Biochemical and Biophysical Research Communications 443 (2014) 1092–1096

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Insulin like growth factor 2 regulation of aryl hydrocarbon receptor in MCF-7 breast cancer cells

Justin K. Tomblin, Travis B. Salisbury *

Department of Pharmacology, Physiology and Toxicology, Joan C. Edwards School of Medicine, Marshall University, 1 John Marshall Drive, Huntington, WV 25755, USA

ARTICLE INFO

Article history:
Received 16 December 2013
Available online 28 December 2013

Keywords:
Aryl hydrocarbon receptor
IGF-2
CCND1
Breast cancer cells

ABSTRACT

Insulin like growth factor (IGF)-1 and IGF-2 stimulate normal growth, development and breast cancer cell proliferation. Cyclin D1 (CCND1) promotes cell cycle by inhibiting retinoblastoma protein (RB1). The aryl hydrocarbon receptor (AHR) is a major xenobiotic receptor that also regulates cell cycle. The purpose of this study was to investigate whether IGF-2 promotes MCF-7 breast cancer proliferation by inducing AHR. Western blot and quantitative real time PCR (Q-PCR) analysis revealed that IGF-2 induced an approximately 2-fold increase ($P < .001$) in the expression of AHR and CCND1. Chromatin immunoprecipitation (ChIP), followed by Q-PCR indicated that IGF-2 promoted ($P < .001$) a 7-fold increase in AHR binding on the CCND1 promoter. AHR knockdown significantly ($P < .001$) inhibited IGF-2 stimulated increases in CCND1 mRNA and protein. AHR knockdown cells were less ($P < .001$) responsive to the proliferative effects of IGF-2 than control cells. Collectively, our findings have revealed a new regulatory mechanism by which IGF-2 induction of AHR promotes the expression of CCND1 and the proliferation of MCF-7 cells. This previously uncharacterized pathway could be important for the proliferation of IGF responsive cancer cells that also express AHR.

1. Introduction

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor whose activity is regulated by lipid soluble environmental toxicants [1]. 2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD) is a prototypical AHR agonist which is found in Agent Orange [1]. The binding of TCDD to AHR stimulates the AHR to translocate into the nucleus and stimulate transcription through specific xenobiotic response elements (XREs) in enhancers and promoters of TCDD stimulated genes [1,2]. TCDD through AHR induces the expression of a "battery" of phase I and phase II drug metabolizing enzymes including the prototype TCDD–AHR gene target cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) [1,2].

The AHR also regulates cell cycle in part by binding with Cyclin D1 (CCND1) and cyclin dependent kinase 4 (CDK4) [3,4]. CDK4 phosphorylates retinoblastoma protein 1 (RB1), which inhibits RB1-mediated repression of E2F transcription factors [5–7]. The activation of E2F induces the expression of E2F target genes that are important for DNA synthesis and cell cycle advance [5–7]. Mitogens promote CDK4 activity by increasing the levels of cyclin proteins including CCND1 [5–7]. By functioning as a regulatory subunit on CDK holoenzymes, CCND1 promotes the phosphorylation and inhibition of RB1 to promote cell cycle advance and proliferation [5–7]. The AHR binds to CDK4 during advance through the cell cycle in human MCF-7 breast cancer cells [4]. TCDD binding to AHR attenuates AHR binding with CDK4, which correlated with cell cycle arrest and reductions in RB1 phosphorylation in MCF-7 cells [4]. CCND1 was also present in CDK4-AHR complexes [4].

Insulin like growth factor (IGF)-1 and IGF-2 stimulate growth, development and the proliferation of human cancer cells including breast cancer cells [8,9]. MCF-7 breast cancer cells have been reported to express high levels of IGF-1 receptor (IGF-1R) and insulin receptor subtype A receptor (IR-A) [8,9]. IGF-R1 and IR-A mediate the proliferative effects of IGFs on human breast cancer cells by inducing the phosphoinositide 3-kinase (PI3K)/AKT (protein kinase B) pathway and the mitogen-activated protein kinase (MAPK) pathway [8–10]. IGF-1 and IGF-2 have also been reported to increase levels of CCND1 to induce proliferation [6,8,9]. CCND1 promoter activity is regulated through multiple enhancers including activator protein-1 (AP-1) and T-cell factor-1 (Tcf-1)/lymphoid enhancing factor-1 (Lef-1) sites [11–14]. The transcription factors Jun and Fos bind to the AP-1 response elements [11,12]. The transcriptional co-activator β-catenin confers transcriptional activity to TCF/LEF transcription factors bound to TCF/LEF elements in the CCND1 promoter [13,14].

