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

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

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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**
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
Introduction ~ Aim

Mitochondria, a cell sensor

Fragmented  Tubular  Hyperfused

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)

| 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 |

### 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)

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:

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

- Mitochondrial membrane potential on DU145:

  * significantly different from the control, \( p \leq 0.005 \).

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

- Collaboration with

Fragmented, Tubular, Hyperfused
Forms classification of mitochondrial network

- Collaboration with

**Image**

- Fragmented
- Tubular
- Hyperfused

**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**
  
  - 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

  EDCs effect on prostate cancer cells

  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
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