways that were over-represented in the signature lists relative to their representation in those pathways in the IPA database.

Questions?

- How does the polarity of gene expression alterations, relative to WT, in PXRKO vs hPXR and CARKO vs hCAR mouse liver compare in pathways commonly affected in these models?
- What genes/pathways are uniquely altered in PXRKO and hPXR liver and CARKO or hCAR liver?
- Does investigation of gene/pathway alterations in PXRKO, CARKO or hPXR, hCAR mouse liver, relative to WT mouse liver, offer insight for understanding endogenous roles for these receptors?
- What is the toxicological relevance of gene/pathway alterations in the liver of these KO and transgenic mouse models?

Experimental outline

- RNA isolated from WT, PXRKO, hPXR, CARKO and hCAR mouse liver and labeled with either cyanine 3 (Cy3) or cyanine 5 (Cy5) nucleotides using an Agilent low input RNA labeling kit.
- Test RNA (PXRKO-, hPXR-, CARKO- and hCAR) hybridised against a reference of WT liver RNA on Agilent mouse whole genome 60mer oligo microarrays in a two colour experiment.
- Rosetta Resolver™ software used to identify a signature list of genes significantly ($P < 0.01$) altered, relative to WT, against a set of experimentally derived 'house-keeping' genes.
- Signature lists examined using IPA software.
- Venn diagram analysis used to focus IPA analysis on gene/pathway alterations common or unique to the signature lists for each KO and humanised model.
- Representation of genes in these lists assessed in IPA using a one-tailed Fishers exact test.
- Genes from significantly over-represented functional categories loaded into pathway diagrams and overlaid with fold change values to assess the polarity and biological implications of the changes.

