Novel methods for the assessment of the impact of chemicals on deiodinases as a crucial target in thyroid hormone regulation

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Introduction

- Exposure to **thyroid hormone (TH) disruptors** can result in physiological abnormalities and increased incidence of diseases such as cancer, metabolic syndrome, obesity, or adverse effect on fetal neurodevelopment.
- Key enzymes in the TH regulation pathway are **deiodinases (DIOs)** – enzymes that convert inactive thyroid hormones (thyroxine, T4) into their active forms (triiodothyronine, T3) by inner and outer ring deiodination.
- One of the most severe adverse outcomes of TH disruption could be observed during the early neurodevelopment.
- The most important DIO for the neural system is the DIO 3. It is highly expressed in the developing and adult brain where it regulates the action and availability of the TH.

Methodology

- Whole-cell lysates of HEK-293T DIOs-overexpressing cells are incubated in high-throughput-compatible 96-well plates with artificial DIO substrates (thyroid hormones).
- Iodine ions released by DIO reaction are separated from thyroid hormones by filtration through DOWEX WX-2
- The amount of released iodine is then quantified by Sandell-Kolthoff reaction and ICP-MS analytical method.

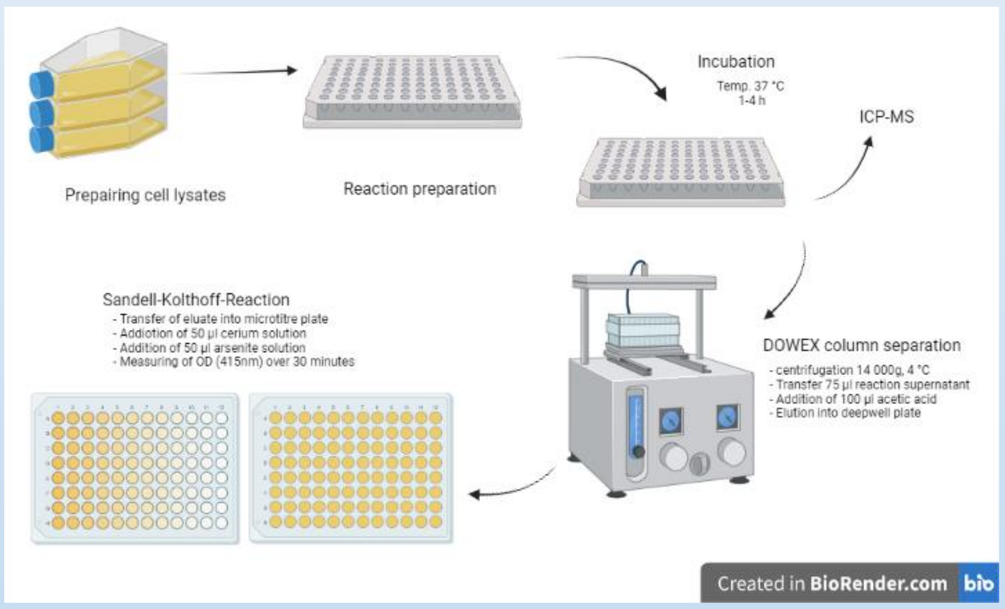

Fig1: Representation of the experimental setup for a quantification of DIO activity through the detection of released iodine by Sandell-Kolthof reaction and verification by ICP-MS.

