The Arylhydrocarbon Receptor Is Only Marginally Involved in the Antileukemic Effects of its Ligand Curcumin

ANDREAS GOERGENS, MARKUS FRERICKS and CHARLOTTE ESSER

Institut für Umweltmedizinische Forschung (IUF), 40225 Düsseldorf, Germany

Abstract. *Background: Acute myeloid leukaemia (AML) continues to present demanding treatment challenges, as in general the prognosis for long-term survival remains dire for the patients. Natural plant-derived substances with antileukemic properties offer new treatment possibilities or may act as by-stander therapy. Their molecular mechanisms of action are often not entirely clear, limiting theory-directed screening and application strategies. The plant substance curcumin is a known activator of the transcription factor aryl hydrocarbon receptor (AhR), and has well-documented antileukemic effects. The AhR regulates cell processes, including cell cycle and apoptosis. We ask here whether direct AhR-activation by curcumin contributes to its antileukemic/apoptotic potential. Materials and Methods: The induction of caspases 3/7, 8, and 9, the breakdown of mitochondrial transmembrane potential, the BCL-2/BAX ratio, and the DNA content of cells were measured as indicators of apoptosis. In addition, the induction of cell cycle inhibitors p21 and p27 were assessed. Results: While triggering of AhR signalling by curcumin in HL-60 cells was confirmed, induction of the above apoptosis parameters was not blocked by two AhR antagonists, α-naphtoflavone (αNF) and 3'-methoxy-4'nitroflavone (MNF). Only a moderate (20%) AhR-dependent induction of caspases 3/7 was detectable. Interestingly, transcriptional changes induced by curcumin and by anticarcinogenic 1,25-dihydroxy vitamin D3 overlapped by one third. Conclusion: We conclude that AhR is only marginally involved in the antileukemic effects of its ligand curcumin.*

Acute myeloid leukaemia (AML) is a frequent type of leukaemia, with a poor prognosis especially in children, where it is responsible for more than half of leukemia deaths. The main strategy to achieve remission or longterm survival for AML patients is aggressive chemotherapy, which is unfortunately accompanied by strong side-effects (1). While the complete remission rates are up to 35-80% depending on the form of AML, the overall 5-year survival rate is only between 5 and 30% (2).

In recent times, plant secondary substances (PSS) present in food or known from traditional medicinal herbs have attracted attention for their pharmaceutical potential or in natural chemoprevention (3). For instance, flavonoids are abundant in food, with a daily intake of about 1 g in humans. Their beneficial effects range from anti-inflammatory and antioxidant to anti-tumorigenic activities (4). Curcumin ((1E,6E)-1,7-*bis*(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) is a compound found in the rhizome of *Curcuma longa*, widely used since ancient times as a spice and pigment. Curcumin has anti-inflammatory, antioxidative, anticancerogenic (5) and antiproliferative effects on many cell types (6). Currently, phase II clinical studies of curcumin in pancreas cancer treatment are underway (7).

Curcumin mediates induction of apoptosis by various mechanisms. Firstly, it is an inhibitor of nuclear factor kappa B (NF-κB), which controls expression of *BCL-2* and *BCL-XL* genes (8, 9). Secondly, it has a strong impact on cell cycle progression: curcumin blocks the G_1/S or G_2/M transition by up-regulating cyclin-dependent kinase (CDK) inhibitors p21 and p27 in a p53-dependent manner (10). We noted that curcumin is a ligand of the aryl hydrocarbon receptor (AhR), a chemical-induced transcription factor (11). The AhR is an evolutionarily ancient member of the basic helix-loop-helix (bHLH), PAS (Per-Arnt-Sim) family of transcription factors. It is abundant in many cell types, including immune cells. The biochemistry of the AhR signalling pathway is well known. Briefly, upon ligand binding, the AhR sheds its chaperone proteins, heatshock protein 90 (hsp90), AhR interacting protein (AIP), p23, and c-src, translocates into the nucleus, where it dimerizes with AhR nuclear translocator (ARNT, another bHLH-PAS protein), and binds to specific DNA sequences known as dioxin or xenobiotic response elements (DREs). The AhR:ARNT complex attracts and coordinates transcription co-factors, resulting eventually in gene expression. Primarily known for its control of detoxification