We have recently shown that adipocytes secrete levels of IGF-2 that are sufficient to stimulate the proliferation of MCF-7 and T-47D breast cancer cells [15]. We also found that AHR knockdown

* Corresponding author. Fax: +1 304/696 7391.
E-mail address: salisburyt@marshall.edu (T.B. Salisbury).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.12.112

MCF-7 cells were less responsive to the proliferative effects of IGF-2 [15]. The purpose of this study was to investigate if: (1) IGF-2 signaling regulates the AHR and (2) IGF-2 induction of CCND1 requires AHR. We provide evidence that IGF-2 signaling activates AHR and that AHR is important for inducing the expression of CCND1 and MCF-7 proliferation. This is a new link between IGF-2 signaling and AHR.

2. Methods

2.1. Materials and MCF-7 cell culture

Dulbecco's Modified Eagle Medium/High glucose (DMEM) with L-glutamine and sodium pyruvate, 10% fetal bovine serum, penicillin, and streptomycin (100 μg/mL) and phosphate buffered saline (PBS) were purchased from Fisher Scientific. Non-specific control RNA (cRNAi) (cat # D-001810-01-20), short interfering RNA against the AHR (AHRi) (J-004990-08-0010) and Dharmafect transfection reagent (#1) were purchased from Thermo Scientific. MCF-7 human breast cancer cells were purchased from ATCC (Manassas) and maintained in DMEM, 10% FBS, with penicillin (100 U/mL) and streptomycin (100 μg/mL) and .01 μg/mL bovine insulin (Cell Applications, Inc.) Insulin like growth factor 2 (IGF-2) was purchased from R & D systems and reconstituted in phosphate buffered solution.

2.2. Western blot analysis to determine IGF-2 induction of AHR and CCND1

200,000 MCF-7 cells plated in 35 mm plates (50% confluent) were serum starved overnight in phenol red-free DMEM and then treated with PBS vehicle or IGF-2 (100 ng/mL) for 3 h. This time point was selected based on our preliminary time course experiments showing that IGF-2 induction of CCND1 mRNA is maximal at 3 h post IGF-2 (data not shown). Total cellular extract was isolated in 200 μL of 2× sample lysis buffer (Bio-RAD; cat #161-0737) and approximately 12.5 μg of protein was subjected to SDS PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad; Hercules, CA). Membranes were blocked in PBS, .01% Tween 20 (Bio-Rad; Hercules, CA) (PBS-T), 5% (wt/vol) lowfat powdered milk for 1 h and incubated overnight with primary antibody at 4 °C with gentle mixing. Membranes were rinsed five times (five minutes each wash) with PBS-T and then incubated with an appropriate HRP-labeled secondary antibody (diluted 1:10,000 in PBS, .01% tween-20, 5% milk) (Thermo Scientific, Pierce) for 1 h, followed with rinsing five times (five minutes each wash) in PBS-T. Membranes were developed with enhanced chemiluminescent substrate (Millipore, Immobilon ECL substrate) and exposure to X-ray film (Midwest Scientific). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody was purchased from Millipore (cat # MAB374), AHR antibody from Santa Cruz (Cat # H-211) and CCND1 antibody from Millipore (cat # 04-1151). Equal protein loading was confirmed by GAPDH Western blots. Normalized levels of AHR, and CCND1 were expressed as a ratio of AHR/GAPDH and CCND1/GAPDH. Densitometry was calculated with ImageJ PC-based software (National Institute of Health).

