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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, 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.

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 and 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)

Experimental workflow
Optimal cell culture protocol
Optimal seeding protocol
Optimal imaging protocol and microscopy tasks automation
Image processing
Feature extraction
Data analysis
Cluster analysis
Machine learning

Image acquisition

Typical software interface of an automated Nikon Microscope plate scan on Nikon XTL. All images are treated for 24h with EDCs then labeled with MitoTracker™ Red to monitor mitochondrial membrane potential. A total of 24 pictures are taken in well. MitoTracker™ Red staining is in red: Nucleus (Dapi staining) is in blue.

Quantitative image based analysis of mitochondrial functions

Effect of 5 EDCs on mitochondrial membrane potential

Effect of 5 EDCs on mitochondrial superoxide anion production

Results obtained using the quantitative image based analysis on an androgen insensitive prostate cancer cell line (DU 145). The left panel shows the results for the mitochondrial membrane potential (MitoTracker™ Red). The right panel shows the results for the superoxide anion production (MitoSox™). The graph bar is representing the percentage of responsive cells. The more bars the higher the concentration of EDCs.

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 PFOS displays an elevated ROS production only at low concentrations (10^-12mol).

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 tethers together into tubular networks.

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.

Finally, we are developing 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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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.