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Cooperation of structurally different aryl hydrocarbon receptor agonists and β-catenin in the regulation of CYP1A expression

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ABSTRACT

The ligand-activated nuclear receptor AhR (aryl hydrocarbon receptor) mediates the response of hepatocytes to various exogenous compounds. AhR is classically activated by planar, aromatic hydrocarbons, but also by other, structurally rather unrelated compounds. Recent data show that the canonical Wnt/β-catenin signaling pathway is also involved in the regulation of hepatic zonal gene expression and drug metabolism in mammalian liver. Previous studies indicate that the loss of β-catenin in hepatocytes diminishes the response to the AhR agonists 3-methylcholanthrene (3MC) *in vivo* and to 2,3,7,8-tetrachlorodibenzo-[*p*]-dioxin *in vitro*. The knockout of β-catenin also impairs the zonal pattern of AhR target gene induction by 3MC. However, it is presently unknown whether the chemical nature of the AhR agonist influences the AhR/β-catenin interaction. Moreover, no information is available about the dose–response curves of AhR activation in the absence or presence of Wnt/β-catenin signaling. In the present study, we have analyzed AhR-dependent responses to different concentrations of structurally unrelated AhR agonists *in vivo* and *in vitro*. The results demonstrate that β-catenin is essential to obtain the maximum AhR response. Moreover, using transgenic mouse models which allow for the ablation of β-catenin at different age of mice, we demonstrate that the presence of β-catenin, not postnatal developmental effects in β-catenin-deficient livers, is responsible for the observed interplay of β-catenin and the AhR.

1. Introduction

The canonical Wnt/β-catenin signaling pathway is an important regulator of adult tissue homeostasis, embryonic development, and tumorigenesis. Extensive reviews of the pathway and its functions can be found in (MacDonald et al., 2009; Nejak-Bowen and Monga, 2008; Takigawa and Brown, 2008). In brief, physiological activation of the pathway by Wnt molecules acting as agonists at so-called Frizzled receptors leads to the stabilization of β-catenin, the crucial protein within this pathway. In the absence of Wnts, free cytosolic β-catenin is phosphorylated by glycogen synthase kinase 3β and casein kinase 1α in a multiprotein complex. This marks β-catenin for subsequent proteasomal degradation. Upon pathway activation by Wnts, the activity of the β-catenin phosphorylation complex is inhibited and β-catenin will accumulate in the cytosol and translocate into the nucleus. There, the protein acts as a transcriptional co-activator of TCF (T cell factor) transcription factors.

During the past few years, an important role of β-catenin in the regulation of gene expression in healthy adult liver has been revealed: transcriptionally active β-catenin is exclusively present in perivenous hepatocytes surrounding the central vein of each liver lobule (Benhamouche et al., 2006; Sekine et al., 2007). The hepatocyte-specific knockout (KO) of the *Ctnnb1* gene, encoding β-catenin, leads to a dramatic loss of the expression of perivenous marker genes such as glutamine synthetase, the model hepatic β-catenin target gene, ammonia metabolism-related genes, and especially genes encoding enzymes involved in the metabolism of drugs and xenobiotics, including many important cytochrome P450 (CYP) enzymes from families 1–3 (Benhamouche et al., 2006; Braeuning et al., 2009; Braeuning and Schwarz, 2010; Sekine et al., 2006; Tan et al., 2006). Inversely, the expression of a transgene encoding a mutant, constitutively active version of β-catenin

Abbreviations: AhR, aryl hydrocarbon receptor; BHA, butylated hydroxyanisole; BNF, β-naphthoflavone; CAR, constitutive androstane receptor; CYP, cytochrome P450; DRE, dioxin response element; GS, glutamine synthetase; GST, glutathione S-transferase; 3MC, 3-methylcholanthrene; KO, knockout; tBHQ, tert-butylhydroquinone; TCDD, 2,3,7,8-tetrachlorodibenzo-[*p*]-dioxin; TCF, T cell factor; WT, wild type.