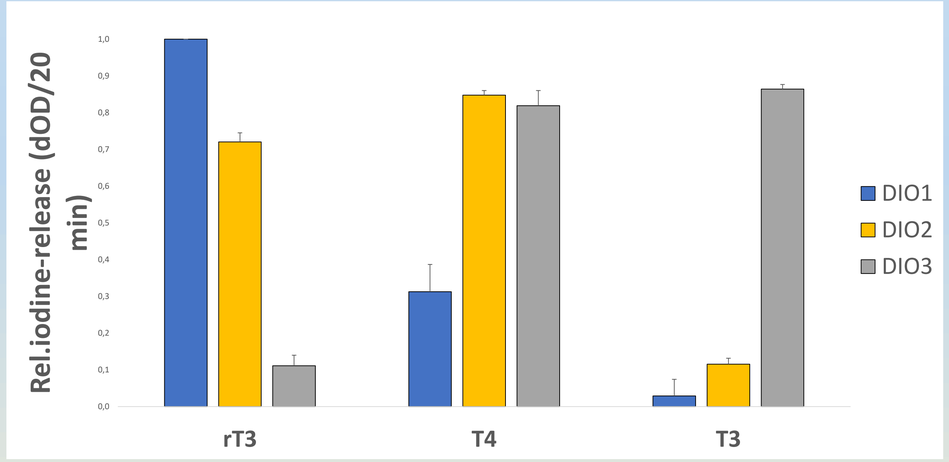

Fig 2: Cell homogenates from HEK-293 transfected cell lines had an isoenzyme-specific deiodination pattern reflecting the known and specific substrate preferences. Reverse triiodothyronine (rT3), thyroxine (T4), and T3 were identified as the preferred substrates for DIO1, DIO2, and DIO3, respectively. Modified from Renko et al., 2015

References

Renko, K. *et al.* An Improved Nonradioactive Screening Method Identifies Genistein and Xanthohumol as Potent Inhibitors of Iodothyronine Deiodinases. *Thyroid* **25,** 962–968 (2015).

Acknowledgement

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Aims

1) Development of *in vitro* HEK-293 based models overexpressing DIO enzymes.
2) Further improvement of the *in vitro* models by selective DIOs knock out by CRISPR/Cas9 together with the elimination of the cross-talks between the three different types of DIOs to increase both the method sensitivity and specificity. Optimization of the DIO activity detection/quantification method.
3) Establishment and optimization of neurodevelopmental model for characterization of TH disruptor's effects.
4) Testing the effects of an endocrine-disrupting chemicals set to identify disruptors of neurodevelopment and thyroid hormone signaling.
5) Characterization of thyroid hormone disruptors' effects in environmental exposure mixtures based on the newly developed and optimized methods.

