Persistent organic pollutants (POPs) induce changes in MCF-10A and in acini structures isolated from pregnant mice exposed *in vivo*

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Introduction

- Persistent organic pollutants (POPs) bio accumulate in living organisms and can be found in high concentrations in animals and humans. Examples of POPs include polychlorinated biphenyls (PCBs), dioxins and flame retardants (BFRs) and perfluorinated compounds (PFCs).
- POPs are found in fat tissue or bind to proteins and are transferred to the offspring through placenta and/or breast milk. Different POPs have been associated with adverse health outcomes like reproductive toxicity, hormone disturbance, DNA damage and cancer.
- Living organisms are usually not exposed to single POPs, but to a complex mixture of substances where adverse health outcomes cannot be predicted based only on knowledge about single components.

Aims

- Investigate how exposure of human breast cells (MCF10A) to single POPs (e.g. PFCs) affect apoptosis and polarization of breast acini.
- Investigate how a complex mixture of POPs (n=29) relevant for human intake, affect polarization of breast acini made from primary mouse cells exposed in vivo.

POPs
+
Breast cells

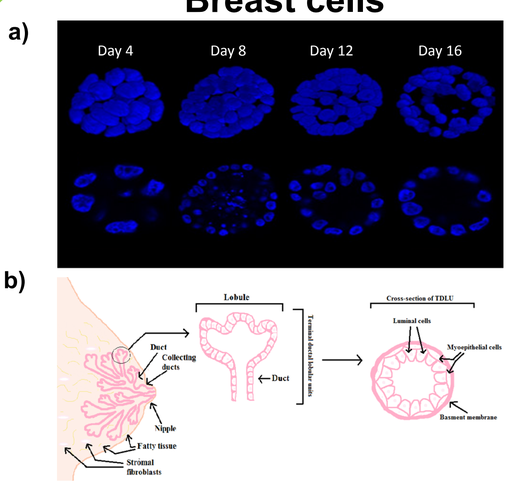


Figure 4: *Breast cells used in exposure studies with POPs and a schematic illustration of the terminal ducts in breast.* ***a)****Confocal microscopic imaging of MCF-10A acini (upper panel) and cross sections on MCF-10A acini (lower panel) DAPI (blue).* ***b)*** *Structure of the mammary gland, the terminal ductal lobular units and cross section of TDLU, showing the primary cells in normal ducts.*

Results

1) Single POPs (PFCs) cause polarisation failure and compromises lumen forma tion in human breast cells (MCF10A)

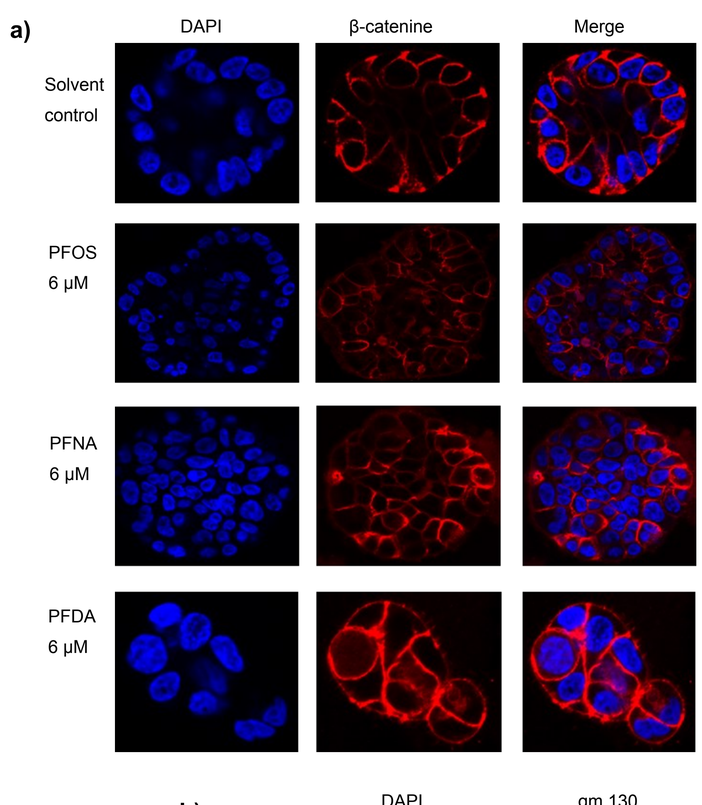
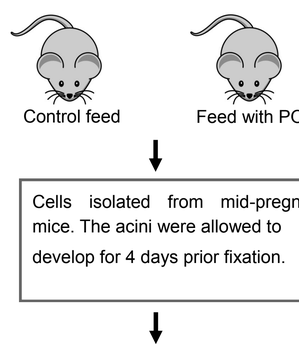


