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Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells.

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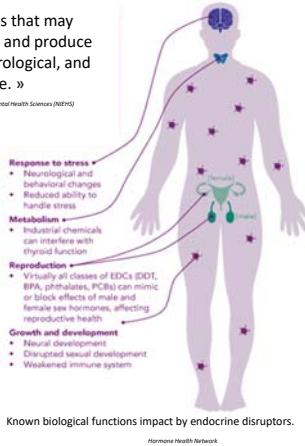
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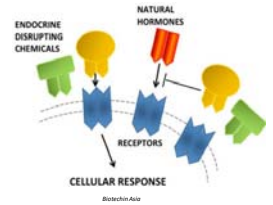
Endocrine disrupting compounds, a global health concern

« 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. »



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.



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 aggressiveness 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 (*High throughput screening*)
- 2) Computational image based analysis of mitochondrial morphology (*Image analysis and classification*)

Quantitative image based analysis of mitochondrial functions

Experimental workflow

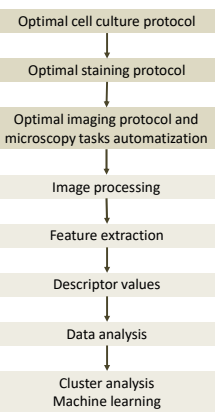
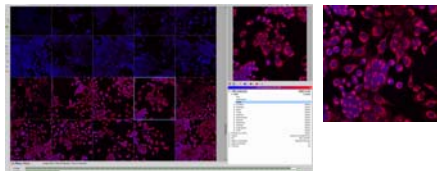
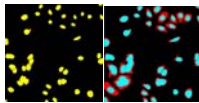


Image acquisition

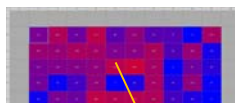


Typical software interface of an automatized 96wells plate scan on Nikon A1R. DU145 are treated for 24h with EDCs, then labelled with MitoTracker™ Red to monitor mitochondrial membrane potential. A total of 12 pictures are taken by well. MitoTracker™ Red staining is in red; Nucleus (Dapi staining) is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

Image processing

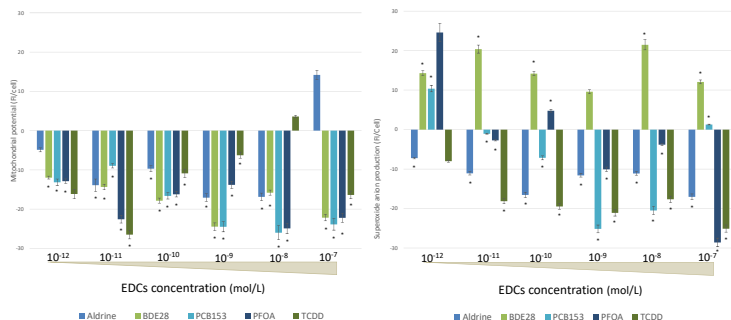


Data analysis



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 shows the results for the mitochondrial membrane potential (MitoTracker™ Red). The right panel shows the result for the superoxide anion production (MitoSox™). The graph baseline represent the 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.

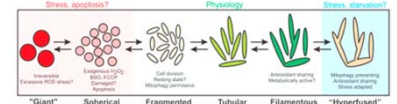
Our results demonstrate that very low concentration (picomolar range) of EDCs affect the mitochondrial function and the production of ROS. Interestingly, we observed a differential effect in ROS production depending on chemical structure of EDCs.

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

Computational image based analysis of mitochondria morphology

Form follows function

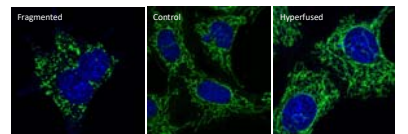
In mitochondria, form and function are intimately linked. They adapt to cellular requirements: energetic, precursor synthesis, stress, apoptosis or growth signaling by changing shape, motility, and tethering together into tubular networks.



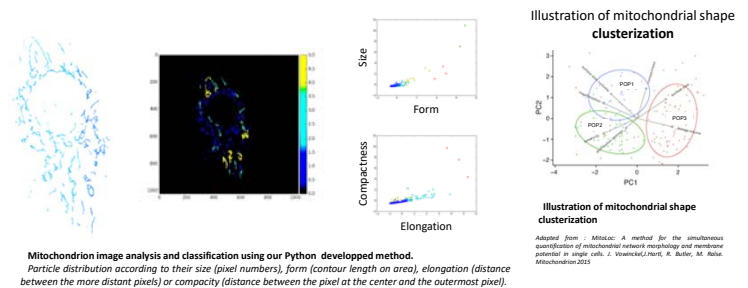
In healthy condition, mitochondria morphology range from fragmented to filamentous. On the contrary, stress condition are associated with giant or hyperfused morphologies that appear to be linked to apoptosis induction and stress adaptation, respectively.

Redox Homeostasis and Mitochondrial Dynamics. P. Williams, R. Bissig, C. Diener, M.P. Murphy, W.J. Kruger. *Exp. Cell Res.* (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.



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

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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