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induces the expression of perivenous markers in periportal hepatocytes (Schreiber et al., 2011).

Drug-metabolizing enzymes such as the CYPs are known to be regulated by a set of nuclear receptors. One of the most extensively studied xenobiotic-sensing receptors is the aryl hydrocarbon receptor (AhR). For a recent review of the AhR and its functions, please refer to (Abel and Haarmann-Stemmann, 2010). The AhR is classically activated by polycyclic aromatic hydrocarbons and dioxins, of which 2,3,7,8,-tetrachloro-[*p*]-dioxin (TCDD) is the most potent and prominent ligand and a potent inducer of members of CYPs from family 1. However, the receptor is also activated by other classes of chemicals, *e.g.*, flavonoids, endogenous tryptophan derivatives, or by the antioxidant tert-butylhydroquinone (tBHQ; (Schreiber et al., 2006)).

Recent studies have demonstrated that β-catenin interacts with the AhR in the induction of drug-metabolizing enzymes: basal expression of the AhR target cytochrome P450 (Cyp) Cyp1a2 is diminished in mice with hepatocyte-specific KO of *Ctnnb1* (Braeuning et al., 2009; Sekine et al., 2006; Tan et al., 2006) and the induction of AhR target Cyps by the AhR agonist 3-methylcholanthrene (3MC) is reduced in this mouse model. Both signaling pathways synergize in the induction of AhR targets by TCDD *in vitro* (Braeuning et al., 2011; Loeppen et al., 2005; Prochazkova et al., 2011). Mechanistic studies indicate that β-catenin and the AhR physically interact and that activation of β-catenin enhances the activity of the AhR at its binding sites on the DNA (Braeuning et al., 2011). Synergistic effects with β-catenin signaling have also been reported for other receptors involved in the induction of drug-metabolizing enzymes, *i.e.*, the constitutive androstane receptor (CAR) and Nrf2 (Braeuning et al., 2009). Interestingly, hepatic zonation of target enzyme induction was lost in mice with KO of *Ctnnb1* in that study. By contrast, the zonated response to CAR activation was preserved when β-catenin was not knocked out in the early postnatal phase as in (Braeuning et al., 2009), but not before adulthood, suggesting β-catenin-dependent postnatal priming of perivenous hepatocytes for the susceptibility to CAR agonists (Ganzenberg et al., 2013).

The AhR is activated by different substances with considerable differences regarding their chemical structures (see above). These differences and especially the differences regarding downstream biological effects (*e.g.*, perivenous induction of CYP1A by the AhR agonist 3MC but periportal induction by the AhR agonist β-naphthoflavone (BNF)) point towards differences in AhR-related cellular signaling following exposure to these different types of agonists. Up to now, it is not known whether the chemical nature of the AhR agonist has an influence on the interaction of the β-catenin pathway with the activated AhR. Furthermore, the observation that AhR target gene induction at a certain concentration of TCDD is lowered in the absence of β-catenin raises questions about the shape of the corresponding dose–response curves, which cannot be answered with the existing studies. In addition, it is unknown whether the zonation of the AhR response solely depends on the presence of β-catenin at the time point of treatment or whether developmental aspects play a role as in the case of CAR activation. Therefore, in the present study, we examined the response of mice with hepatocyte-specific KO of *Ctnnb1* to three different AhR agonists: the polycyclic aromatic hydrocarbon 3MC, the flavonoid BNF, and the antioxidant butylated hydroxyanisole (BHA), the metabolic precursor of tBHQ. Additional studies were conducted *in vitro* to obtain information about the dose–response relationship of AhR activation in the absence or presence of active β-catenin. Moreover, a comparison of 3MC-induced AhR target gene expression in mice with early postnatal and adult KO of β-catenin was performed.