Correspondence to: Charlotte Esser, Ph.D., Auf'm Hennekamp 50, 40225 Düsseldorf, Germany. Tel: +49 2113389253, Fax: +49 2113190910, e-mail: chesser@uni-duesseldorf.de

Key Words: AhR, HL-60, curcumin, cell cycle, apoptosis, AML.

metabolism (in particular phase I and II biotransformation enzymes) (12), recent research has elucidated the role of the AhR in many cellular processes. Thus, the AhR regulates cell cycle genes such as *p21* and *p27* and pro-apoptotic genes such as *BAX* (13-15), which are likewise potential target genes of PSS/flavonoids. Here we tested, whether AhR-activation by curcumin contributes to the pro-apoptotic effect of this substance in the human myeloid cell line HL-60.

Materials and Methods

Cell culture. Cells from the human myeloid cell line HL-60 (16) were cultured in RPMI-1640 medium with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin and 0.1 mg/ml streptomycin (PAA Laboratories GmbH, Pasching, Austria) under standard conditions. curcumin, α-naphthoflavone (αNF) and 3'-methoxy-4'-nitro-flavone (MNF; Sigma, St. Louis, USA), dissolved in 0.5% or 0.1% dimethylsulfoxid (DMSO; Merck, Darmstadt, Germany) were added for different times and concentrations. Cultures treated only with DMSO served as control.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) viability assay and caspase assay. Cell viability and caspase activation in the presence of AhR ligands was assessed photometrically by commercial detection agents and kit tests, according to the instructions of the manufacturers. Briefly, cells were cultivated at a density of 5×10^5/ml in 96-well plates in the presence or absence of curcumin/DMSO, or curcumin plus AhR antagonist. Treatment was followed by addition of MTT (Sigma, Taufkirchen, Germany) and determination of absorbance at 570 nm, or addition of caspase substrates (Promega, Madison, USA) and determination of luminescence with a MICRO LUMAT Plus LB96V luminometer (PerkinElmer, Regensdorf, Switzerland).

Real-time polymerase chain reaction (PCR). Total RNA was isolated with Trizol™ (Sigma), reversely transcribed into cDNA with MMLV reverse transcriptase and 1 μg oligo(dT)15 primers (Bioline, Luckenwalde, Germany) in a final volume of 40 μl. Real-time PCR analysis was performed with Sensimix SYBR-green from Bioline in a Rotor-Gene 3000 thermo cycler (LTF Labortechnik, Wasserburg, Germany), using a 15 μl reaction volume. PCR conditions were 15 min at 94˚C, 20 s at 94˚C, 15 s at 56 or 60˚C, 20 s at 72˚C (45 cycles), and 3 min at 72˚C. Primer sequences can be provided on request. The difference between crossing points for genes of interest and HPRT as housekeeping gene (ΔCp) were determined in the linear range of the fluorescent signal with Corbett analysis software version 6.1 (Corbett, LTF Labortechnik, Wasserburg). The difference between treated and untreated probe (ΔΔCp) was calculated as log2 fold induction of the gene.

Flow cytometric analysis of cell cycle, reactive oxygen species (ROS) content and mitochondria membrane potential. Determination of cell cycle, reactive oxygen species (ROS) content and mitochondria potential was carried out flow cytometrically. Cells were cultured at 5×10^5 cells/ml in 2 ml in 24-well plates. For visualizing treatment effects on the cell cycle, cells were washed with phosphate-buffered saline (PBS) after 24 hours exposure time, and fixed with 70% ethanol at 4˚C for 30 minutes, followed by additional washing with PBS. Cells were incubated for 1 hour with 10 μg/ml RNAse (Sigma) in PBS at 37˚C, washed again, then stained with 10 μg/ml propidium iodide (Sigma) in PBS for 15 minutes in the dark.

ROS content was determined by incubation of washed cells with for 20 minutes with 10 μM 2',7'-dichlorofluorescein diacetate (DCFH-DA; Sigma) in PBS at 37˚C in the dark, followed by a washing step with PBS. Measurement of mitochondrial membrane potential was performed by adding 10 μM tetramethyl rhodamine methyl ester (TMRM) in PBS (Sigma) and incubating for 20 minutes at 37˚C in the dark. After washing twice with PBS, membrane potential-dependent fluorescence was measured flow cytometrically. Live cells were gated according to scatter characteristics. Cells were analysed in list-mode on a FACSCalibur™ (Becton-Dickinson, Franklin Lakes, NJ, USA) and WinMDI 2.8 software (biology software net).

