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

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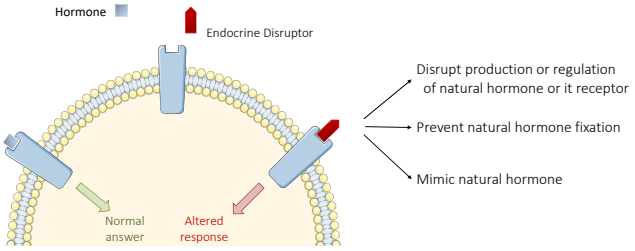
Endocrine disruptors

According to the World Health Organization, an endocrine disruptors may be describe as an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism or its progeny, or (sub) populations.



They are found in many everyday products like food packaging, food preservatives or additives, pesticides residues, everyday plastic objects, etc.

Widely distributed throughout the environment and bioaccumulable in living organisms, **P**ersistent **O**rganic **P**ollutants are a specific class of EDC that accumulate in fat deposit. Some of them have been recognized as causing adverse effects on wildlife and also human's health such as diabetes and cancer…

Working hypothesis

Cancer cells undergo of genetic modifications that lead to a phenotype characterized by a high metabolic flexibility allowing them to grow in various cellular environments and favoring their proliferative and invasive capacities as well as death resistance. Mitochondria are complex organelles possessing a central role in metabolism, apoptosis, signaling, etc.

AIM

Develop screening procedures to analyze the effects of various endocrine disruptors at different concentrations and in combination on:

- **Mitochondrial morpho-function** *(Hight throughput screening + image analysis and classification)*
- **Metabolism** *(XF analyzer, SeaHorse)*
- **Migration/Invasion/Proliferation** *(Videomicroscopy, IncuCyte)*
- **Signaling pathways** *(Luminex)*

Workflow for microscopy based screening

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

Microscopy based analysis of mitochondria functional parameters

Mitochondrial membrane potential (MitoTracker Red™ staining)

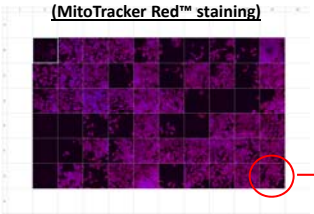
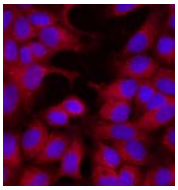


Image of a 96 wells plate (20X, Nikon A1R, automatization process)



Magnification 20X

Superoxide anion production (MitoSox™ staining)

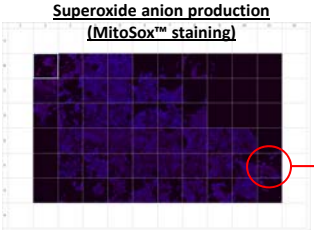
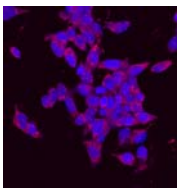
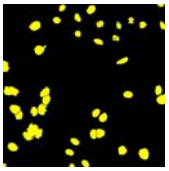


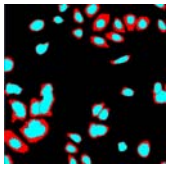
Image of a 96 wells plate (20X, Nikon A1R, automatization process)



Magnification 20X

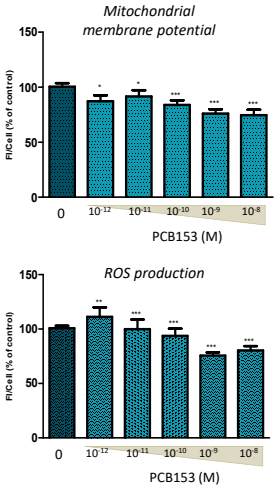
1) Nuclear masking for cell counting



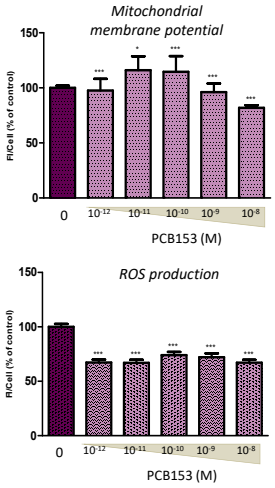


2) Dye masking for cell fluorescence intensity analysis

Androgen insensitive prostate cancer cell line (DU145)

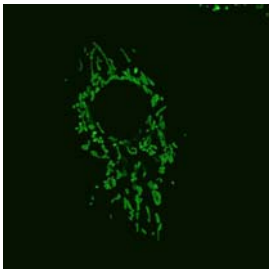


Androgen sensitive prostate cancer cell line (22RV1)



Exemple of results obtained using this quantitative image based analysis on DU145 and 22RV1 prostate cancer cell lines after treatment with a range doses of PCB153, an ED non inflammable electric isolant.

Mitochondrial topology analysis and classification



Original picture (60X, Nikon A1R)

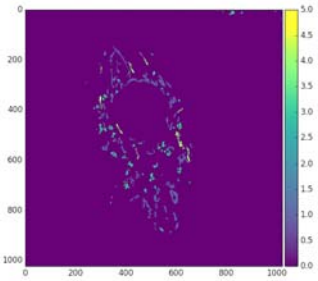
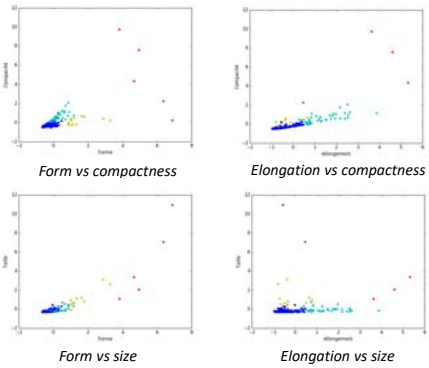


Image with developed method (Python program)

Classification



Form vs compactness — *Elongation vs compactness* — *Form vs size* — *Elongation vs size*

Clusterization of mitochondrial shape

The developed method using Python will classified all image's region according to the highest gain leading to no loose of information or noisy image. After detection, this method will automatically partitioning regions using K-means methods leading to the clusters classifications thanks to following criterias : form, compactness, size, elongation, fragmentation.

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 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. In the future, this can lead to a high-throughput screening method useful to industrial and regulatory agencies.

Don't hesitate to contact us for collaboration!

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