

THE EFFECTS OF AMMONIUM PERFLUOROCTANOATE (APFO) ON THE TRANSCRIPTIONAL PROFILE OF PANCREAS AND LIVER OF MALE RATS

S. Plummer¹; O. Vassieva¹; C. Elcombe¹

¹ CXR Biosciences Ltd, Dundee, United Kingdom.

Introduction

Ammonium perfluorooctanoate (APFO) is used in the emulsion polymerisation of fluoropolymers. Two-year carcinogenicity studies in rats have shown an increased incidence of liver, pancreatic (acinar cell) and testicular (Leydig cell) tumours (1). APFO is a peroxisome proliferator activated receptor (PPAR) ligand and previous studies have shown that several agents that fall into this class induce a characteristic profile of liver, pancreatic and testicular tumours. The mechanism(s) of pancreatic carcinogenesis induced by APFO poorly understood but evidence suggests that direct genotoxicity is not involved. This study was designed to determine the profile of gene expression changes taking place in the pancreas (and liver) when rats are exposed to APFO in the diet in order to gain insight into the potential mechanisms of carcinogenicity in this organ.

Study design

6 control rats and 6 rats were fed PPC (300ppm) in the diet for 28 days. The pancreas and liver were removed into RNA later. RNA was extracted with Tri-Reagent and RNeasy columns. RT synthesis of Cy5 (red) and Cy3 (green) labelled cDNA from test and control RNA, respectively. The labelled cDNAs were combined and hybridised on Agilent rat cDNA arrays containing 14,841 rat genes. RNA from three individual rats were analysed in duplicate in order to incorporate a 'dye swap', Figure 1.

The arrays were scanned on an Agilent microarray scanner and data extracted using Agilent Feature Extraction software. Tertiary analysis of the data to generate a 'signature' gene list derived from the combined data from six arrays was performed using Rosetta Resolver software. Functional annotation of the 'regulated' genes (i.e. genes for which there was a significant alteration in the amount of expression relative to the control (reference RNA derived from untreated rat pancreas/liver) was performed using Dragon Annotate software.

Supervised clustering analysis was used to define pathways that were significantly affected by the treatment. These pathways were used to formulate a hypothesis to explain the carcinogenic effects of the compound in pancreas.

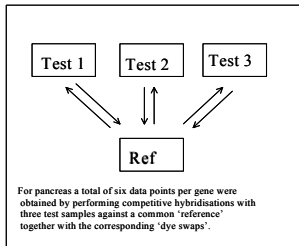


Figure 1. Structure of microarray experiment.

Liver profile

There was significant induction of a battery of genes regulated by PPAR α , including several genes involved in peroxisomal β oxidation and proliferation e.g. **Acyl-CoA-oxidase** (~10 fold), **peroxisomal membrane protein** (~10 fold) and **Cyp IVA2/3** (~50 fold). There was a Down-regulation of genes associated with lipid trafficking e.g. **Apolipoproteins (C-IV, AII, B)**

Table 3. Differentially expressed genes in liver in response to APFO treatment of male SD rats. Table includes gene categories discussed in the report. Genes are grouped by functional category. The absolute expression intensity (AEI) was used to calculate the fold changes. The 'P' value is calculated using a cumulative error model developed for Agilent cDNA arrays using Rosetta Resolver software. The rank value (RV= fold change*(max AEI-minAEI)) is used to assign 'importance' to the gene changes. There is a higher probability of gene changes being positive in subsequent QRT-PCR analysis if the rank value is high (Muth et al 2002)