2. Material and methods

2.1. Animal breeding

Mice with loxP site-flanked *Ctnnb1* (encoding β-catenin) alleles (Huelsken et al., 2001) were interbred either with Alb-Cre or TTR-Cre mice as previously described (Ganzenberg et al., 2013). This resulted in two different mouse models for Cre recombinase-mediated genetic ablation of β-catenin. First, mice with albumin (Alb-Cre model) promoter-driven hepatocyte-specific knockout (KO) of *Ctnnb1* (Braeuning et al., 2009), where Cre recombinase accomplishes target gene recombination shortly after birth (Postic and Magnuson, 2000). Second, mice with transthyretin (TTR-Cre model) promoter-driven hepatocyte-specific KO of *Ctnnb1* (Ganzenberg et al., 2013), where a modified Cre enzyme can be activated by tamoxifen at the desired point in time. Genotyping was performed by standard PCR using the following primer pairs: *Ctnnb1*^{loxP}_fwd 5′-ACTGCCTTTGTTCTCTTCCCTTCTG-3′; *Ctnnb1*^{loxP}_rev 5′-CAGCCAAGGAGAGCAGGTGAGG-3′; Cre_fwd 5′-TCCATGAGTGAACGAACCTGGTCG-3′; Cre_rev 5′-TTTGCCTGCATTACCGGTCGATGC-3′. Only male mice were used in the study. The genetic background of the animals was C3H/He.

2.2. Animal experimentation

The group size for treatment was 5–6 mice per genotype. Treatment followed the experimental outline described in (Ganzenberg et al., 2013) and (Braeuning et al., 2009). At 8 weeks of age, mice with Alb-Cre KO of *Ctnnb1* (genotype: *Ctnnb1*$^{loxP/loxP}$, Alb-Cre^{+}) and age-matched Cre-negative controls (WT; *Ctnnb1*$^{loxP/loxP}$, Alb-Cre^{-}) were treated with different AhR activators by intraperitoneal injection as follows: 3MC (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 10 or 100 mg/kg body weight (single injection); BNF (Sigma, Taufkirchen, Germany) at 20 or 150 mg/kg body weight (single injection for 20 mg/kg, 2 × 75 mg in 24 h intervals for 150 mg/kg), or BHA (Sigma) at 350 mg/kg body weight (single injection). Mice with TTR-Cre KO of *Ctnnb1* (*Ctnnb1*$^{loxP/loxP}$, TTR-Cre^{+}) and age-matched WT (*Ctnnb1*$^{loxP/loxP}$, TTR-Cre^{-}) controls were treated at 10 weeks of age at five consecutive days by intraperitoneal injections with 1.5 mg tamoxifen per mouse per day. Following a treatment-free interval of 4 weeks, mice were treated with 3MC by single intraperitoneal injection at 10, 25, or 50 mg/kg body weight. All AhR activators were dissolved in corn oil. Control mice received an injection with corn oil alone. Tamoxifen was dissolved in a mixture of 1:7 EtOH/corn oil. Mice were sacrificed 48 h (BHA: 72 h) after start of inducer treatment, between 9 a.m. and 11 a.m. to reduce circadian variation. Livers were excised and tissue aliquots were either immediately frozen in liquid nitrogen or fixed in Carnoy's solution. Mice had access to tap water and standard chow *ad libitum*, received humane care, and protocols complied with institutional guidelines.

2.3. Immunostaining

Carnoy-fixed liver tissue was embedded in paraffin. Slices of 5 μm thickness were stained for glutamine synthetase (GS) and cytochrome P450 1A (CYP1A) using standard methodology as previously described (Ganzenberg et al., 2013) and antibodies against GS (1:1000 dilution; Sigma) or CYP1A (1:1000; gift from Dr. R. Wolf, University of Dundee, Dundee, UK) in combination with appropriate horseradish peroxidase-conjugated secondary antibodies (1:100; Dako, Glostrup, Denmark) with the substrate 3-amino-9-ethylcarbazole/H_2O_2. Counterstaining of nuclei was done by hematoxylin staining. An Axio Imager. M1 light microscope (Zeiss, Göttingen, Germany) was used for image acquisition.