2.3. Q-PCR analysis to determine IGF-2 induction of AHR, CYP1A1 and CCND1

200,000 MCF-7 cells plated in 35 mm plates were serum starved overnight in phenol red-free DMEM, and then treated with PBS vehicle or IGF-2 (100 ng/mL) for 3 h. Total RNA was isolated in TRI-Reagent and quantitated by Nanodrop spectrophotometry. RNA was reverse transcribed to complementary DNA (cDNA) (Verso cDNA kit; Thermo Fisher Scientific; cat # AB-1453/B). Resulting cDNAs were subjected to quantitative real-time PCR (Q-PCR) using gene specific primers (300 nM per reaction) and 40 cycles of PCR in accordance with Absolute Blue SYBR Green Rox Mix (Thermo Fisher Scientific; cat # AB-4162/B) protocols. Relative gene expression between controls and IGF-2 treated cells was calculated using the formula $2-\Delta\Delta CT$, as described by Livak and Schmittgen [16]. Glyceraldehyde-3-phosphate (GAPDH) mRNA levels served as the internal control. Primer sequences for GAPDH, AHR, CCND1 and CYP1A1 were: GAPDH (forward, 5′-CATGAGAAGTATG ACAACAGCCT-3′; reverse, 5′-AGTCCTTCCACGATACCAAAGT-3′), AHR (forward, 5′-ACATCACCTACGCCAGTGG-3′; reverse, 5′-CTCTAT GCCGCTTGGAAGGAT-3′), CCND1 (forward, 5′-CCGCAATGACCCCGC ACGAT-3′; reverse, 5′-AGGGCAACGAAGGTCTGCGC-3′) and CYP1A1 (forward, 5′-CTTCACCCTCATCAGTAATGGTC-3′; reverse, 5′-AGGCTG GGTCAGAGGCAAT′-3). The Harvard Primer Bank http://pga.mgh.harvard.edu/primerbank/ was used to design primers. Primer specificity was verified with melt curve analysis and NIH primer blast search engines http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome.

2.4. Chromatin immunoprecipitation

MCF-7 cells (500,000 per 60 mm plate) were serum starved overnight in phenol red-free DMEM, and then treated with PBS vehicle or IGF-2 (100 ng/mL) for 3 h. Formaldehyde (1%) was then added to medium for 10 min, followed by Glycine (.5 M) for 5 min. Cells were rinsed with PBS, collected in PBS, pelleted by centrifugation, and lysed in 300 μL of lysis buffer (1% SDS; 5 mM EDTA; 50 mM Tris–HCl, pH 8) per 60 mm plate plus protease inhibitors (Thermo Scientific) for 15 min on ice. Cell extracts were sonicated (5 times, each time 10 s) and diluted 1:10 in dilution buffer (16.7 mM Tris–HCl, pH 8; 167 mM NaCl; 1.2 mM EDTA; 0.01% SDS; 1.1% Triton X-100), rotated overnight at 4 °C with 1 μg of non-specific IgG (Santa Cruz; cat # 2027) or anti-AHR antibody (Santa cruz; cat # H-211). Antibody-chromatin complexes were collected using 5 μL of magnetic protein A beads (life technologies; cat # 100.01D) with rotation at 4 °C for 90 min. Using magnetic separation (life-technologies; part # 49-2025), beads were washed sequentially with buffer 1 (20 mM Tris–HCl, pH 8; 150 mM NaCl; 2.0 mM EDTA; 0.1% SDS), buffer 2 (20 mM Tris–HCl, pH 8; 500 mM NaCl; 2.0 mM EDTA; 0.1% SDS), buffer 3 (10 mM Tris–HCl (pH 8); 0.25 M LiCl; 1 mM EDTA; 1% NP-40; 1% deoxycholate), and then 1× TE buffer for five minutes each, and incubated at 65 °C for 4–6 h in elution buffer (1% SDS, 0.1 M $NaHCO_3$) with proteinase K. DNA was purified (Qiagen; cat # 28204) and analyzed using real time PCR. Primers spanning AP-1 and TCF/LEF response elements in the CCND1 promoter and xenobiotic response elements (XREs) in the CYP1A1 promoter were used: AP-1 (forward, 5′-GGCAGAG GGGACTAATATTTCCAGCA-3′; reverse, 5′-GAATGGAAAGCTGAGAA ACAGTGATCTCC-3′) [17], TCF/LEF (forward, 5′-GCTCCCATTCTCTGC CGG-3′; reverse, 5′-CGGAGCGTGCGGACTCTG-3′) [18] and XRE (forward, 5′-ACGCAGACCTAGACCCTTTGC-3′, reverse, 5′-CGGGTGCGC GATTGAA-3′) [19]. ChiP data was expressed as % input, in which signals obtained from the ChIP are divided by signals obtained from an input sample.