Category	Gene	Rank value	Function		
lipid metabolism	U20033	17.8	1.92E-32	25274	acyl-CoA oxidase
	AJ224120	11.2	0	84127	peroxisomal membrane protein
	AF180801	11.0	3.96E-35	33724	peroxisomal membrane protein
	K02782	10.5	0	421660	acyl-CoA oxidase
	K02763	10.1	0	22555	acyl-CoA oxidase
	AB010428	7.6	5.16E-17	225730	acyl-CoA oxidase
	D00689	6.6	5.48E-20	208055	acyl-CoA oxidase
	K03248	5.8	0	89496	acyl-CoA oxidase
	AF044674	3.9	7.11E-10	41231	acyl-CoA oxidase
	AF200367	3.3	6.82E-18	34858	acyl-CoA oxidase
glutamate metabolism	K65083	2.8	3.67E-10	1606	glutamate decarboxylase
	M57719	46.1	0	2021556	glutamate decarboxylase
	K43308	35.1	0	285579	glutamate decarboxylase
	D43623	6.5	7.32E-34	10273	glutamate decarboxylase
	AJ007704	2.4	1.92E-04	6795	glutamate decarboxylase
	L07736	1.5	1.40E-05	993	glutamate decarboxylase
	X97831	3.1	3.46E-09	20609	glutamate decarboxylase
	D17895	12.7	6.17E-31	27912	glutamate decarboxylase
	AJ001118	5.9	8.61E-10	18686	glutamate decarboxylase
	AF228917	4.9	7.24E-15	2755	glutamate decarboxylase
mitochondrial	L03294	4.1	2.16E-04	6747	mitochondrial
	AF113914	4.1	2.21E-35	13228	mitochondrial
	K08100	2.7	5.69E-06	145339	mitochondrial
	M14201	2.7	9.18E-25	145530	mitochondrial
	K03488	-1.6	0.01	95	mitochondrial
	J12888	-1.9	4.74E-04	7877	mitochondrial
	M13508	-13.1	6.42E-06	18058	mitochondrial
	AJ005046	4.6	8.72E-10	2469	mitochondrial
	K03243	-2.2	0	-630	mitochondrial
	D03834	2.8	5.00E-15	15977	mitochondrial
D12158	1.5	7.93E-05	469	mitochondrial	
X03430	-3.0	9.06E-41	-6503	mitochondrial	

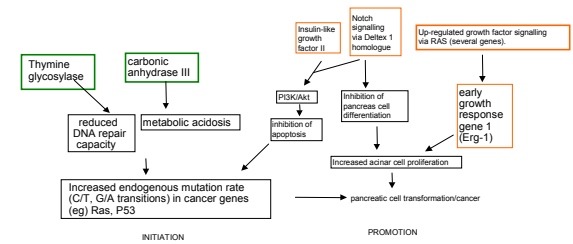
Pancreas profile

There was an absence of PPAR α regulated genes. Up-regulation of genes associated with gluconeogenesis and glutamate metabolism was seen. e.g. **Phosphoenolpyruvate carboxykinase** (up~ 6 fold), **Glutamine aminohydrolase**, **glutamate decarboxylase** (up~2fold). There was up-regulation of genes associated with cell proliferation and inhibition of apoptosis e.g. **Early growth response gene 1**, **RAS/MAPKinase signalling**, **notch signalling**, **Insulin-like growth factor II**. Down-regulation of **carbonic anhydrase** (~ 4 fold) and **thymine glycosylase** was seen.

Table 2. Differentially expressed genes in pancreas in response to APFO treatment of male SD rats. Table includes gene categories discussed in the manuscript. Genes are grouped by functional category. The absolute expression intensity (AEI) was used to calculate the fold changes. The 'P' value is calculated using a cumulative error model developed for Agilent cDNA arrays using Rosetta Resolver software. The rank value (RV= fold change*(max AEI-minAEI)) is used to assign 'importance' to the gene changes.

