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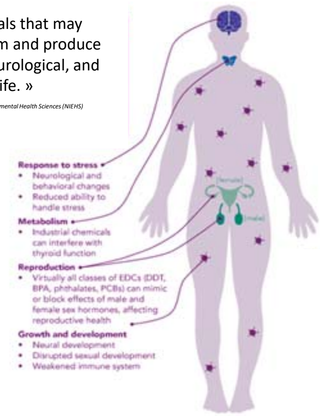
Endocrine disrupting compounds, a global health concern

« Endocrine disruptors (EDCs) are chemicals that may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. »

National Institute of Environmental Health Sciences (NIEHS)

A wide range of substances, both natural and man-made, are thought to cause endocrine disruption, including pharmaceuticals, dioxin and dioxin-like compounds, polychlorinated biphenyls, DDT and other pesticides, and plasticizers such as bisphenol A. EDCs may be found in many everyday products: plastic bottles, metal food cans, detergents, flame retardants, food, toys, cosmetics, and pesticides.

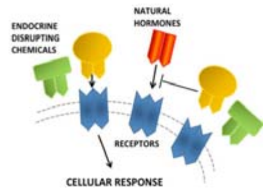


Known biological functions impact by endocrine disruptors.

Hormone Health Network

Which mechanisms of action ?

- Mimic or partially mimic hormones like estrogens or androgens and thyroid hormones.
- Bind to a receptor within a cell and block the action of the endogenous hormones. The normal signal fails to occur and the body does not properly respond.
- Interfere or block the synthesis of natural hormones or their receptors for example, by altering their metabolism in the liver.



Biotechin Asia

Major challenge of the field

The list of potential EDCs comprises a large and growing number of individual compounds or mixtures and their metabolic and environmental derivatives. These compounds have diverse chemical structure and may not appear to share any structural similarity. Thus, there is an urgent need **for multiparametric, robust, and high throughput cell-based assay** that can investigate the complex mechanisms underlying the adverse effects of known EDCs and **identify new compounds with endocrine-disrupting potential**.

Mitochondria : a cell sensor … Mitochondria play a major role in cancer cell metabolism and recent data demonstrate that they are implicated in cancer progression. Our hypothesis is that ED may promote cancer cell agressiveness through modifications of cancer cell metabolism.

AIM

We aimed to understand whether EDCs alter **mitochondrial functions**. To achieve this aim we used several **unbiased quantitative image-based assays** with simple read-out and we **developed computational image based analysis** to evaluate the effects of various endocrine disruptors on mitochondrial topology.

1) Quantitative image based analysis of mitochondrial functions *(Hight throughput screening)*

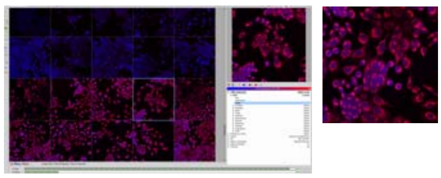
2) Computational image based analysis of mitochondrial morphology *(Image analysis and classification)*

Quantitative image based analysis of mitochondrial functions

Experimental workflow

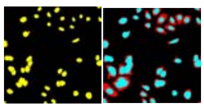
Optimal cell culture protocol
↓
Optimal staining protocol
↓
Optimal imaging protocol and microscopy tasks automatization
↓
Image processing
↓
Feature extraction
↓
Descriptor values
↓
Data analysis
↓
Cluster analysis
Machine learning

Image acquisition



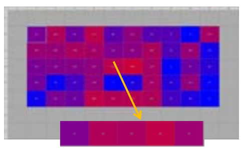
Typical software interface of an automatized 96wells plate scan on Nikon A1R.
DU145 are treated for 24h with EDCs, then labelled with MitoTracker™ Red to monitor mitochondrial membrane potential . A total of 12 pictures are taken by well. MitoTracker™ Red staining is in red; Nucleus (Dapi staining) is in blue.

Image processing



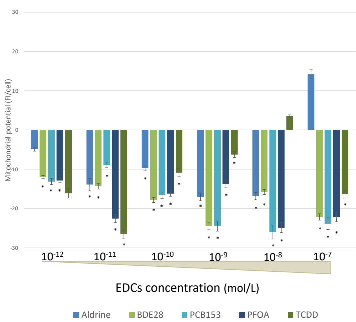
Nuclear masking for cell counting (yellow/cyan) and dye masking (red) for fluorescence intensity per images.