Results

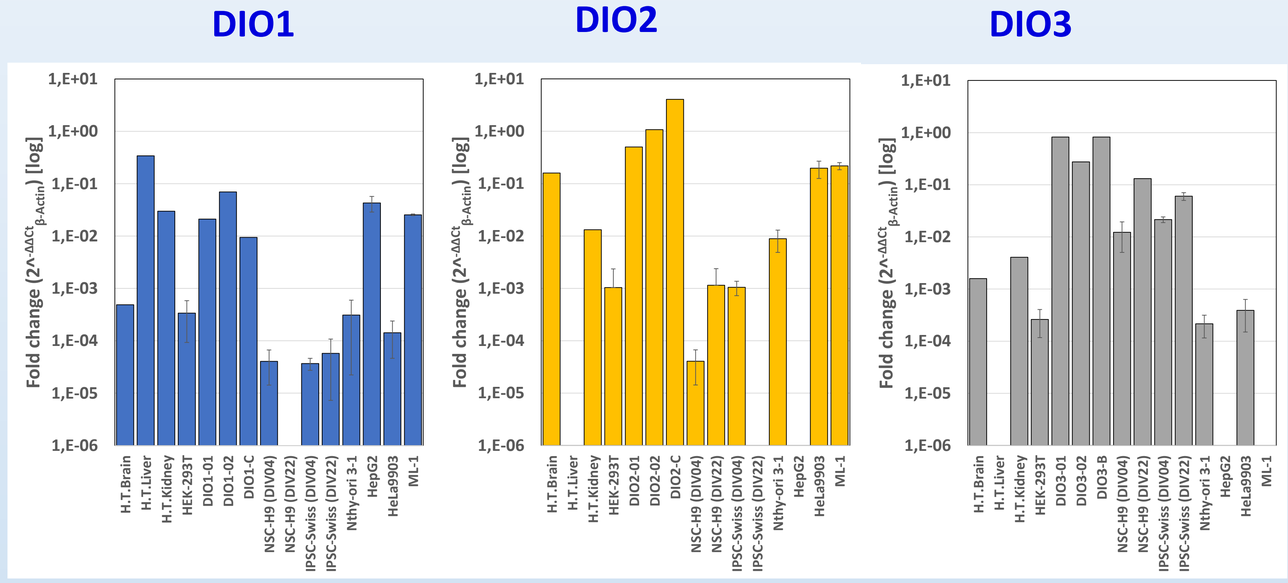

Fig 3: A comparison of DIOs expression in the newly developed transfection models and established cell lines with endogenous expression. Data are plotted as fold change over expression in Human Total Thyroid (Fold = 1.0). Bars represent average of biological replicates +- SD. Samples without detected gene expression are displayed as empty bars.

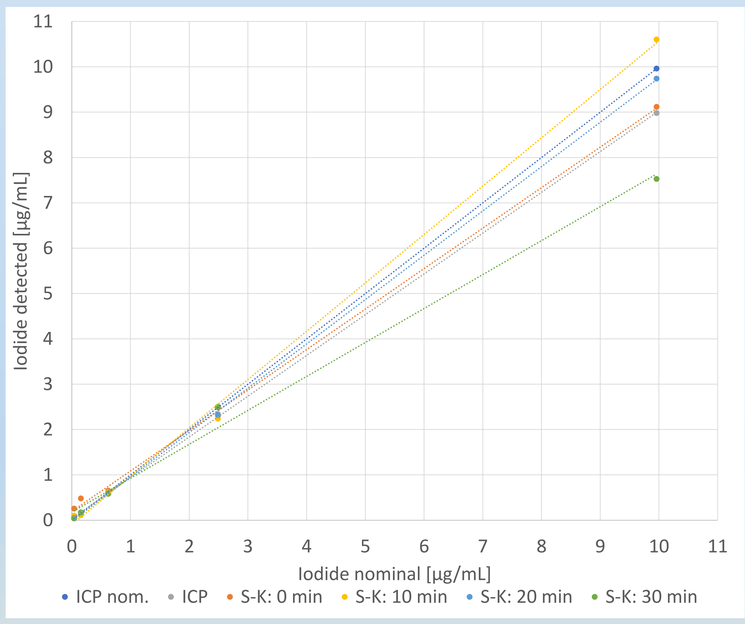

Fig 4: Correlations of nominal potassium iodide calibration to values measured by ICP-MS and SK reaction absorbance kinetics at 415 nm (measured from the start of the SK reaction with 10-minute intervals up to 30 min. Data are plotted as fold change over Human Total Thyroid (Fold = 1.0). Bars represent average of biological replicates +- SD. Samples without detected gene expression are displayed as empty bars.

Expected outcomes

- Development of new approach methodologies respecting the 3R principles that would identify EDs in high throughput and higher predictivity for humans
- *In vitro* characterization of the effects of chemicals (including environmental pollutants and their mixtures) on thyroid hormone regulation in the context of early neurodevelopment

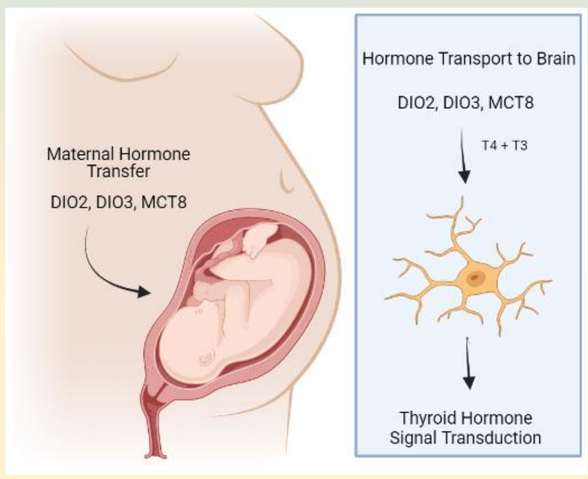

Fig 5: Principle of fetal dependence on maternal thyroid hormone transfer and its influence on early neurodevelopment. Created in Biorender.

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