Category	GeneBank	Description	log2 change	P value	Rank value	Function
lipid metabolism	L0292	Acyl-CoA oxidase	-1.05	4.41E-07	-123	acyl-CoA oxidase
	D00100	Acyl-CoA oxidase	-1.3	0	-75	acyl-CoA oxidase
	X03415	Acyl-CoA oxidase	-1.54	2.46E-08	-370	acyl-CoA oxidase
	AJ132130	Acyl-CoA oxidase	-1.19	0.01	-113	acyl-CoA oxidase
	L02934	Acyl-CoA oxidase	1.41	4.67E-04	383	acyl-CoA oxidase
	M16767	Acyl-CoA oxidase	1.68	0	334	acyl-CoA oxidase
	K05489	Acyl-CoA oxidase	2.15	1.46E-22	1122	acyl-CoA oxidase
	D29773	Acyl-CoA oxidase	1.44	0	152	acyl-CoA oxidase
	D11548	Acyl-CoA oxidase	1.5	0	123	acyl-CoA oxidase
	AF01890	Acyl-CoA oxidase	-1.47	2.19E-04	-169	acyl-CoA oxidase
glutamate metabolism	L04277	Glutamate decarboxylase	1.93	1.64E-02	93	glutamate decarboxylase
	L15193	Glutamate decarboxylase	1.3	0	84	glutamate decarboxylase
	X57405	Glutamate decarboxylase	1.48	0	234	glutamate decarboxylase
	L18822	Glutamate decarboxylase	1.44	0	183	glutamate decarboxylase
	L57439	Glutamate decarboxylase	1.21	3.00E-04	104	glutamate decarboxylase
	AF038333	Glutamate decarboxylase	-1.21	0	-276	glutamate decarboxylase
	M14256	Glutamate decarboxylase	-1.25	0.01	-275	glutamate decarboxylase
	M88804	Glutamate decarboxylase	-1.13	0.01	-137	glutamate decarboxylase
	X07286	Glutamate decarboxylase	-1.33	6.94E-04	-123	glutamate decarboxylase
	M18416	Glutamate decarboxylase	6.5	1.26E-12	1100	glutamate decarboxylase
mitochondrial	L04277	Glutamate decarboxylase	2.35	3.89E-18	202	glutamate decarboxylase
	L10483	Glutamate decarboxylase	2.34	4.33E-08	163	glutamate decarboxylase
	X18334	Glutamate decarboxylase	1.93	8.29E-04	553	glutamate decarboxylase
	AF104223	Glutamate decarboxylase	1.48	1.00E-04	233	glutamate decarboxylase
	M8895	Glutamate decarboxylase	1.34	3.29E-07	189	glutamate decarboxylase
	M89933	Glutamate decarboxylase	1.33	0	131	glutamate decarboxylase
	AF006510	Glutamate decarboxylase	-1.17	7.89E-04	-213	glutamate decarboxylase
	L04333	Glutamate decarboxylase	-1.59	0.01	-145	glutamate decarboxylase
	AF022447	Glutamate decarboxylase	2.33	1.19E-14	9930	glutamate decarboxylase
	AF003239	Glutamate decarboxylase	1.78	3.19E-14	2903	glutamate decarboxylase
cellular processes	L00036	Early growth response gene 1	1.46	1.51E-02	377	early growth response gene 1
	L00343	Early growth response gene 1	5.75	5.72E-28	2003	early growth response gene 1

Discussion and Conclusions



Postulated mechanism of APFO carcinogenesis in the pancreas.

Summary of cancer hypotheses with gene targets:

- Increased endogenous mutation rate through hydrolytic deamination of 5-methyl cytosine caused by metabolic acidosis (**carbonic anhydrase**) and decreased efficiency of G/T mismatch repair (**thymine glycosylase**).
- Increased rate of cell proliferation through growth factor signalling via Ras/MAP kinase pathway (**Insulin-like growth factor II**, **ets related protein 81**, **Farnesyltransferase**, **EGR1**).
- Decreased rate of apoptosis and differentiation through induction of Notch signalling pathway (**R.rattus mRNA homologue of Drosophila Notch protein**, **deltex 1 homologue**).

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