Figure 1: *Acini structures (8days) exposed with single PFCs and stained with markers for polarisation.* ***a)*** *β-catenine and* ***b)*** *gm 130 by use of konfocal microscopy.*

2) A mixture of POPs affects acini development in primary mouse breast cells



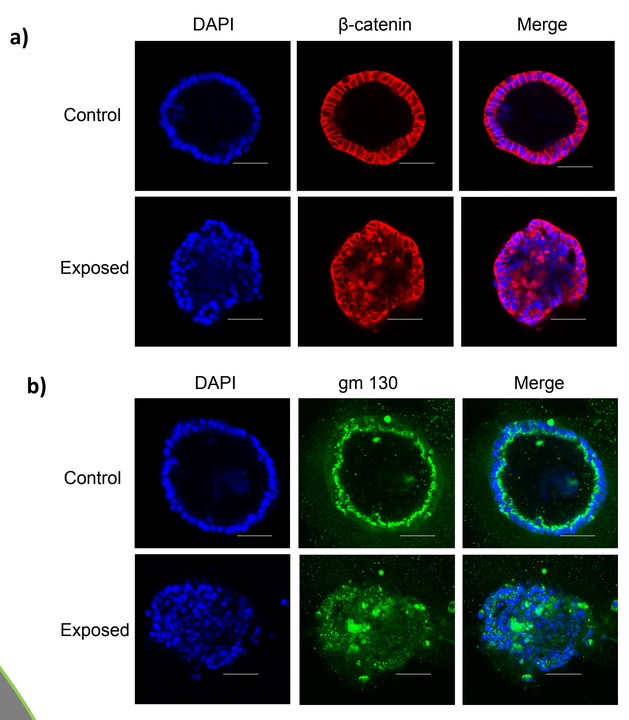


Figure 2. *Mouse primary mammary cells from exposed and control mice. a) Acini structures at day 4 stained for β-catenine. b) Acini structures at day 4 stained for gm130.*

Methods

POPs

Perfluorinated compounds (PFCs): PFOA, PFOS, PFNA, PFDA and PFuNA (Sigma Aldrich). A complex mixture of POPs (N=29) relevant to human exposure was prepared in the lab (unpubl. data) and used in mouse feed.

MCF-10A

The human epithelial cell line, MCF-10A, was a kind gift from Professor Finian Martin, University College Dublin. The cells were seeded on chamberslides precoated with matrigel, and allowed to devlop into acinistructures for 4-12 days. The cells were exposed at seeding and whenever the medium was changed.

Primary eptihelial cells

Mouse mammary epithelial cells were harvested from mid– to late-pregnant CD-1 mice. The cells were seeded as described for MCF-10A and allowed to develop for 4 days.

Antibodies and Microscopy

Primary antibodies were purchased from BD Bioscience; mouse anti-β-catenin and mouse anti-GM130. The acini were analyzed using a Zeiss LSM 710 confocal microscope.

RNA extraction and gene expression

RNA was extracted from 8 days acini structures, using RNA-STAT 60 (Amsbio). cDNA synthesis was performed using the iSript cDNA synthesis kit (Biorad). RT Profiler PCR Array (SA Biosciences) was used to analyse expression of apoptotic genes.

3) Transcription of apoptosis regulating genes in MCF10A is upregulated after PFC exposure

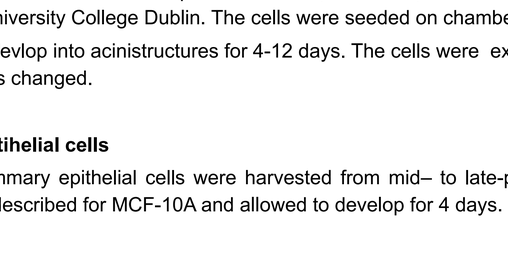


Figure 3: *Transcript levels of apoptosis regulating genes (TNFRSF21, DFFA and IGF1R) in exposed MCF10A acini structures. Cells were exposed with 6 µM of PFC compounds (PFOS, PFOA, PFDA) and RNA was harvested at day 8.*

Conclusions

- PFCs impaired polarisation of MCF-10A acini structures.
- TNFRSF21, DFFA and IGF1R were upregulated after PFC exposure, with the most prominent change in IGF1R which is anti-apoptotic
- A complex mixture of POPs, relevant to human exposure impaired polarisation of acini structures from murine primary breast cells.
- Since luminal filling is often observed in breast cancer, these results suggest that POPs may play a role in breast cancer development.
- The current methodology can be useful in studies of breast development and breast cancer in other species, for example in dogs.

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