Microarray analysis. A total of 5×10^5 cells/ml in 5 ml were treated with 100 μM curcumin or 0.5% of DMSO for 24 h. Total RNA was isolated, transcribed in biotinylated RNA (Enzo Bio Array High Yield RNA transcript labelling kit; Affymetrix, High Wycombe, USA) and hybridized to Affymetrix HG-U133_A2 chips after ascertaining integrity. Data were analyzed with the Bioconductor Affy package provided by the manufacturer (two-fold cut-off point). Two independent experiments were performed. Gene ontology analysis was carried out with GOToolBox™ (http://burgundy.cmmt.ubc.ca/GOToolBox/) (17) and Bonferroni correction. For comparison, raw data from an experimental series of HL-60 cells treated with 20 different chemicals (such as 1,25-dihydroxyvitamin D3 (VitD), 16-ketoestradiol and 1,10-phenanthroline) hybridized to the same platform were downloaded from the GEO database (ncbi.nlm.nih.gov/geo/projects, accession number GSE995) and analyzed in parallel. Biological functions (processes) of differentially expressed genes were classified with the GOToolBox™ .

Statistics. Experiments were performed three times independently if not otherwise stated. Data were analyzed by Student's *t*-test with GraphPad Prism® software (GraphPad Software, Inc., La Jolla, CA, USA). Means±SD were calculated.

Results

Curcumin reduces viability of HL-60 leukemia cells. Exposure to curcumin for 24 h significantly dreduced HL-60 viability (Figure 1); 100% cell death was not achieved within the solubility-dependent limits of concentrations used for curcumin. For all further experiments, the concentration of 50 μM curcumin was chosen.

Parameters of apoptosis in HL-60 cells after flavonoid treatment. Curcumin induced activity of caspase-8 (mediator of the extrinsic receptor-mediated apoptosis pathway) and caspase-9 (mediator of the intrinsic mitochondria-mediated pathway), as well as effector caspase-3 and -7 (Figure 2 A) approximately 5- to 6-fold.

Apoptosis can be initiated and balanced by the pro- and anti-apoptotic proteins of the BCL-2 family (18). We determined the *BCL-2*/*BAX* and *BCL-XL*/*BAD* mRNA ratios in HL-60 cells. Both ratios shifted in favour of the pro-

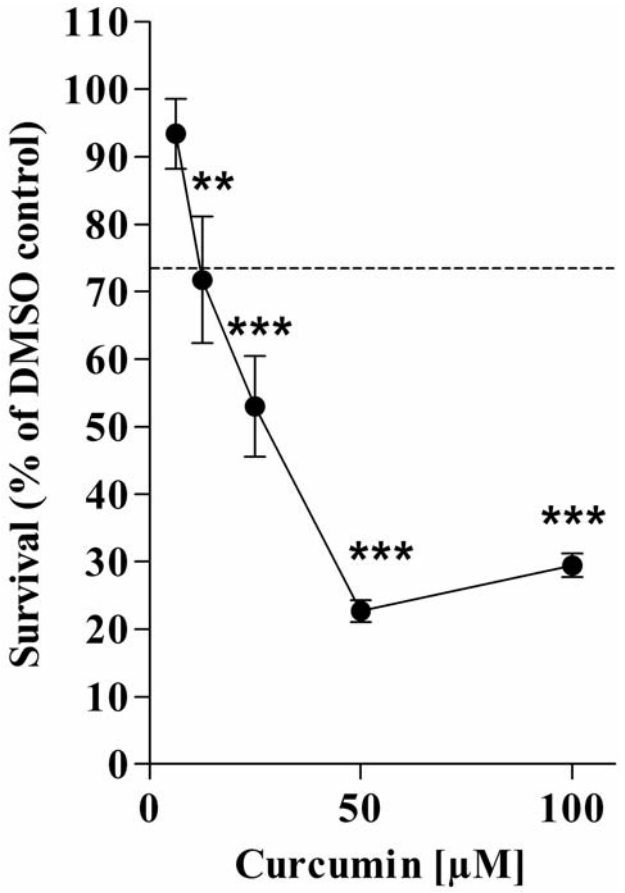

Figure 1. *Curcumin reduces viability of HL-60 cells. HL-60 cells were exposed to different doses of curcumin for 24 h. Cell viability was measured by MTT assay at 570 nm. DMSO (0.5%)-treated cells were considered as exhibiting 100% viability. *p<0.05, **p<0.01, *p<0.001.*