Data analysis

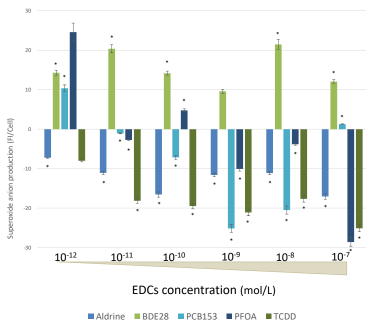


'Heat map' representing MitoTracker™ Red fluorescence intensity per well.

Effect of 5 EDCs on mitochondrial membrane potential



Effect of 5 EDCs on mitochondrial superoxide anion production



Results obtained using this quantitative image based analysis on an androgen insensitive prostate cancer cell line (DU145). The left panel show the results for the mitochondrial membrane potential (MitoTracker™ Red). The right panel show the result for the superoxide anion production (MitoSox™). The graph baseline represent control values arbitrarily set to 0. Thus, the data represent the deviation of the fluorescence intensity/cell as compared to the control.
* significantly different from the control, p≤0,005.

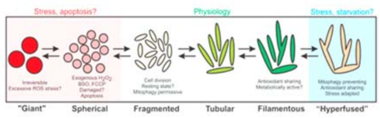
Our results demonstrate that very low concentration (picomolar range) of EDCs affect the mitochondrial function and the production of ROS. Interestingly, we observed a differential effect in ROS production depending on chemical structure of EDCs.

In particular, BDE28 increases ROS production over a wide range of concentrations and PFOA displays an elevated ROS production only at low concentrations (10^{-12}mol)

Computational image based analysis of mitochondria morphology

Form follows function

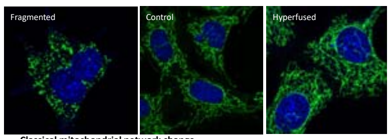
In mitochondria, form and function are intimately linked. They adapt to cellular requirements: energetic, precursor synthesis, stress, apoptosis or growth signaling by changing shape, motility, and tethering together into tubular networks.



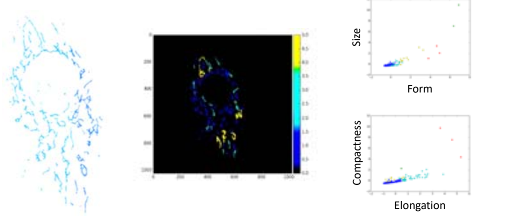
In healthy condition, mitochondria morphology range from fragmented to filamentous. On the contrary, stress condition are associated with giant or hyperfused morphologies that appear to be linked to apoptosis induction and stress adaptation, respectively.

Redox Homeostasis and Mitochondrial Dynamics. P Willems, R Rossignol, C Dieteren, MP. Murphy, W.J.H. Koopman Cell Metabolism (2015)

Our image classification method using Python allows us to classify all images region according to the highest gain leading to no loose of information or noisy image. After detection, this method automatically partitions regions using K-means methods leading to the clusters classifications.



Classical mitochondrial network change.
Prostate cancer cell (DU145) were treated over night with rotenone (100nM), left untreated or treated with compound C (20µM) -an AMPK inhibitor mimicking an energy stress- from left to right respectively. In green, mitochondria are immunostained using Tom20 (green) and with dapi for nucleus. Zeiss LSM500, 63X.



Mitochondrion image analysis and classification using our Python developped method.
Particle distribution according to their size (pixel numbers), form (contour length on area), elongation (distance between the more distant pixels) or compacity (distance between the pixel at the center and the outermost pixel).

Illustration of mitochondrial shape **clusterization**

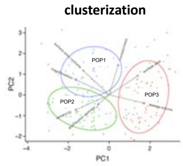


Illustration of mitochondrial shape clusterization

Adapted from : MitoLoc: A method for the simultaneous quantification of mitochondrial network morphology and membrane potential in single cells. J. Vowinckel, J. Hartl, R. Butler, M. Ralser. Mitochondrion 2015

This single-cell image based analysis of the mitochondrial network allow us to classify mitochondrial morphology based on form clusterization : size, form, elongation and compacity.

Final goal

When combined, **morphological and functional parameters** allow us to discriminate subtle perturbations of the mitochondrial structure-function induced by endocrine disruptors in prostate cancer cells. We are performing a multiparametric profile for each EDC, which will allow us to cluster these pollutants in respect to their mitochondrial effects rather than to their classes. This clustering will be crucial to predict whether the combination of several EDCs will have additive or synergic effects. We are confident that this multiparameter analysis strategy could represent a new perspective in **identification and characterization of endocrine disruptors** based on their effects on cell metabolism in order to estimate their potential risk on human health.

Don't hesitate to contact us for collaboration!

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