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

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

To cite this version:
Aurélie Charazac, Célia Decondé Le Butor, Kévin G. Giulietti, Jean-Marc A. Lobaccaro, Silvère Baron, et al.. Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells. International Congress of Translational Research in Human Nutrition, Jun 2017, Clermont-Ferrand, France. hal-01548783

HAL Id: hal-01548783
https://hal.archives-ouvertes.fr/hal-01548783
Submitted on 12 Jul 2017

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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, Descombes 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
Introduction ~ EDC

Major challenge

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<th>DANISH EPA</th>
<th>TEDX</th>
<th>EU REACH SVHC</th>
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Registration, Evaluation and Authorization 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

Registration, Evaluation and Autorization of Chemicals

Promotes alternative methods for the hazard assessment of substances.

Adapted from EndocrineScience.org
Mitochondria, a cell sensor

Introduction ~ Aim

Mitochondria, a cell sensor

Fragmented

Tubular

Hyperfused

Apoptosis

Healthy

Stress

Mitochondria, a cell sensor

Introduction ~ Aim

Mitochondria, a cell sensor

Aim

Mitochondria, a cell sensor

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)

| A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

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](image)

*significantly different from the control, p≤0.005.*
EDCs affect the mitochondrial function

- Mitochondrial membrane potential on DU145:

![Graph showing the effect of EDCs on mitochondrial membrane potential.](image)

*significantly different from the control, p≤0.005.
Forms classification of mitochondrial network

- Collaboration with

 Fragmented, Tubular, Hyperfused
Forms classification of mitochondrial network

- Collaboration with

![Image of mitochondrial network classification](image)

**Image**
- Fragmented
- Tubular
- Hyperfused

**Basic thresholding method**

**Developed method**

Fragmented, Tubular, Hyperfused
Exemple of developed method

Tubular

Aggregates Tubular Hyperfilamentous
Exemple of developed method

Fragmented (Rotenone, 100nM)

Tubular

Fused (Compound C, 20µM)

Aggregates Tubular Hyperfilamentous

Centre Méditerranéen de Médecine Moléculaire Inserm U1065
Final Goal

- Development of a multiparametric high throughput cell based screening:

Mitochondrial functional parameters

- Mitochondrial morphological parameters

Proliferation

Cytotoxicity

EDCs effect on prostate cancer cells

- Apoptosis

- Maximal glycolitic 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 glycolytic 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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