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

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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.

A wide range of substances, both natural and man-made, are thought to cause endocrine disruption, including pharmaceuticals, endocrine disruptors, and plasticizers such as bisphenol A. EDCs may be found in many everyday products: plastic bottles, metal food cans, detergents, flame retardants, personal care products: plastic bottles, metal food cans, detergents, flame retardants, etc.

*Significantly different from the control, p<0.005.

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!
Project supervisor : chevalier.n@chu-nice.fr
Post-doctoral investigator : aurelie.charazac@unice.fr

Final goal

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Experimental workflow

- Quantitative image based analysis of mitochondrial functions
- Computational image based analysis of mitochondrial morphology

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 a semi-automated 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 cell culture protocol
- Optimal staining protocol
- Image processing
- Feature extraction
- Data analysis
- Cluster analysis

Computational image based analysis of mitochondrial 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 in tubular networks.

Our image classification method using Python allows us to classify all images regions 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.

Results obtained from this quantitative image-based analysis on 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 (PQ1Tracker™). The graph bar represent the mean fluorescence intensity in the region of interest. Red indicates increased mitochondrial membrane potential and green indicates the decrease of mitochondrial membrane potential.

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 (10⁻¹⁰ mol/L).

For a single cell image analysis of the mitochondrial network allow us to classify mitochondrial morphology based on form clustering - size, form, elongation and compacity.

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

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