apoptotic *BAX* and *BAD*, respectively (Figure 2 B). Flow cytometric data demonstrated the resulting collapse of the mitochondrial membrane potential after treatment with curcumin (Figure 2 C).

Influence of curcumin on the cell cycle of HL-60 cells. Reduced cell viability may reflect blockade in the cell cycle. AhR target genes include CDK2 inhibitors, in particular *p21* and *p27*(15). RT-PCR showed significant induction (16-fold) of these cell cycle inhibitors in HL-60 cells by curcumin (Figure 3 A). In the cell cycle without treatment, about 60% of the cells were in the G_1 phase, and almost no cells in the apoptotic subG_1 peak (19). Curcumin treatment led to a formation of a subG_1 peak (47% of all cells) and led further to a strong arrest of living cells in the S-phase, as only 6% of the living cells were detected in the G_2/M-phase compared to 18% under control conditions (Figure 3 B).

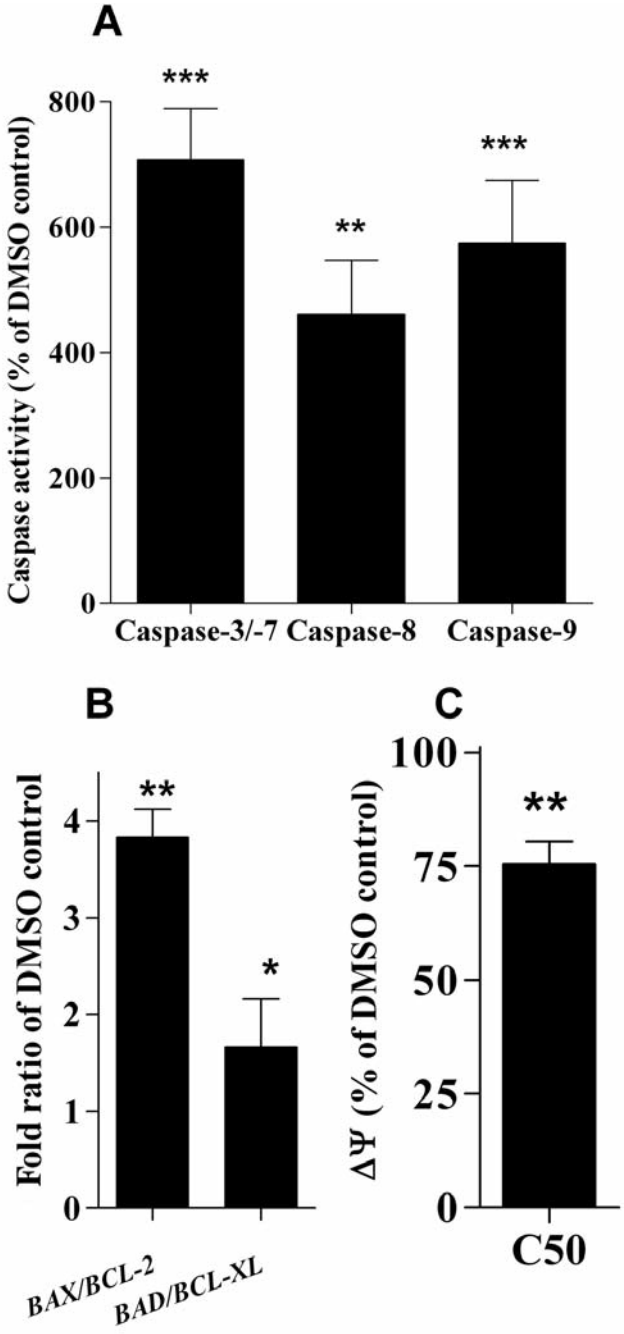

Figure 2. *Curcumin induces apoptosis in HL-60 cells that is mediated by the extrinsic and intrinsic pathways. HL-60 cells (5×10^5/ml) were treated for 24 h with 50 µM of curcumin (C50) or DMSO (0.5%) as control. A, Activity of caspase-8 (as mediator of the extrinsic apoptosis pathway), caspase-9 (as mediator