A Multi-Receptor and Multi-Species Assay for Potential Endocrine Disruptor Targets

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OVERVIEW

- A multiplexed, multi-receptor transactivation assay was developed to target nuclear receptors from diverse ecological species.
- Screening a targeted library showed correlation between receptor sequence similarity and chemical response.

INTRODUCTION

Screening technologies have been developed to identify xenobiotic chemicals that bind nuclear receptors and thus have potential for adverse health effects through disruption of endocrine function. However, the focus has been on human receptors despite environmental exposure to a huge diversity of other species. We evaluated a multiplexed transactivation assay providing the ability to screen for effects across multiple species and receptors.

METHODS

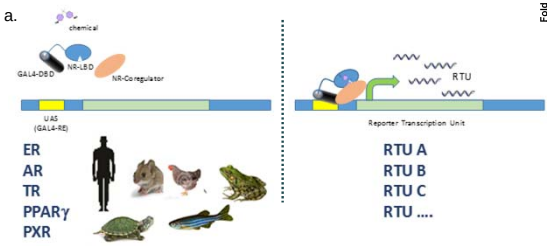

Fig 1a. The ligand-binding domains of nuclear receptors shown in Table 1 were cloned in to a mammalian one-hybrid system. Receptors were co-transfected with unique RTU reporter genes in to HepG2 cells, treated with chemicals for 24 hr, and reporter expression quantitated as shown in **b)**.

Table 1 Receptors & Species

Abbr	Gene	ID	Species
ER1-Zf	Estrogen receptor -1 Zebrafish	BC162466	*Danio rerio*
ER2a-Zf	Estrogen receptor -2A Zebrafish	BC044349	*Danio rerio*
ER2b-Zf	Estrogen receptor -2B Zebrafish	BC086848	*Danio rerio*
ER1-Fr	Estrogen receptor -1 Frog	NM_001089617	*Xenopus laevis*
ER2-Fr	Estrogen receptor -2 Frog	NM_001130954	*Xenopus laevis*
ER1-Tr	Estrogen receptor -1 Turtle	NM_001282246	*Chrysemys picta*
ER1-Ch	Estrogen receptor -1 Chicken	NM_205183	*Gallus gallus*
ERa-Hu	Estrogen receptor -alpha Human	NM_000125	*Homo sapiens*
Erb-Hu	Estrogen receptor -beta Human	NM_001437	*Homo sapiens*
AR-Zf	Androgen receptor -Zebrafish	NM_001083123	*Danio rerio*
AR-Fr	Androgen receptor -Frog	NM_001090884	*Xenopus laevis*
AR-Tr	Androgen receptor -Turtle	XM_005279527	*Chrysemys picta*
AR-Ch	Androgen receptor -Chicken	NM_001040090	*Gallus gallus*
AR-Hu	Androgen receptor -Human	NM_000044	*Homo sapiens*
TRa-Zf	Thyroid receptor-alpha Zebrashish	BC096778	*Danio rerio*
TRb-Zf	Thyroid receptor-beta Zebrashish	BC163114	*Danio rerio*
TRa-Fr	Thyroid receptor-alpha Frog	NM_001088126	*Xenopus laevis*
TRa-Tr	Thyroid receptor-alpha Turtle	XM_005294120	*Chrysemys picta*
THRa	Thyroid receptor-alpha Human	NM_199334	*Homo sapiens*
THRb	Thyroid receptor-beta Human	NM_000461	*Homo sapiens*
PPARg-Zf	PPARg-Zebrafish	NM_131467	*Danio rerio*
PPARg-Ms	PPARg-Mouse	NM_001127330	*Mus musculus*
PPARg-Hu	PPARg-Human	BC006811	*Homo sapiens*
PXR-Hu	Pregnane X Human	O75469	*Homo sapiens*
PXR-Ms	Pregnane X Mouse	O54915	*Mus musculus*
GAL4	GAL4 Yeast DBD	P04386	*Saccharomyces cerevisiae*

