

Comparison of Gene Expression Changes in Pancreatic Acinar Cells of Rats Fed Diets Containing Wy14,643, or Ammonium Perfluorooctanoate (APFO).

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Introduction

Two-year carcinogenicity studies in rats with ammonium perfluorooctanoate (APFO) have shown an increased incidence of liver, pancreatic (acinar cell) and testicular (Leydig cell) tumours. Our previous transcription profiling (TP) studies in whole pancreas of rats treated with APFO (300ppm), procarcinogenic and non-carcinogenic doses of Wy14,643 (Wy) (50ppm and 20ppm, respectively) or the non-carcinogen di(2-ethylhexyl)phthalate (DEHP) (12000ppm) in the diet for 1, 7 or 28 days have identified carcinogenesis-associated gene expression changes with these PPAR alpha agonists leading to formulation of a hypothesis for pancreatic carcinogenesis (Figure 1 and Plummer *et al* 2005). In order to assess whether or not the procarcinogenic gene expression changes are taking place in 'target' cells we have performed TP analysis in purified acinar cells from rats treated with APFO (300ppm) or Wy (50ppm) for 1, 7 or 28 days.

Study Design

Groups of rats (n = 5) were fed diets containing either APFO (300ppm), Wy (50ppm) or control diet for 1, 7 or 28 days. The pancreas was removed and acinar cells isolated by a method involving collagenase digestion and centrifugation through BSA (4%). Purified acinar cells were lysed with Tri-Reagent and snap frozen. RNA was purified on RNeasyTM columns, labelled with Cy5 and Cy3 and hybridised against labelled RNA (Cy3 or Cy5) pooled from controls on Agilent Whole Rat Genome oligo microarrays. Hybridisations were made with RNA from 3 different rats and included 'dye swap' replicates to control for dye bias.

Expression ratio (log ratio) values from the individual arrays were combined to make an error weighted mean (n=5) and 'signature' gene lists of significantly (P<0.01) altered genes generated using Rosetta LuminatorTM software. The compare biosets function of Luminator was used to make Venn diagrams identifying genes that were either unique or common to the two treatments, (Figure 3). Trend plots showing changes in expression of genes across time (1, 7 and 28 days) were constructed in Luminator (Figure 4).

Treatment-induced changes in gene expression in whole pancreas were compared with those in isolated acinar cells.

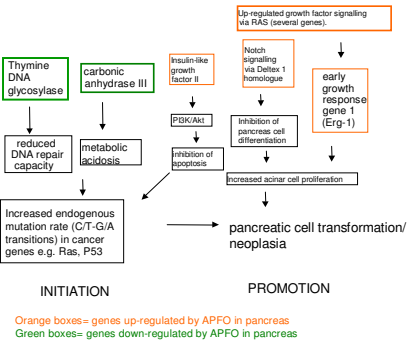


Figure 1. Postulated mechanism of APFO carcinogenesis in the pancreas.

Results

Immunohistochemistry of fixed smears of isolated acinar cells, using α -amylase (acinar cell specific) or cytokeratin 19 (duct cell-specific) antibodies, showed that the isolated cell preparations contained greater than 90% acinar cells (Figure 2).

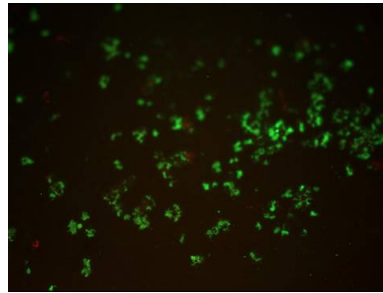


Figure 2. Immunostaining of isolated rat pancreas acinar cells. Acinar cell are green (FITC), duct cells are red (rhodamine).

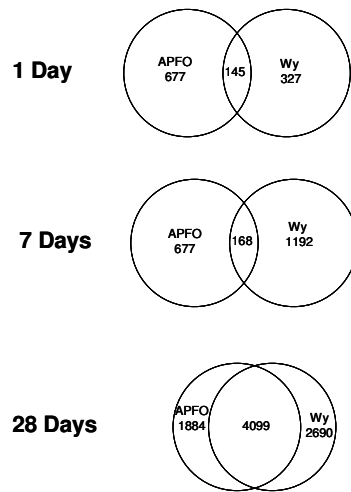
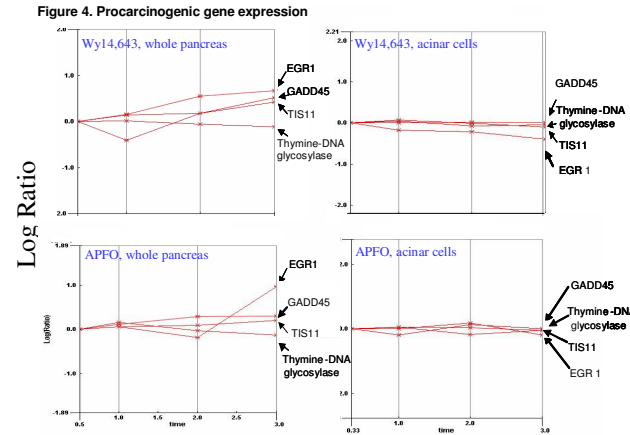