2.5. AHR knockdown experiments

MCF-7 cells were reverse transfected using methods we have used previously to selectively target genes for knockdown [20]. Briefly, 200,000 MCF-7 cells were plated in phenol red-free DMEM, 5% charcoal treated FBS, 50 nM cRNAi or AHRi, 2 μL of Dharmafect #1 per well of a 6 well plate for 12 h and then new media was applied for 24 h. Cells were then serum starved overnight in phenol

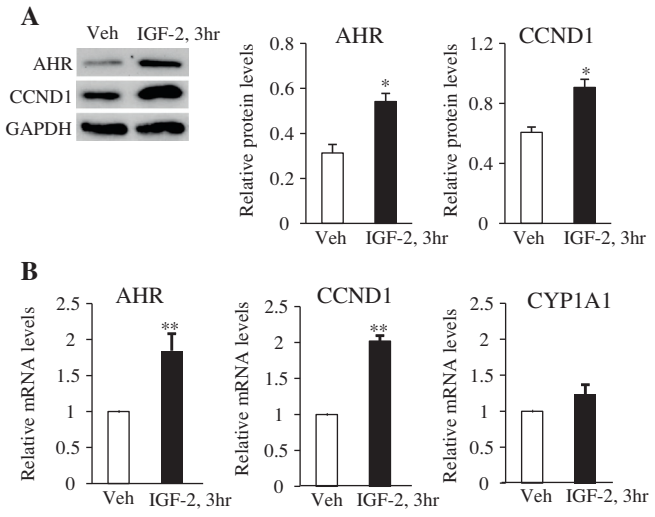

Fig. 1. IGF-2 stimulates AHR. MCF-7 cells were treated with vehicle (Veh) or IGF-2 (100 ng/mL) for 3 h. (A) Total cellular protein was then isolated and subjected to Western blot analysis. The blot was then probed with the indicated antibodies. Relative level of AHR and CCND1 protein was expressed as a ratio of AHR/GAPDH and CCND1/GAPDH. Significant ($P < .01$) increases in AHR, CCND1 protein by IGF-2 are indicated (∗). (B) Quantitative real-time quantitative polymerase chain reaction (Q-PCR) analysis of relative mRNA levels of CCND1, AHR and CYP1A1, with normalization to GAPDH internal control. The value in vehicle treated cells was set to 1. Significant ($P < .01$) increases in AHR and CCND1 mRNA by IGF-2 are indicated (∗∗). CYP1A1 mRNA was not significantly induced by IGF-2. Data shown are the means ± S.E. of (A) three and (B) four independent experiments.

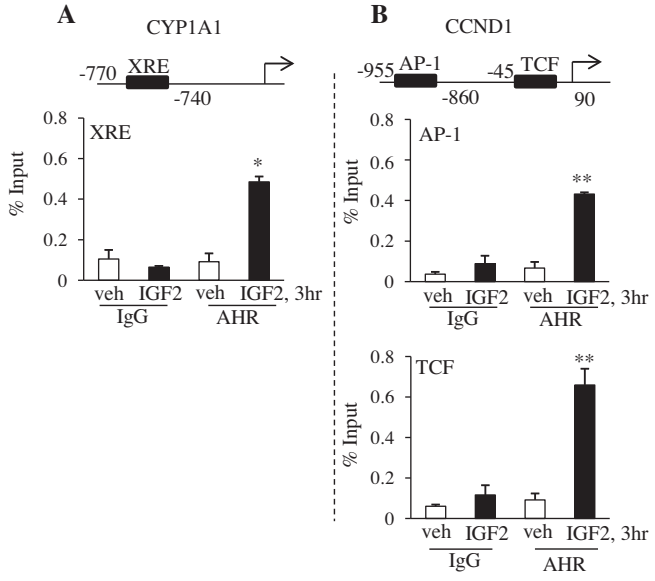

Fig. 2. IGF-2 stimulates AHR binding on CYP1A1 and CCND1 gene promoters. MCF-7 cells were treated with vehicle (Veh) or IGF-2 (100 ng/mL) for 3 h and chromatin immunoprecipitation (ChIP) experiments were conducted, followed by Q-PCR. (A) A significant ($P < .0001$) increase in AHR binding to the XRE on the CYP1A1 promoter induced by IGF-2 is indicated (∗). (B) A significant ($P < .0001$) increase in AHR binding to the AP-1 and TCF/LEF site induced by IGF-2 is indicated as (∗∗). Data shown are the means ± S.E. of three independent experiments.

red-free DMEM, followed by treatment with PBS vehicle or IGF-2 (100 ng/mL) for 3 h. Treatments were stopped and cellular protein or mRNA was isolated for Western blot and Q-PCR analysis, respectively, as detailed in Sections 2.2 and 2.3.

