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

Aurélie Charazac, Célia Decondé Le Butor, Kévin Giulietti, Jean-Marc A. Lobaccaro, Silvère Baron, Jérôme Gilleron, Patrick Fenichel, Xavier Descombes, Frédéric Bost, Clavel Stéphan, et al.

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

Charazac Aurélie, Decondé le Butor Célia, Giulietti Kévin, Lobaccaro Jean Marc, Baron Silvère, Gilleron Jérôme, Fénichel Patrick, Descombès Xavier, Bost Frédéric, Clavel Stéphan & Chevalier Nicolas.
« An endocrine disruptor is 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. »

World Health Organization

Endocrine Disruptor Compounds

- Pesticides
  - Aldrine...
- Perfluorinated Compounds
  - PFOA...
- Flame Retardants
  - BDE 28...
- Polychlorinated Biphenyls
  - PCB 153...
- Dioxins
  - TCDD...
Registration, Evaluation and Autorization of CHemicals

Promotes alternative methods for the hazard assessment of substances.

Adapted from EndocrineScience.org
Urgent need for multiparametric, robust and high throughput cell-based assay:

- To investigate the mechanisms underlying the adverse effects of known EDCs
- To identify new compounds with endocrine-disrupting potential


Adapted from EndocrineScience.org
Mitochondria, a cell sensor

Introduction ~ Aim

Apoptosis

Healthy

Stress

Mitochondria, a cell sensor

Introduction ~ Aim

Apoptosis

Healthy

Stress

Calcium signalling

Cell growth and differentiation

Cell cycle control

Cell death

Mitochondria, a cell sensor

Alteration of **mitochondrial forms and functions** by EDCs?

1) Quantitative image based analysis of mitochondrial functions
   - High throughput screening

2) Computational image based analysis of mitochondrial morphology
   - Image analysis and classification
Multiparametric cell-based assay

Automated image acquisition
(Nikon A1R-20X)

Image processing

1) Dapi masking:
   cell counting

2) Dye masking:
   fluorescence intensity
Quantitative image based analysis of mitochondrial functions

Multiparametric cell-based assay

Automated image acquisition
(Nikon A1R-20X)

Image processing

1) Dapi masking:
cell counting

2) Dye masking:
fluorescence intensity

- Multiparameter:
  - mitochondrial membrane potential (MitoTracker Red™)
  - superoxide anion production (MitoSox™)
  - mitophagy (Keima probe)
EDCs affect the mitochondrial function

- **ROS production on DU145:**

![Graph showing ROS production on DU145](image)

- Superoxide anion production (FI/cell)
- EDCs concentration (mol/L)

<table>
<thead>
<tr>
<th>EDCs concentration (mol/L)</th>
<th>Aldrine</th>
<th>TCDD</th>
<th>PCB153</th>
<th>PFOA</th>
<th>BDE28</th>
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<tr>
<td>$10^{-12}$</td>
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<td>$10^{-11}$</td>
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<td>$10^{-10}$</td>
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<td>$10^{-9}$</td>
<td></td>
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<tr>
<td>$10^{-8}$</td>
<td></td>
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</tbody>
</table>

* significantly different from the control, $p \leq 0.005$. 
EDCs affect the mitochondrial function

- **Mitochondrial membrane potential on DU145**:

![Graph showing mitochondrial membrane potential on DU145](image)

* significantly different from the control, \( p \leq 0.005 \).
Forms classification of mitochondrial network

- Collaboration with

Fragmented, Tubular, Hyperfused
Computational image based analysis of mitochondrial morphology

Forms classification of mitochondrial network

- Collaboration with

Image

Basic thresholding method

Developed method

Fragmented, Tubular, Hyperfused
Exemple of developed method

Tubular

Aggregates Tubular Hyperfilamentous
Computational image based analysis of mitochondrial morphology

Exemple of developed method

Fragmented
(Rotenone, 100nM)

Tubular

Fused
(Compound C, 20µM)

Aggregates Tubular Hyperfilamentous
Final Goal

- Development of a multiparametric high throughput cell based screening:

**Mitochondrial functional parameters**
- Proliferation
- Cytotoxicity

**Mitochondrial morphological parameters**
- EDCs effect on prostate cancer cells
- Apoptosis
- Maximal glycolytic and respiratory capacity
- Migration/invasion
Final Goal

- Development of a multiparametric high throughput cell based screening:
  - Mitochondrial functional parameters
  - Mitochondrial morphological parameters
  - Proliferation
  - Cytotoxicity
  - Apoptosis
  - Migration/invasion
  - Maximal glycolitic and respiratory capacity

Signature of EDCs based on their effects on cell metabolism
New perspective in identification and characterization of EDCs
Thanks...

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