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Novel cell-based assay for detection of thyroid receptor beta-interacting environmental contaminants

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ABSTRACT

Even though the presence of endocrine disrupting chemicals (EDCs) with thyroid hormone (TH)-like activities in the environment is a major health concern, the methods for their efficient detection and monitoring are still limited. Here we describe a novel cell assay, based on the translocation of a green fluorescent protein (GFP)—tagged chimeric molecule of glucocorticoid receptor (GR) and the thyroid receptor beta (TRβ) from the cytoplasm to the nucleus in the presence of TR ligands. Unlike the constitutively nuclear TRβ, this GFP-GR-TRβ chimera is cytoplasmic in the absence of hormone while translocating to the nucleus in a time- and concentration-dependent manner upon stimulation with triiodothyronine (T3) and thyroid hormone analogue, TRIAC, while the reverse triiodothyronine (3,3′,5′-triiodothyronine, or rT3) was inactive. Moreover, GFP-GR-TRβ chimera does not show any cross-reactivity with the GR-activating hormones, thus providing a clean system for the screening of TR beta-interacting EDCs. Using this assay, we demonstrated that Bisphenol A (BPA) and 3,3′,5,5′-Tetrabromobisphenol (TBBPA) induced GFP-GR-TRβ translocation at micro molar concentrations. We screened over 100 concentrated water samples from different geographic locations in the United States and detected a low, but reproducible contamination in 53% of the samples. This system provides a novel high-throughput approach for screening for endocrine disrupting chemicals (EDCs) interacting with TR beta.
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1. Introduction

Thyroid hormones are critical for normal development, growth, and metabolism of all vertebrates, including mammals (Grimaldi et al., 2013; Lopez-Juarez et al., 2012; Pascual and Aranda, 2013; Sirakov et al., 2013; Zoeller et al., 2002). They are involved in important physiological functions including, but not limited to neurogenesis and brain function (Horn and Heuer, 2010; Reinehr, 2010), homeostasis (Warner and Mittag, 2012), thermo-regulation (Ribeiro, 2008), cardiovascular health (Danzi and Klein, 2012; Vargas et al., 2012), reproductive health (Krassas et al., 2010; Wagner et al., 2008), osmoregulation and renal function (Vargas et al., 2006; Vargas et al., 2012).

The predominant TH in the circulation is 3,3′,5,5′-tetraiodothyronine (thyroxine, T4), which is the precursor for the active T3 (3,3′,5-triiodothyronine) form of the hormone. The functions of TH are mediated by the interaction of T3 with the thyroid hormone receptors (TRα and TRβ). These TRs are present in most tissues and their expression begins early in development

Abbreviations: EDCs, endocrine disrupting chemicals; TH, thyroid hormone; GR, glucocorticoid receptor; TR, thyroid receptor; T3, 3,3′,5-triiodothyronine; TRIAC, Tiratricol or triiodothyroacetic acid; rT3, reverse triiodothyronine or 3,3′,5′-triiodothyronine; T4, thyroxine; BPA, Bisphenol A; TBBPA, 3,3′,5,5′-tetrabromobisphenol A; TREs, TH-response elements; RXR, retinoid X receptor; NCoR, nuclear corepressor; SMRT, silencing mediator of retinoid and thyroid hormone receptor; HDAC, histone deacetylase; HAT, histone acetyl transferase; POCIS, polar organic chemical integrative samplers; DBD, DNA binding domain; LBD, ligand binding domain; hPCK1, phosphoenolpyruvate carboxykinase 1; COQ10A, coenzyme Q10 homolog A; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; T3-EQ, T3-equivalent.

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(Zoeller et al., 2002). They are localized in the cell nucleus and interact with specific DNA sequences called TH-response elements (TREs). TRs bind to TRE as heterodimers with retinoid X receptor (RXR) and recruit a number of cofactors such as corepressors and coactivators (Zhang and Lazar, 2000). Unliganded TR/RXR complex recruits corepressor complex containing NCoR (nuclear receptor corepressor) or SMRT (silencing mediator of retinoid and thyroid hormone receptor), which exhibits histone deacetylase (HDAC) activity. In this state, the TR/RXR heterodimer represses transcription by restricting the accessibility of basal transcription factors to targeted promoters. In the presence of T3, the corepressors are replaced by coactivator complexes, which contain histone acetylase (HAT) activity. A recent study demonstrated that in addition to the hormone-independent TR occupancy there is a significant hormone-induced TR recruitment to chromatin associated with chromatin remodeling and activated gene transcription in mouse liver tissue (Grontved et al., 2015). It was demonstrated that the T3 regulated genes can also respond to other hormonal signals. Thus, the action of the TH is often described as 'permissive' hormone action, indicating that the TH effects at the cellular, tissue, and organismal levels provide a platform for other biological signals. However, this 'permissive' action of the TH is crucial for body development and homeostasis (Grimaldi et al., 2013; Konig and Moura Neto, 2002; Oetting and Yen, 2007; Warner and Mittag, 2012). Thyroid signaling disruption could arise due to altered hormone production, transport and metabolism, as well as by disruption of the existing feed-back mechanisms, or untimely receptor activation/deactivation (Gilbert et al., 2012). In addition, mistiming of TH-modulated events may have permanent effects on neurodevelopment, whereas in adults changes in TH signaling are typically easily treatable by pharmaceuticals with no permanent deleterious effects (Murk et al., 2013).