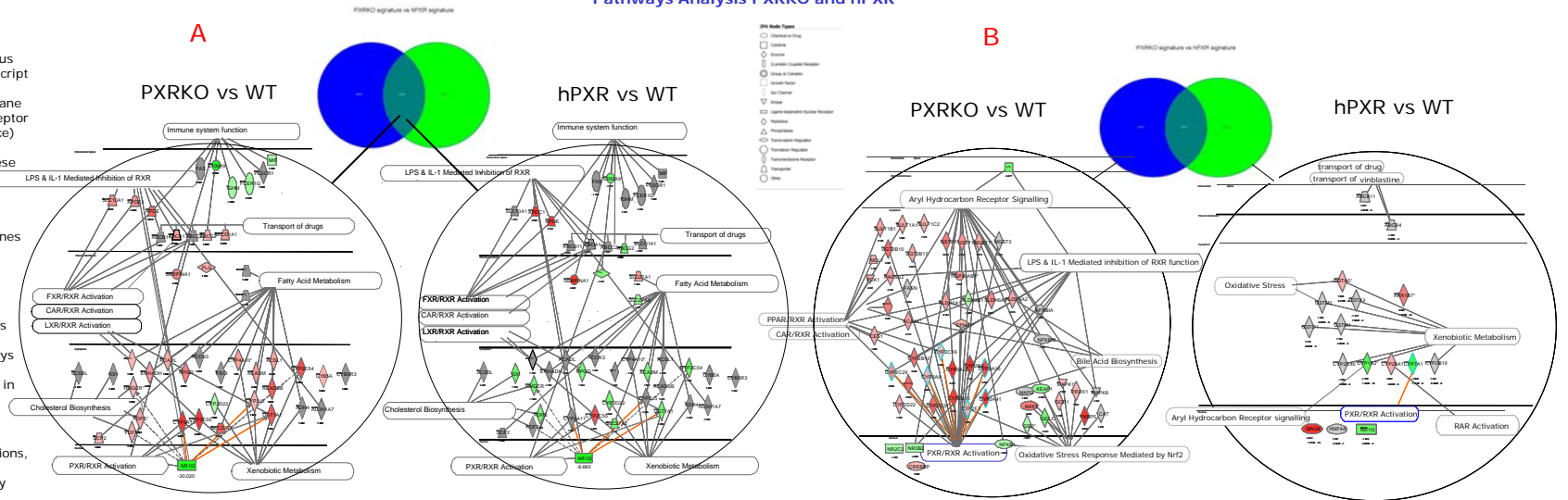


Figure 1. (A) Network of genes overrepresented in the intersect between the hPXR and PXRKO signature gene lists; (B) Networks of gene uniquely altered in PXRKO and hPXR mouse liver. Red nodes= up-regulated; green nodes= down-regulated; Grey nodes: genes that were altered by < 1.5 fold. Grey lines indicate genes associated with a particular functional category

Gene/ID	Name	Sequence Description	CAR PXR Fold Change	CAR PXR Fold Change
Lipid Metabolism				
NM_025797	CYP5B	Cytochrome P450 family 5, subfamily B, polypeptide 55	-2.3	1.7
NM_025806	CYP5C2	Cytochrome P450 family 5, subfamily C, polypeptide 55	-2.3	1.7
NM_025837	CYP5C4	Cytochrome P450 family 5, subfamily C, polypeptide 54	-1.2	4.0
NM_019494	CYP5C11	Cytochrome P450 family 5, subfamily C, polypeptide 52	-1.2	4.0
NM_019507	CYP5C12	Cytochrome P450 family 5, subfamily C, polypeptide 53	-1.2	4.0
NM_020411	CYP5C11	Cytochrome P450 family 5, subfamily C, polypeptide 11	-1.2	4.0
NP_177876	CYP5C10	Cytochrome P450 family 5, subfamily C, polypeptide 10	-1.2	4.0
NM_020555	HNF1P	Hepatocyte nuclear factor 1, group 1, member 2	-1.2	4.0
NP_201246	HNF1A1	HNF1 hepatocyte nuclear factor 1, group 1, polypeptide 41	-1.7	3.4
Other transport				
NM_015454	MRKX1	MRKX protein expressed, subfamily A, member 1	-2.0	2.0
NM_015500	MRKX2	MRKX protein expressed, subfamily A, member 2	-1.7	1.0
Fatty acid/lipid metabolism				
NM_021018	ACOX1	Acyl-Coenzyme A oxidase/Coenzyme A oxidase	-1.2	1.2
NM_007382	ACOX10	Acyl-Coenzyme A oxidase/Coenzyme A oxidase, C-4 to C-12 straight chain	-1.4	2.3
NM_020528	ACOX10B	Acyl-Coenzyme A oxidase/Coenzyme A oxidase, straight chain	-1.2	3.7
NM_019729	ACOX11	Acyl-Coenzyme A oxidase 1, gamma	-1.2	1.7
BC009689	ACOX11	Acyl-Coenzyme A oxidase 1, gamma	-1.2	1.7
NM_021737	ACOX12H1	Acyl-Coenzyme A oxidase/Coenzyme A oxidase, gamma	-1.2	1.7
NM_019139	ACOX12	Acyl-Coenzyme A oxidase/Coenzyme A oxidase, gamma	-1.2	1.7
Cholesterol biosynthesis				
NM_020010	ERG1	Ergosterol 14-reductase, C-14 sterol 14-reductase 1	-2.3	2.3
NM_019131	ERG11	Ergosterol 14-reductase, long-chain sterol 14-reductase 1	-1.2	1.8
NM_134650	ERG2	Ergosterol 14-reductase, gamma	-1.2	1.8
NP_207766	ERG2R	Ergosterol 14-reductase, gamma	-1.2	1.8
NM_117660	ERG3	Ergosterol 14-reductase, delta	-1.2	1.8
Immune response				
NM_021067	IFIT1	Interferon induced protein 1, member 1	-1.2	1.8
NM_021068	IFIT2	Interferon induced protein 2, member 2	-1.2	1.8
NM_021069	IFIT3	Interferon induced protein 3, member 3	-1.2	1.8
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