of the intrinsic apoptosis pathway), caspase-3 and -7 (as effector caspases) as determined by Caspase-Glo™ Assays (Promega). Caspase activity is shown as % of DMSO control. B, Ratio changes of BAX/BCL-2 and BAD/BCL-XL mRNA by curcumin. RNA content was determined semi-quantitatively against HPRT. Results are depicted in comparison to DMSO solvent control. C, Mitochondrial membrane potential in HL-60 cells treated with curcumin. Values are shown as % of DMSO solvent control (0.5%). *p<0.05, **p<0.01, *p<0.001.*

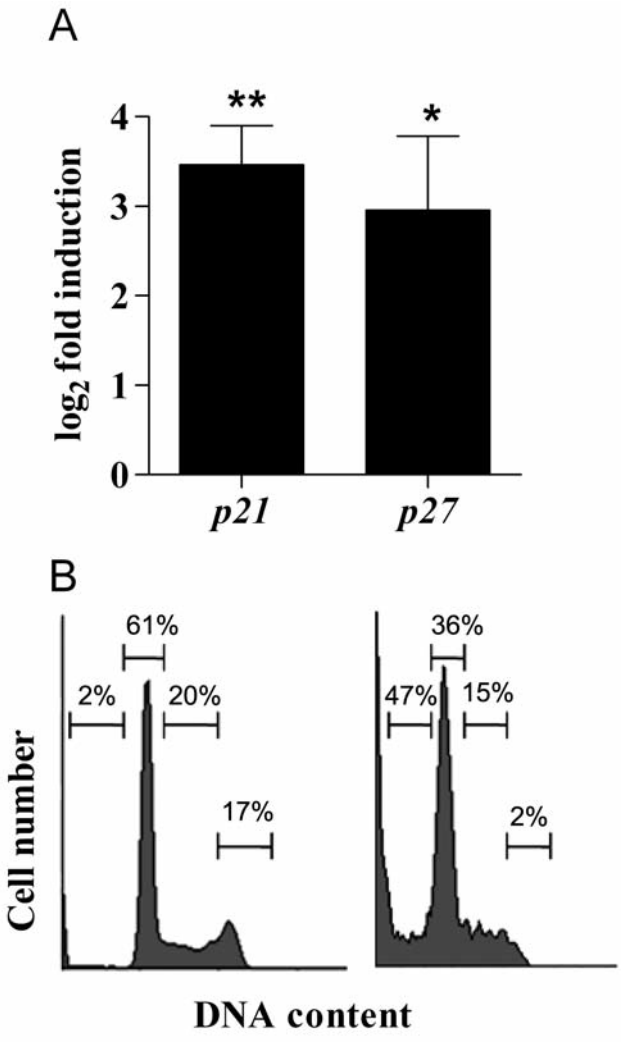

Figure 3. *Curcumin induces cell cycle arrest in HL-60 cells accompanied by induction of the cell cycle inhibitors p21 and p27. HL-60 cells (5×10^5/ml) were treated for 24 h with 50 μM of curcumin (C50) or DMSO (0.5%) as control. A, Total RNA was isolated and transcribed cDNA used in semi-quantitative RT-PCR. P21 and p27 levels were measured as log(2)-fold induction of expression in DMSO (0.5%) treated cells. B, HL-60 cells were stained with propidium iodide and analyzed by FACS. Histograms show DNA content. G_1-phase, S-phase, and G_2/M-phase of cells are indicated. Apoptotic cells are in the sub G_1 peak. Data are from one representative experiment out of three independent experiments.*

