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

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) (1000ppm) 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 2000). 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 RNA (Cy3 or Cy5) pooled from controls on Agilent Whole Rat genesets to make an error weighted mean (n=5) and 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 commonly 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-blobular 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 differentiates 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.

Results

Intromolecular histochemistry of fixed arrays of isolated acinar cells, using α-amylase (acrinal cell specific) or cytokeratin 19 (duct specific) antibodies, showed that the isolated cell preparations contained greater than 96% acinar cells (Figure 2).

Trend analysis of time-dependent changes 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).

Discussion

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 commonly 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.

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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 glucosoneogenesis/pentastar2 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.
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