A growing number of studies indicate that contamination of the environment with endocrine disrupting chemicals, including chemicals interfering with the thyroid signaling, may have deleterious health effects. According to the World Health Organization an endocrine disruptor as an "exogenous substance or a mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny, or (sub) populations". The Scientific Statements of the Endocrine Society from 2009 and 2015 postulate that in addition to the EDCs' effects on reproduction, breast development and cancer, prostate cancer, neuroendocrinology, obesity, and cardiovascular endocrinology, EDCs can also negatively impact thyroid metabolism (Diamanti-Kandarakis et al., 2009; Gore et al., 2015). Therefore, there is a rising need to develop novel approaches for identification of EDCs interfering with the thyroid hormone signaling [summarized in (Murk et al., 2013)].

Here we describe the development and implementation of a rapid high-throughput cell-based screening assay for detection of EDCs with thyroid hormone-like activities.

2. Materials and methods

2.1. Chemicals

Bisphenol A (BPA),3,3′,5,5′-Tetrabromobisphenol A (TBBPA), 3,3′,5-Triiodo-L-thyronine sodium salt powder (T3), triiodothyroacetic acid (TRIAC), reverse triiodothyronine (rT3), and thyroxine (T4) were purchased from Sigma and their purity was above 95% (catalog Nu: 239658-50G, 330396-100G, T6397, 51-24-1, 5817-39-0, and 51-48-9, respectively). The organic solvent DMSO (catalog Nu: D2650) was also purchased from Sigma.

2.2. Water samples collection

Environmental water samples were collected as part of ongoing U.S. Geological Survey (USGS) projects that were implemented to monitor the presence and effects of endocrine-disruptors and other contaminants of emerging concern. They were collected between 2010 and 2013 from different geographic locations in the United States (Supplemental Table 1) and included discrete grab water samples and samples collected via polar organic chemical integrative samplers (POCIS). Grab water samples were processed at the USGS, Leetown Science Center as described below.

2.3. POCIS samples

The POCIS membranes were shipped to the USGS, Columbia Environmental Research Center for analyte recovery. The procedures for preparing the POCIS samples for analysis deviated slightly from those described earlier (Alvarez et al., 2009). Briefly, chemicals were extracted from the POCIS sorbent using 25 ml of 80:20 (V:V) dichloromethane:methyl-*tert*-butyl-ether. The extracts were reduced by rotary evaporation, filtered, and composited into 2-POCIS equivalent samples thereby concentrating the amount of chemical present in each sample to aid in the detection.

2.4. Grab water samples

Grab water samples were collected in 1 l pre-cleaned amber glass bottles with Teflon lined caps (C&G Containers Scientific Supplies, Lafayette, LA). Water was acidified to pH3, held on ice, and stored at 4 °C. Within one week of collection, the preserved water samples were filtered through a GF/F filter (0.7 μm) using a solvent rinsed all-glass apparatus. Filters were rinsed with 1 ml of methanol to liberate soluble compounds from the retained suspended solids. Filtered samples and blanks were subjected to solid phase extraction (SPE) using OASIS® HLB (200 mg) glass cartridges (Waters Corporation, Milford, MA), following an existing protocol (Ciparis et al., 2012). In short, cartridges were sequentially pre-conditioned and 800 ml of filtered samples were loaded onto the cartridge at a flow rate of 5–6 ml/min (continuous vacuum). Analytes were eluted from the cartridge with 100% methanol and concentrated by evaporation. For biological testing, samples were reconstituted in DMSO and diluted in growth media to a final 1000× concentration from the original water volume while maintaining DMSO at <0.2%. Samples were added to cells for 3 h at 100 x concentration or as indicated in the text.

2.5. Construction of the GFP-GR-TRβ chimeric receptors

GFP-GR-TR TRβ construct was generated by fusing the human GR N-terminus, DNA binding domain (DBD) and hinge regions upstream of a hybrid ligand binding domain (LBD) composed of hGR helix 1 and partial loop 1–3 sequences linked to portions of the hTRβ LBD. The eGFP-GR-TR216 chimera containing eGFP-linker-hGR (2-552)-hTR LBD (216-end) and the eGFP-GR-TR226 chimera containing eGFP-linker-hGR (2-552)-hTR LBD (226-end) were prepared in spectinomycin resistant GATEway (Thermo Fisher Scientific, Waltham, MA) Entry clones. All entry clones were sequenced throughout the entire cloned region and were found to completely match the expected DNA sequence. Ampicillin resistant pFUGW lentiviral vectors expressing clones were generated with hygromycin selection marker and a Tet-regulated pTRE-tight promoter. Transfection-ready DNA was prepared using the Sigma GenElute XP Maxiprep kit and verified by agarose gel electrophoresis and restriction digest.