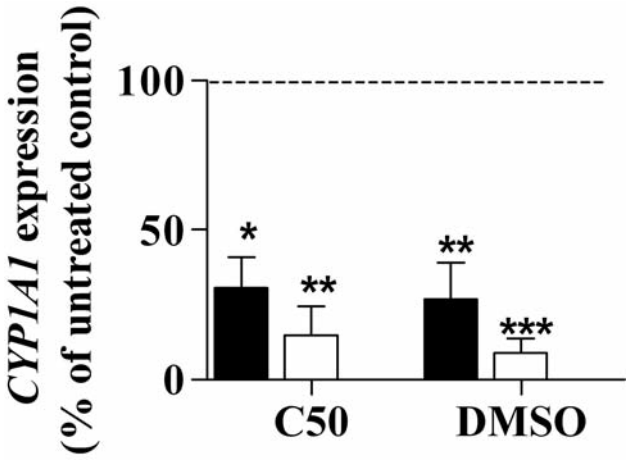

Figure 4. *Pretreatment with AhR antagonists reduces curcumin induced CYP1A1 expression. HL-60 cells were preincubated for 1h with either α-naphthoflavone (αNF, black bars), 3'-methoxy-4'-nitroflavone (MNF, white bars) at 1 μM, or DMSO as vehicle, followed by 24 h with 50 μM of curcumin (C50), or DMSO. CYP1A1 expression was determined by RT-PCR and calculated as % of DMSO-only control. Curcumin alone induced CYP1A1 at least six-fold (data not shown).*

The AhR is expressed and functional in HL-60 cells. AhR presence in HL-60 cells was confirmed by RT-PCR. To assess functional competence, HL-60 cells were incubated with 10 nM of AhR-high-affinity agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and tested for up-regulation of the prototypical target gene *CYP1A1*. The exposure led to time and dose-dependent increase of *CYP1A1* transcription (data not shown).

Blocking of the AhR in HL-60 cells by α-naphthoflavone (αNF) and MNF. Similarly to TCDD, curcumin induced the AhR target gene *CYP1A1* in HL-60 cells (data not shown). In agreement with concentrations used in the literature (20), the concentration of 1 μM αNF or MNF inhibited the maximum *CYP1A1* induction at 50 μM curcumin optimally (by about 70%, Figure 4). In further experiments, we therefore pre-treated cell cultures for 60 min with 1 μM of antagonists αNF or MNF.

AhR dependency of the effects of curcumin. Except for caspase-3/7 induction, none of the pro-apoptotic effects of curcumin was blocked by AhR-antagonists αNF and MNF (Table I). However, blocking AhR activity with αNF and MNF reduced curcumin induced effector caspase-3 and -7 by only 20%. Neither the effects on caspase-8 and -9, the breakdown in membrane potential, nor the inhibition of ROS (*i.e.* antioxidant activity) was blocked by antagonists, *i.e.* these were not AhR-mediated effects. Cell viability remained low in αNF and MNF pre-treated cultures subsequently treated with curcumin.

Involvement of other mechanisms in the effects of curcumin. Curcumin is a pleiotropic agent, thus its pro-apoptotic and antileukemic actions on HL-60 cells may involve various cellular signaling pathways. Depending on the cell type, the AhR affects other signaling pathways, such as the NF-κB pathway (21). ROS content was also reduced (Table I). We tested for the involvement of vitamin D receptor (*VDR*)