RESULTS

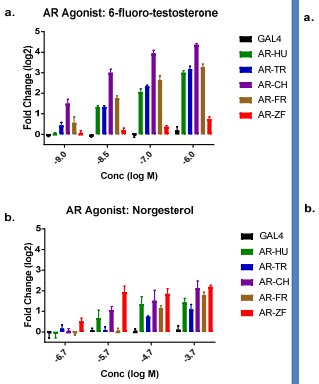

Fig. 2 AR Response. a) Initial testing included 2 nM 6-fluoro-testosterone in order to allow AR antagonist detection but zebrafish was found to be weakly responsive. **b)** Norgesterol was shown to stimulate zebrafish AR and subsequent assays included both norgestrel and 6FT.

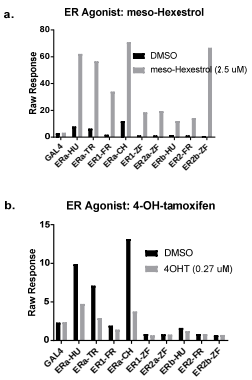

Fig. 3 ER Response. a) All ER's responded to meso-Hexestrol as an agonist. **b)** 4OH-tamoxifen reduced basal ER activity for some but not all of the ER's permitting detection of ER antagonists without added agonist.

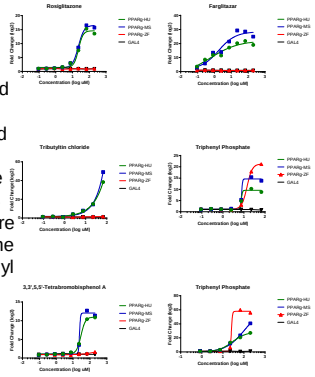

Fig. 4. PPARγ Response. Human and mouse receptors responded as expected to TZD's and an organotin; however the zebrafish receptor was unresponsive. All 3 were strongly activated by the flame retardant triphenyl phosphate.

RESULTS

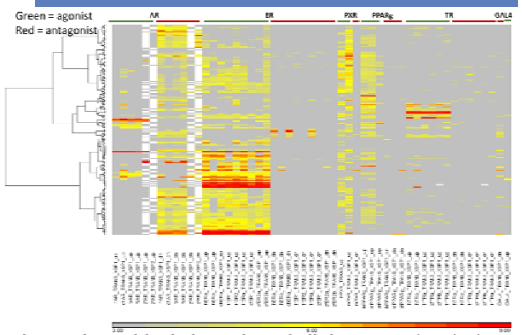

Fig. 5. Hierarchical clustering of all data. 189 chemicals selected for enrichment of receptor ligands were tested in concentration response. Hit calls were made and AC50 values calculated for actives and plotted as –logM values. Gray indicates negative activity call.

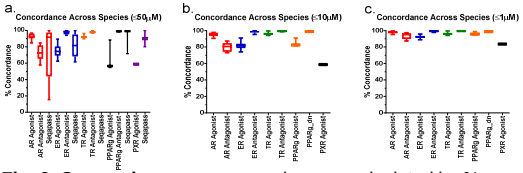

Fig. 6. Concordance across species was calculated by % agreement (hit or inactive) at the designated potency cutoff **a)** 50 μM, **b)** 10 μM and **c)** 1μM.

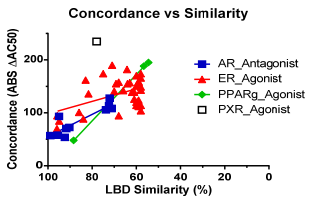

Fig. 7. Amino acid sequence similarity between species was calculated and compared to Concordance Value calculated by summing the absolute difference of AC50 values for each chemical.

CONCLUSIONS

- All receptors responded to reference compounds indicating human host cell was competent for diverse species
- High potency compounds similar across species; less so for lower potencies
- Distinct differences found for some potent compounds, particularly for zebrafish
- LBD similarity correlates with compound sensitivity
- Flexible platform readily adaptable to screen multiple receptors/species of interest

Acknowledgements:
- Screening work was done under EPA contract EP-D-12-054 by Attagene, Inc.
- Parth Kothiya for data processing