Disruption of cell cycle control in rat liver epithelial 'stem-like' cells by AhR ligands

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Introduction

The aryl hydrocarbon receptor (AhR) ligands are efficient liver tumor promoters, which can alter cell cycle control in various hepatic models [1-3]. However, the role AhR in regulating cell proliferation is still far from being elucidated, since the ligand -dependent activation of AhR can induce both cell cycle arrest or proliferation, depending on the cellular model. Diverse AhR ligands, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) or flavonoids have been shown to disrupt contact inhibition and to stimulate cell proliferation in rat liver epithelial WB -F344 cell line, considered to represent an in vitro model of liver progenitor cells [4-7]. The release from contact inhibition induced by TCDD or PCBs is associated with upregulation of cyclin A expression and cyclin A/cdk2 activity [4, 6].

TCDD and PCB 126 induce cholangiocarcinomas in rats, suggesting that undifferentiated liver cells might be a direct target of carcinogenic effects of dioxin-like compounds in liver [8]. Therefore, there is a need to define the role of AhR ligands in cell cycle regulation in liver progenitor cells. Using model non-persistent AhR ligands, we have investigated their impact on cell cycle regulation in confluent WB-F344 cells. In order to define the role of AhR and its dimerization partner ARNT in the disruption of contact inhibition, we developed WB-F344 cells stably expressing dominant negative AhR and ARNT variants and studied effects of model AhR ligands in these cells.

Methods and Materials

Cells, detection of cell proliferation and cell cycle, cell transfections. WB-F344 rat liver epithelial cells were grown in modified E-MEM (Sigma-Aldrich, St. Louis, MO) and the proliferative effects of test compounds on confluent cells were determined as described previously [5,6]. WB-F344 cells were transfected with truncated AhR pAHR?495-805 [9], ARNT deletion mutant pArnt? b/CMV4 [10] and/or with resistance plasmids, using Lipofectamine transfection reagent (Invitrogene, Carlsbad, CA). Stable transfectants were developed using puromycin or G418 as selection antibiotics (Invitrogene). The clones used in this study were selected based on inducibility of CYP1A1 levels.

Detection of CYP1A1 and cyclin A mRNA and protein levels. Total RNA was isolated using the RNeasy mini kit (Qiagen, Valencia, CA) including treatment with DNase I (Qiagen). The amplifications of the samples were carried out using QuantiTect Probe RT-PCR kit (Qiagen) according to manufacturer's specifications. The primers and probe for rat CYP1A1 and for the reference gene porphobilinogen deaminase have been described preciously [7]. For cyclin A2 (GenBank accession number XM_342229), we used the following primers and probe: forward 5 '-CACGTACCTTAGGGAAATGGAGGTTA-3', reverse 5'-ATTCTTCTCCCACTTCAACTAGCCAG-3', probe 5'-CACAAGGATGGCCC GCATACTGTTAGTG-3'. CYP1A1 and cyclin A protein levels were determined by Western blotting.

Results and Discussion

Both the flavonoid 3'-methoxy-4'-nitroflavone (3'M4'NF) and PAHs with low genotoxicity, benzo[b]fluoranthene and benz[a]

anthracene, increased expression of cyclin A and/or cyclin A/cdk2 complex activity, which corresponded with an increased percentage of cells entering S-phase (Table 1). However, PAHs did not stimulate pRB hyperphosphorylation, suggesting that respective cdks are not active in these conditions. 3'M4'NF induced nuclear translocation of AhR to the same extent as TCDD, however, the levels of CYP1A1 mRNA and protein induced by 3'M4'NF were significantly lower. In a marked contrast, benzo[a] pyrene, which is a powerful genotoxic PAH and induced apoptosis in WB-F344 cells, stimulated strong cyclin A accumulation and pRb hyperphosphorylation.

Our data thus suggested that various types of compounds behaving as AhR ligands induce the same type of effect in confluent WB-F344 cells, the release from contact inhibition leading to significantly increased cell numbers. However, the direct mechanistic evidence for involvement of AhR and ARNT in this effect is still missing. Therefore we have tested the effects of model AhR ligand, PCB 126, on cells stably expressing dnAhR and dnARNT constructs. We found that only dnAhR efficiently suppressed the release from contact inhibition. Using CYP1A1 as marker for AhR activation, and cyclin A as a marker for induced proliferation, we found that dnAhR suppressed both induction of CYP1A1 and cyclin A mRNAs (Table 2) and protein. In contrast, dnARNT only suppressed CYP1A1 induction. Thus, the role for ARNT in the release from contact inhibition is still questionable. Further studies should elucidate its contribution to this effect, which might be an important mode of action associated with tumor promotion.

Table 1: Effects of AhR ligands on cell cycle regulation in contact-inhibited rat liver epithelial 'stem-like' WB-F344 cells

Compound	Cell numbers	S-phase cells	pRb phosphorylation	Cyclin A levels and/or cyclin A/cdk2 activity
TCDD	↑	↑	-	↑
PCB 126	↑	↑	**not analyzed**	↑
Benzo[b] fluoranthene	↑	↑	-	↑
Benz[a]anthracene	↑	↑	-	↑
Benzo[a]pyrene	-/↓	↑↑	↑↑	↑↑
3'M4'NF	↑	↑	**not analyzed**	↑

Table 2: Effects of 24-h exposure to PCB 126 (100 nM) on induction of CYP1A1 and cyclin A mRNA levels in wild-type and transfected WB-F344 variants.

Treatment	CYP1A1	Cyclin A
Wild type WB-F344 cells + DMSO	**1.0 ± 0.2**	**1.0 ± 0.2**
Wild type WB-F344 cells + PCB 126	**156.5 ± 8.9**	**2.3 ± 0.4**
pAHR?495-805/WB-F344 + DMSO	**1.0 ± 0.3**	**1.0 ± 0.1**
pAHR?495-805/WB-F344 + PCB 126	**32.4 ± 11.1**	**0.9 ± 0.3**
vector control/WB-F344 cells + DMSO	**1.0 ± 0.4**	**1.0 ± 0.3**
vector control/WB-F344 cells + PCB 126	**162.9 ± 14.1**	**2.3 ± 0.2**
pArnt?b/CMV4/WB-F344 + DMSO	**1.0 ± 0.2**	**1.0 ± 0.2**
pArnt?b/CMV4/WB-F344 + PCB 126	**5.9 ± 3.3**	**2.2 ± 0.3**
vector control/WB-F344 cells + DMSO	**1.0 ± 0.2**	**1.0 ± 0.1**
vector control/WB-F344 cells + PCB 126	**148.6 ± 0.1**	**2.5 ± 0.5**

Results are expressed relative to respective DMSO-treated controls.

Acknowledgements:

Authors thank Drs. Thomas A. Gasiewicz (University of Rochester, NY), Alvaro Puga (University of Cincinnati Medical Center, OH) and Roland H. Wenger (University of Leipzig, Germany) for kindly providing 3'-methoxy-4'-nitroflavone, dnAhR and dnARNT constructs, respectively. This work was supported by grant No. B6004407 (Grant Agency of the Academy of Sciences of the Czech Republic), Czech Ministry of Agriculture (grant No. 0002716201) and Research Plan No. AVOZ 5000507 of the ASCR.

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