Aryl hydrocarbon receptor (AhR)- and nuclear factor E2-related factor-2 (Nrf2)-mediated regulation of the multidrug resistance-associated protein 4 (Mrp4) transport activity in killifish (*Fundulus heteroclitus*) renal proximal tubules

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Toxic and persistent environmental pollutants accumulate in the food chain. Many of these chemicals act through specific receptors, *e.g.,* AhR and Nrf2, to alter cellular function. Here we show that such chemicals alter the activity of an ATP-binding cassette (ABC) transporter in killifish renal tubules. Ligands for AhR and Nrf2 increased the transport activity of Mrp4, a proximal tubule transporter that drives foreign chemicals into forming urine.

In the aquatic environment, persistent pollutants have the potential to alter tissue function and thus disrupt normal physiological processes. Many of basic biological processes are shared with higher vertebrates. This is especially true for the kidney, where marine teleost and human proximal tubules share many functional similarities[1]. Previously, we showed that exposing killifish renal tubules to AhR ligands that are polycyclic aromatic hydrocarbons increased expression and transport function of multiple ABC transporters, including Mrp2, P-glycoprotein and Breast cancer resistance protein[4]. In the present study, we extended this work by examining the AhR-dependent Mrp4, an efflux transporter that handles both endogenous molecules involved in cellular signaling as well as xenobiotics[6]. We also examined the effects of sulforaphane (SFN), a ligand for Nrf2, a receptor that responds to cellular oxidative stress[2].

For these experiments, isolated renal tubules were incubated for 3 h in medium with the AhR agonists, β-naphthoflavone (BNF) or 2,3,7,8-Tetrachlorodibenzodioxin (TCDD). Fluo-cAMP, a fluorescent analogue of cyclic AMP and specific Mrp4 substrate[5] was then added. After 1 h luminal fluorescence was quantified by confocal microscopy. Concentration-dependent increases in luminal fluorescence were found with 0.2-1.0 μM BNF and with 0.1-1.0 nM TCDD. Inhibiting transcription or translation with actinomycin D or cycloheximide respectively prevented the increase in fluo-cAMP transport by BNF. SFN, an isothiocyanate found in broccoli, is an Nrf2 ligand and has anti-cancer properties[7]. Incubating killifish kidney tubules for 3 h with 1-2 μM SFN increased transport of fluo-cAMP into the tubule lumens. Exposing tubules to SFN and TCDD increased transport above that found with TCDD alone (Fig 1).

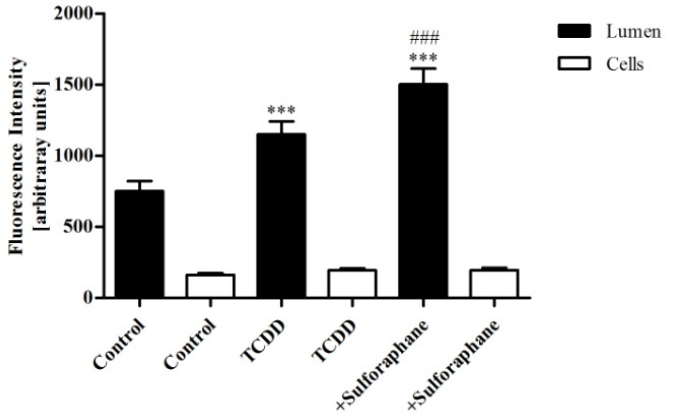

Figure 1. Exposing killifish tubules to 1 nM TCDD and 2 μM SFN together increased transporter activity above that found with TCDD alone.

The present study with killifish renal proximal tubules demonstrates the upregulation of Mrp4-mediated transport by BNF, TCDD and SFN, indicating both AhR and Nrf2 regulate Mrp4. So far, Mrp4 upregulation *via* AhR and Nrf2 has been shown on mRNA and protein level for human and mouse hepatocytes[3,8]. Future studies are planned to understand the mechanism of AhR- and Nrf2-mediated regulation using immunostaining and qRT-PCR and to determine the involvement of protein phosphorylation in receptor signaling.

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