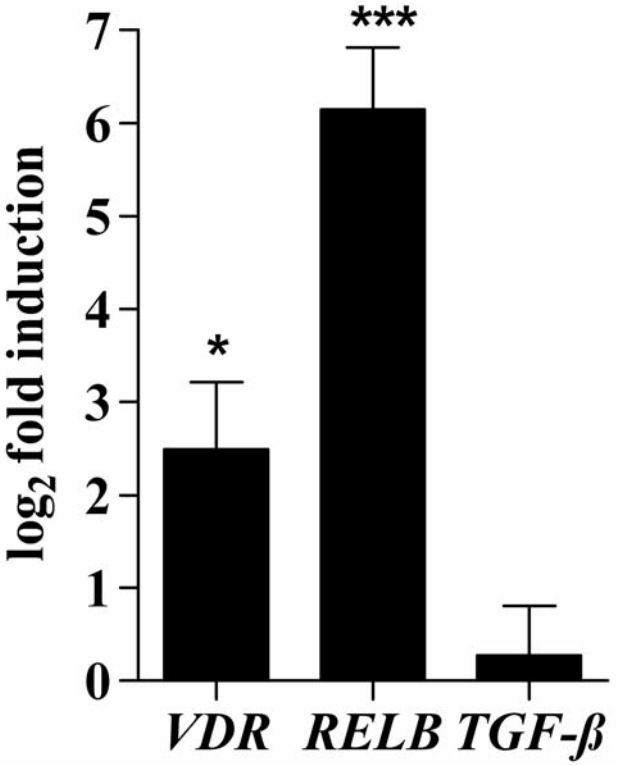

Figure 5. *Curcumin induces key genes of other apoptosis relevant pathways in HL-60 cells. HL-60 cells (5×10^5/ml) were treated for 24 h with 50 μM of curcumin (C50) or DMSO (0.5%) as control. Total RNA was isolated, transcribed into cDNA and used in RT-PCR. Gene induction was measured as log2 fold induction of DMSO (0.5%) treated cells.*

pathway, tumor growth factor beta (TGF-β), and NF-κB as three other potential antileukemic pathways in HL-60 cells (22). Confirming and extending literature results, *RELB* mRNA was up-regulated by more than 60-fold and *VDR* more than 4-fold in HL-60 cells after curcumin treatment, while *TGF-β* mRNA content remained unchanged (Figure 5). In order to better assess this, we analyzed global gene expression profiles of HL-60 cells after 24 h of curcumin exposure. A total of 771 genes were differentially expressed; 550 were up- and 221 were down-regulated. We compared our array data to the published transcriptional profile of HL-60 cells treated for 120 hours with 0.1 μM VitD (PubMed, accession number GSE 995). Interestingly, 205 of the curcumin-modulated genes were reported as changed by VitD.

Discussion

Plant secondary substances (PSS) hold promise in cancer prevention and cancer therapy. In order to screen for natural substances, or to rationally develop designer molecules, it is pivotal to fully understand their (cell-specific) mechanisms of action (23).

We provide here, for the first time, evidence for partial participation of the AhR in the pro-apoptotic action of PSS flavonoid/AhR agonist curcumin in AML cells. The PSS curcumin has anticarcinogenic and antileukemic activity (6). Why curcumin effects are limited to cancer cells is not known. Primary cells (*e.g.* hepatocytes or leukocytes) are not affected (24). We showed that curcumin induced *CYP1A1* transcription, and this activity was inhibited by AhR antagonists. However, curcumin-induced apoptotic mechanisms were not diminished by blocking AhR signaling, except for that of a moderate AhR-dependent induction of caspase-3/7.

The AhR is a sensor of small molecules and well known to react to the presence of many PSS, with transcriptional reprogramming of cells, including genes of the cell cycle and apoptosis. Therefore, the AhR has been suggested as a target for pharmacological interventions (25). Curcumin increased the ratio of *BAX*/*BCL2*, thought to be associated with increased apoptosis. It also inhibits the NF-κB pathway, which drives BCL-2 (26). Beyond this, *BAX, p21* and *p27* could be potential direct target genes of the AhR, and are up-regulated by AhR ligands in human vascular endothelial cells (27). Notably, *p21* and *p27* have at least one putative DRE in their human promoters. Two putative DREs in the mouse promoter of *BAX* have been reported. These DREs were shown to be functional by luciferase reporter gene assays (27, 28). Confirming and extending previous work, we showed that curcumin reduced viability in HL-60 acute myelocytic leukemia cells by induction of apoptosis *via* the extrinsic and intrinsic pathway. The antioxidant capacities of curcumin, although strong, did not protect HL-60 cells from apoptosis. This is in agreement with previous findings in other cancer types such as liver cancer (8, 29).

To our surprise, inhibition of the AhR signaling pathway by two specific AhR antagonists, αNF or MNF abrogated none of the parameters measured. In contrast to other cell types, such as 5L cells (dedifferentiated descendents of the rat hepatoma line H4IIEC3), induction of cell cycle genes *p21* and *p27* and of *BAX*/*BCL2* were AhR independent in AML cells under the conditions used here (15).