2.6. Cell growth experiments

MCF-7 cells were reverse transfected with 50 nM cRNAi or AHRi as detailed in Section 2.4 for 36 h and then serum starved overnight in phenol red-free DMEM. Cells were treated with PBS vehicle or IGF-2 (100 ng/mL) for three additional days. Cells were collected in trypsin and manual cell counting using a hemocytometer and trypan blue was used to determine live cell number.

2.7. Statistics

Two-tailed, paired *t* tests with confidence intervals of 95% were used to determine statistically significant differences between two groups (vehicle versus IGF-2 treatment) in Fig. 1. The Newman–Keuls (SNK) post hoc test was used to determine statistically significant differences among groups following one-way analysis of variance (ANOVA) in Fig. 2–4. Specific *P* values are indicated in Section 3.

3. Results

3.1. IGF-2 increases AHR expression

To investigate whether IGF-2 stimulates AHR expression, overnight serum starved MCF-7 cells were treated with vehicle or IGF-2 (100 ng/mL) for 3 h. Western blot analysis revealed that IGF-2 induced 1.7- and 1.5-fold increases ($P < .01$) in AHR and CCND1 protein, respectively, compared with vehicle (Fig. 1A). The observed increases in AHR and CCND1 protein correlated with significant increases ($P < .01$) in AHR mRNA (1.9-fold) and CCND1 mRNA (2-fold) in IGF-2 treated cells compared with control cells (Fig. 1B). The levels of CYP1A1 mRNA, which is a TCDD-AHR gene target, were not induced by IGF-2 treatment (Fig. 1B). This result suggests that IGF-2 signaling does not induce rapid increases in the levels of a lipophilic endogenous AHR ligand capable of inducing CYP1A1 expression.

3.2. IGF-2 stimulates AHR binding to CYP1A1 and CCND1 gene promoters

To determine if IGF-2 promotes AHR binding to gene promoters, we performed chromatin immunoprecipitation experiments followed by real-time quantitative PCR (Q-PCR) (ChIP–qPCR). We first focused on AHR binding XREs in the CYP1A1 promoter [19]. AHR–ChIP–qPCR experiments revealed that AHR binding to the XREs on the CYP1A1 promoter in vehicle treated cells was low and not significantly higher than non-specific IgG (Fig 2A). In contrast, IGF-2 promoted a 5-fold increase ($P < .0001$) in AHR binding to the XREs on the CYP1A1 promoter compared to vehicle and this was significantly higher than non-specific IgG (Fig. 2A).

To determine if IGF-2 stimulates the AHR to bind to the promoter of an induced gene, we examined AHR binding to the AP-1 and TCF/LEF response elements on the CCND1 promoter. These transcription factor binding sites were selected because AP-1 and TCF/LEF sites promote CCND1 transcription [12–14]. The binding of AHR to AP-1 and TCF/LEF sites on the CCND1 promoter was minimal in vehicle treated cells and not significantly different than non-specific IgG (Fig. 2B). In contrast, the binding of AHR to AP-1 and TCF/LEF response elements on the CCND1 promoter was substantially increased (approximately 7-fold) by IGF-2 and this was significantly ($P < .0001$) greater than non-specific IgG (Fig. 2B). Collectively, these results indicate that IGF-2 promotes AHR binding to CYP1A1 and CCND1 gene promoters.

