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, 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

Endocrine Disruptor Compounds

Pesticides
Aldrine...

Perfluorinated Compounds
PFOA...

Flame Retardants
BDE 28...

Polychlorinated Biphenyls
PCB 153...

Dioxins
TCDD...

Pineal gland
Hypothalamus
Pituitary gland
Thyroid gland
Parathyroid gland
Thymus
Adrenal gland
Pancreas
Testis

Centre Méditerranéen de Médecine Moléculaire Inserm U1065
Major challenge

Acceptation of WHO definition

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Based on risk assessment

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List updating

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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


Adapted from EndocrineScience.org
Introduction ~ Aim

Mitochondria, a cell sensor

Apoptosis
Healthy
Stress

Introduction ~ 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
Automated image acquisition
(Nikon A1R-20X)

Image processing

1) Dapi masking:
cell counting

2) Dye masking:
fluorescence intensity
I ~ 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 | W | X | Y | Z |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

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

Image processing

1) Dapi masking:
   cell counting

2) Dye masking:
   fluorescence intensity
EDCs affect the mitochondrial function

- ROS production on DU145:

- Significantly different from the control, \( p \leq 0.005 \).

* * *
EDCs affect the mitochondrial function

**Mitochondrial membrane potential on DU145:**

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

- Collaboration with

[Images of mitochondrial morphology with labels: Fragmented, Tubular, Hyperfused] → [Morphological representations: Fragmented, Tubular, Hyperfused]
Forms classification of mitochondrial network

- Collaboration with

Fragmented, Tubular, Hyperfused

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
  - Mitochondrial morphological parameters

  - EDCs effect on prostate cancer cells

  - Proliferation
  - Cytotoxicity
  - 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
  - EDCs effect on prostate cancer cells
  - Signature of EDCs based on their effects on cell metabolism
  - New perspective in identification and characterization of EDCs
Thanks...

Team 7
Frédéric Bost
Jean-François Tanti
Mireille Cormont
Stephan Clavel
Célia Deconde Le Butor
Lisa Kaminski
Kathiane Laurent
Nicolas Chevallier
Jérôme Gilleron
Jade Evrard
Bastien Vergoni
Gwenaëlle Bouget
Karine Dumas
Marine Bourcier
Mounia Tannour-Louet
Calypso Vacher-Chicane
Sophie Giorgetti-Péraldi
Sophia Fazio
Jean-François Louet
Stéphanie Torrino
Faustine Pastor