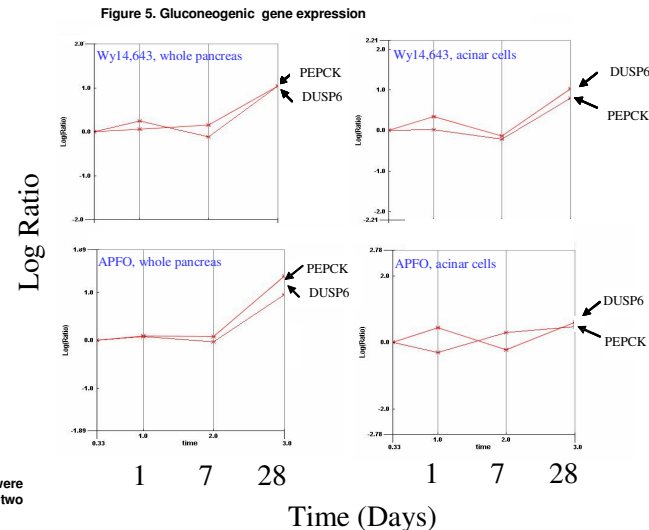
Figure 3. Venn diagrams showing the numbers of genes that were significantly regulated (p<0.01) either uniquely or commonly by the two treatments (Wy50ppm, and APFO (300ppm) at 1, 7 and 28 days.

Results

Trend analysis of time-dependent changes in EGR1, TIS11, GADD45 α and thymine-DNA glycosylase in whole pancreas versus acinar cells showed that the procarcinogenic gene expression changes observed in whole pancreas did not occur in isolated acinar cells (Figure 4).



Trends Analysis of time-dependent gene expression changes in genes associated with gluconeogenesis, namely phosphoenolpyruvate carboxykinase (PEPCK) and dual specificity phosphatase 6 (DUSP6), showed similar increases in expression in response to both Wy14,643 and APFO in both whole pancreas and acinar cells (Fig. 5).



Summary and Conclusions

★ Procarcinogenic gene expression changes observed previously (Plummer *et al* 2005) in response to dietary treatment with Wy 14,643 (50ppm) and APFO (300ppm) did not occur in pancreatic acinar cells isolated from rats treated with these agents.

★ By contrast, gene expression changes in the gluconeogenesis/pancreatitis genes phosphoenolpyruvate carboxykinase (PEPCK), dual specificity phosphatase 6 (DUSP6) showed similar time-dependent changes in both whole pancreas and isolated acinar cells in response to these treatments.

★ The number of genes commonly altered by both APFO and Wy14,643 treatments was proportionally higher at 28 days (70%) than it was at 1 and 7 days (5-10%) suggesting commonality in the mechanism(s) involved in mediating the effect of these two treatments on pancreas gene expression at the 28 day time point.

★ Either the workup procedure for isolation of acinar cells selectively negated the procarcinogenic gene expression changes in isolated acinar cells or these gene expression changes are occurring in another cell type in the pancreas.

★ The pancreas contains inter- and intra-lobular ducts which contain centroacinar cells (Figure 6). These cells are more sensitive to proliferative stimuli caused by dietary modulation of gut hormone secretion and are thought to represent a precursor cell population that differentiate into acinar cells. These characteristics render the centroacinar cells susceptible to factors leading to cell transformation. Hence these cells are a possible target for carcinogenesis with these agents.

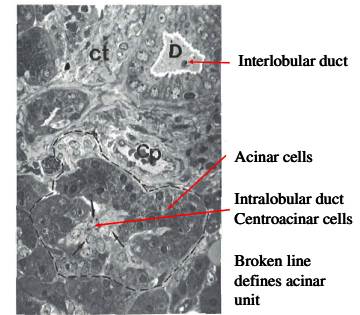


Figure 6. The exocrine pancreas

References

Plummer, S.M., Vassieva, O. and Elcombe, C. (2005) Abstract # 761, Proceeding of the SOT 44th Annual Meeting p 108.