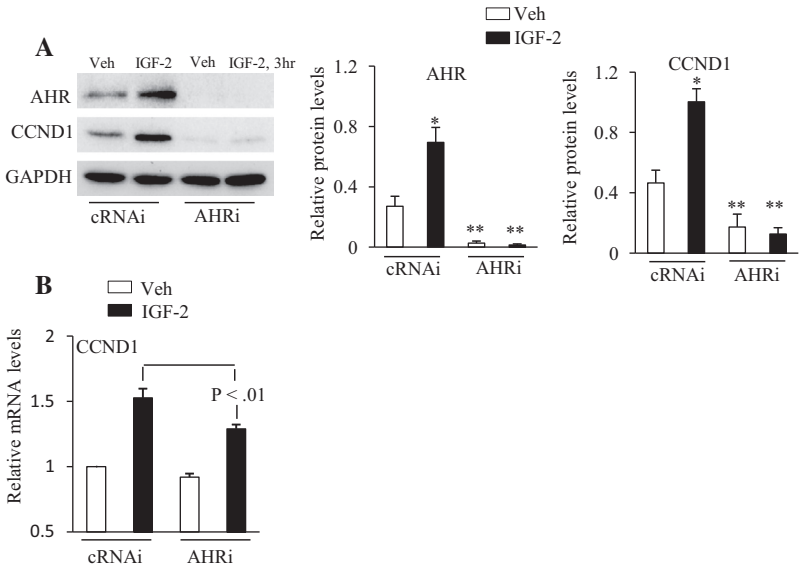

Fig. 3. IGF-2 induction of CCND1 requires AHR. MCF-7 cells were reverse transfected (see Section 2 for details) with cRNAi or AHRi prior to treatment with vehicle (Veh) or IGF-2 (100 ng/mL) for 3 h. (A) Total cellular protein was then isolated and subjected to Western blot analysis. The blot was then probed with the indicated antibodies. Relative level of CCND1 and AHR protein was expressed as a ratio of AHR/GAPDH and CCND1/GAPDH. A significant ($P < .001$) increase in AHR and CCND1 by IGF-2 is indicated by (*). A significant ($P < .001$) decrease in AHR and CCND1 by AHRi is indicated by (**). (B) Q-PCR analyses of relative levels of CCND1 mRNA, expressed normalized to GAPDH. A significant decrease in CCND1 by AHRi is indicated by ($P < .01$). Data shown are the means ± S.E. of three independent experiments.

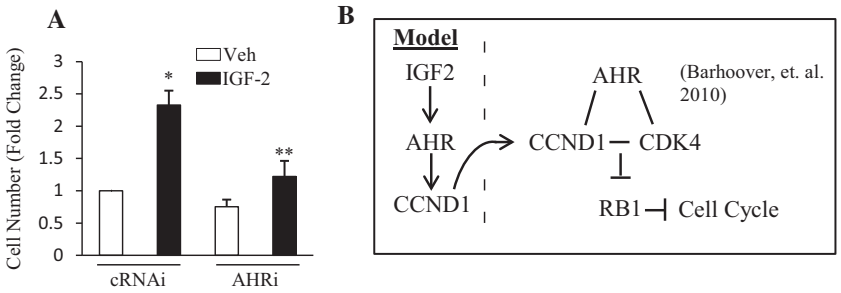

Fig. 4. IGF-2 induction of MCF-7 proliferation requires AHR. (A) MCF-7 cells were reverse transfected (see Section 2 for details) with cRNAi or AHRi prior to treatment with vehicle (Veh) or IGF-2 (100 ng/mL). After three days in culture, cells were collected and live cell number was determined. Cell number (Fold change) was expressed relative to the number of live cells in the cRNAi plus Veh group, which was arbitrarily assigned a value of 1. (B) Model of AHR roles in IGF-2 signaling. Dotted lines distinguish our data herein and data from Barhoover et al. 2010. IGF-2 signaling increases AHR, increases in AHR stimulate increases in CCND1 protein. The increased levels of AHR in IGF-2 treated cells functions as a scaffold that bridges CCND1 and CDK4, which in turn stimulates the phosphorylation and inhibition of RB1. Inhibition of RB1 promotes cell cycle and MCF-7 proliferation.

3.3. AHR knockdown inhibits IGF-2 induction of CCND1

Next, experiments were conducted to investigate if IGF-2 induction of CCND1 requires AHR. Overnight serum starved control and AHR knockdown MCF-7 cells were treated with vehicle or IGF-2 (100 ng/mL) for 3 h. As shown in Fig. 3A, AHR protein levels were lower ($P < .0001$) in AHR knockdown cells (by 10-fold) than control cells. IGF-2 stimulated ($P < .001$) approximately 2-fold increases in AHR and CCND1 protein in control cells, which was completely abrogated in AHR knockdown cells (Fig. 3A). Analysis of mRNA revealed that IGF-2 stimulated a 1.5-fold increase in CCND1 mRNA, which was reduced ($P < .01$) to 1.3-fold in AHR knockdown cells (Fig. 3B). These findings indicate that AHR knockdown cells are less responsive to IGF-2-stimulated increases in CCND1 mRNA and CCND1 protein.

3.4. AHR knockdown compromises MCF-7 proliferation

To determine the role of AHR in cell proliferation, MCF-7 control and AHR knockdown cells were treated with vehicle or IGF-2 (100 ng/mL) for 3 days. IGF-2 stimulated a 2.3-fold increase the number of live MCF-7 cells, which was reduced ($P < .0001$) to 1.2-fold by AHR knockdown (Fig. 4A). Reduced proliferation is consistent with observed reductions in CCND1 levels in AHR knockdown cells compared with control cells (Fig. 3A), given that CCND1 induces cell cycle progression.

4. Discussion

Our data indicates that IGF-2 signaling increases the levels of AHR mRNA and protein (Fig. 1) as well as the binding of AHR to AP-1 and TCF/LEF response elements on the CCND1 promoter (Fig. 2). We propose that the observed increase in AHR binding to the CCND1 promoter is important for the induction of CCND1 expression. Indeed, we found that AHR knockdown completely abrogated IGF-2 stimulated increases in CCND1 protein and significantly inhibited the induction of CCND1 mRNA compared to control cells (Fig. 3).

Modeled in Fig. 4B are our findings herein and the findings of Barhoover et al. 2010. We show that IGF-2 increases AHR, which

in turn stimulates increases in CCND1 protein (Figs. 1–3). Based on the results of Barhoover et al. 2010 [4], the observed increases in AHR and CCND1 would bind to CDK4, which would promote the phosphorylation and inhibition of RB1 to promote cell cycle advance and MCF-7 proliferation (Fig. 4B). Our results showing that AHR knockdown cells are significantly less responsive to the proliferative effects of IGF-2 (Fig. 4A) support a requirement of AHR for the induction of CCND1 and CDK4-induced phosphorylation of RB1 in MCF-7 cells [4]. Collectively, our results and the findings of Barhoover et al. 2010 [4], provide two different but complementary mechanisms of action by which AHR may mediate the proliferative effects of IGF proteins and perhaps other mitogens that induce CCND1. These new findings suggest that human cancer cells that are highly responsive to IGF growth factors may require the AHR for maximal proliferation.

Specific transcriptional proteins have been reported to stimulate the expression of AHR. Nuclear Factor, Erythroid 2-Like 2 (NRF-2) through the activation of an antioxidant response element (ARE) in the promoter of AHR, induces AHR transcription [21]. Overexpression of constitutively active β-catenin stimulated increases in AHR mRNA and AHR protein in human colon cancer cells [22]. A prior report showed that the application of medium containing 10% calf serum, platelet-derived growth factor (PDFG) or basic fibroblast growth factor (bFGF) to overnight serum starved murine 3T3 fibroblasts stimulated increases in the levels of AHR protein and the activity of a murine AHR promoter reporter construct, which correlated with the onset of DNA synthesis [23]. Our finding that IGF-2 induced increases in AHR in MCF-7 cells further links growth factor signaling with endogenous AHR regulation. Our result showing that IGF-2 induction of AHR is important for the induction of CCND1 provides insight as to the mechanism by which endogenous AHR regulation stimulates proliferation.

In conclusion, we provide evidence that IGF-2 induction of AHR is important for the induction of CCND1 and MCF-7 proliferation. Barhoover et al. 2010 have shown that TCDD inhibits MCF-7 cell cycle by disrupting interactions between CDK4 and AHR in MCF-7 cells [4]. We postulate that TCDD and other exogenous AHR ligands may impact cell proliferation by interfering with AHR-protein interactions on the CCND1 promoter.

Acknowledgments

This work was supported (in part) by NIH Grants P20RR016477 and P20GM103434 to the WV – INBRE program and Research Starter Grant from the PhRMA Foundation, Washington DC to Travis Salisbury. We would like to acknowledge the Marshall University School of Medicine Genomics Core Facility. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

[1] M.S. Denison, A.A. Soshilov, G. He, D.E. DeGroot, B. Zhao, Exactly the same but different: promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor, Toxicol. Sci. 124 (2011) 1–22.
[2] R.L. Yeager, S.A. Reisman, L.M. Aleksunes, C.D. Klaassen, Introducing the "TCDD-inducible AhR-Nrf2 gene battery", Toxicol. Sci. 111 (2009) 238–246.
[3] A. Puga, Y. Xia, C. Elferink, Role of the aryl hydrocarbon receptor in cell cycle regulation, Chem. Biol. Interact. 141 (2002) 117–130.
[4] M.A. Barhoover, J.M. Hall, W.F. Greenlee, R.S. Thomas, Aryl hydrocarbon receptor regulates cell cycle progression in human breast cancer cells via a functional interaction with cyclin-dependent kinase 4, Mol. Pharmacol. 77 (2010) 195–201.
[5] C.J. Sherr, The Pezcoller lecture: cancer cell cycles revisited, Cancer Res. 60 (2000) 3689–3695.
[6] M. Fu, C. Wang, Z. Li, T. Sakamaki, R.G. Pestell, Minireview: Cyclin D1: normal and abnormal functions, Endocrinology 145 (2004) 5439–5447.
[7] S.A. Henley, F.A. Dick, The retinoblastoma family of proteins and their regulatory functions in the mammalian cell division cycle, Cell Div. 7 (2012) 10.
[8] E.J. Gallagher, D. LeRoith, Minireview: IGF, insulin, and cancer, Endocrinology 152 (2011) 2546–2551.
[9] D.H. Cohen, D. LeRoith, Obesity, type 2 diabetes, and cancer: the insulin and IGF connection, Endocr. Relat. Cancer 19 (2012) F27–F45.
[10] B. Dufourny, J. Alblas, H.A. van Teeffelen, F.M. van Schaik, B. van der Burg, P.H. Steenbergh, J.S. Sussenbach, Mitogenic signaling of insulin-like growth factor I in MCF-7 human breast cancer cells requires phosphatidylinositol 3-kinase and is independent of mitogen-activated protein kinase, J. Biol. Chem. 272 (1997) 31163–31171.
[11] E.A. Klein, R.K. Assoian, Transcriptional regulation of the cyclin D1 gene at a glance, J. Cell Sci. 121 (2008) 3853–3857.
[12] C. Albanese, J. Johnson, G. Watanabe, N. Eklund, D. Vu, A. Arnold, R.G. Pestell, Transforming p21ras mutants and c-Ets-2 activate the cyclin D1 promoter through distinguishable regions, J. Biol. Chem. 270 (1995) 23589–23597.
[13] S.Y. Lin, W. Xia, J.C. Wang, K.Y. Kwong, B. Spohn, Y. Wen, R.G. Pestell, M.C. Hung, Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression, Proc. Natl. Acad. Sci. USA 97 (2000) 4262–4266.
[14] O. Tetsu, F. McCormick, Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells, Nature 398 (1999) 422–426.
[15] T.B. Salisbury, G.Z. Morris, J.K. Tomblin, A.R. Chaudhry, C.R. Cook, N. Santanam, Aryl hydrocarbon receptor ligands inhibit igf-ii and adipokine stimulated breast cancer cell proliferation, ISRN Endocrinol. 2013 (2013) 104850.
[16] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method, Methods 25 (2001) 402–408.
[17] K. Wu, Z. Li, S. Cai, L. Tian, K. Chen, J. Wang, J. Hu, Y. Sun, X. Li, A. Ertel, R.G. Pestell, EYA1 phosphatase function is essential to drive breast cancer cell proliferation through cyclin D1, Cancer Res. 73 (2013) 4488–4499.
[18] S. Takayama, I. Rogatsky, L.E. Schwarcz, B.D. Darimont, The glucocorticoid receptor represses cyclin D1 by targeting the Tcf-beta-catenin complex, J. Biol. Chem. 281 (2006) 17856–17863.
[19] E.V. Hestermann, M. Brown, Agonist and chemopreventative ligands induce differential transcriptional cofactor recruitment by aryl hydrocarbon receptor, Mol. Cell Biol. 23 (2003) 7920–7925.
[20] T.B. Salisbury, A.K. Binder, J.C. Grammer, J.H. Nilson, Maximal activity of the luteinizing hormone beta-subunit gene requires beta-catenin, Mol. Endocrinol. 21 (2007) 963–971.
[21] S. Shin, N. Wakabayashi, V. Misra, S. Biswal, G.H. Lee, E.S. Agoston, M. Yamamoto, T.W. Kensler, NRF2 modulates aryl hydrocarbon receptor signaling: influence on adipogenesis, Mol. Cell Biol. 27 (2007) 7188–7197.
[22] D.R. Chesire, T.A. Dunn, C.M. Ewing, J. Luo, W.B. Isaacs, Identification of aryl hydrocarbon receptor as a putative Wnt/beta-catenin pathway target gene in prostate cancer cells, Cancer Res. 64 (2004) 2523–2533.
[23] C. Vaziri, A. Schneider, D.H. Sherr, D.V. Faller, Expression of the aryl hydrocarbon receptor is regulated by serum and mitogenic growth factors in murine 3T3 fibroblasts, J. Biol. Chem. 271 (1996) 25921–25927.