High cell type specificity and flexibility of response is a characteristic feature of the AhR signaling pathway. Indeed, it is probably a necessary part of its physiological function and cellular control (13). For instance, the AhR signal pathway crosstalks with NF-κB, estrogen receptor, and hypoxia signaling (21). Cell type, differentiation stage, metabolic capacities, exposure dose, ligand, and the cell-specific signal pathway profile all contribute to shaping the outcome of AhR triggering (30, 31). The parameters which determine ligand-dependent outcome of AhR action are poorly understood. Affinity to the AhR is important, and has been used for the determination of toxic equivalency factors of poorly degradable environmental pollutants (14, 32). However, many chemical parameters of the ligands, such as

Table I. *Pre-treatment with the AhR antagonists αNF and MNF has no influence on the observed effects that are induced by curcumin. HL-60 cells (5×10^5/ml) were pretreated in triplicate for 1 h with 1 μM of the AhR antagonists αNF or MNF or DMSO (0.1%) as control. Cells were then treated for 24 h with 50 μM of curcumin (dissolved in 0.5% DMSO) and assays were performed as described in Materials and Methods. Data are means±SD; no significant differences were observed between antagonist-treated cultures and agonist-only samples.*

	Curcumin		
Assay	+ DMSO	+ 1 μM αNF	+ 1 μM MNF
Viability (% of control)	26±3	27±2	23±2
Caspase 3/7 activity (% of control)	642±66	570±64	507±43
Caspase 8 activity (% of control)	461±86	517±54	465±61
Caspase 9 activity (% of control)	575±100	606±96	585±162
BAX/BCL-2 ratio	4.0±2.2	3.9±1.7	3.2±0.9
Mitochondrial membrane potential (MFI)	1299±118	1378±136	1361±156
ROS (MFI)	32±2	30±1	30±4
p21 mRNA expression (% of control)	100	92±36	114±36
p27 mRNA expression (% of control)	100	94±55	107±42
Cell cycle distribution (% of living cells)			
G_1-phase	68±1	72±2	74±6
S-phase	28±3	24±2	21±5
G_2/M-phase	5±3	4±1	5±1

MFI, Mean fluorescence index; MFI of untreated cells was 60.

their degradation kinetics are important factors as well; for instance, the flavonoid indirubin has a similar affinity to the AhR as does TCDD (33), but in contrast to the latter is quickly degraded and no systemic toxicity is known (34).

Small molecular differences can turn agonists into antagonists. Good examples of this are α- and β-NF, which have been used as controls in research studies on AhR effects for many years. The β form is an agonist of AhR and the α form an antagonist or partial agonist of AhR (35). Other parameters might be subtle, yet decisive, ligand-dependent conformation changes upon binding, bioavailability within the cell. Moreover, a substance may at the same time bind to the AhR, interact with other cell components and increase or decrease oxidative stress. The latter might be especially important in ROS scavengers such as the PSS we investigated here (36).

Interestingly, although curcumin reduced ROS content by about 50%, these antioxidant properties did not override the apoptotic effects (data not shown). In general, dietary flavonoids are likely to interact with more than one signaling pathway, *e.g.* curcumin activates mitogen-activated protein (MAP) kinases, NF-κB, protein kinase C, c-Jun, activator protein 1 (AP-1) and the extracellular signal-regulated kinase ERK (37). While a challenge in many ways, this complexity, once better understood, is an opportunity for pharmaceutical exploitation with the goal of cell-specific intervention. For instance, selective AhR modulators have developed to treat estrogen-dependent breast cancer (38).

Exploring curcumin-dependent transcriptional changes in more depth, we analyzed the gene expression profile of HL-60 cells after 24 h curcumin treatment. The transcription of many genes changed upon curcumin treatment, conceivably reflecting the multitude of signaling pathways triggered by this molecule (see above). As has been observed before for other AhR ligands, genes can be up- or down-regulated (30). A GO analysis and comparison with published microarray information for HL-60 cells revealed strong overlap of genes modulated by curcumin with those by VitD: 205 out of 771 modulated genes overlapped. The extent of similarity between transcriptome changes by two different substances is unusual, and suggests an involvement of VDR in the effects of curcumin.

In conclusion, curcumin activates the AhR and induces apoptosis in HL-60 cells, but the pro-apoptotic effects are largely not due to this AhR ligand activity. However, as the AhR is involved in cell cycle and proliferation in other cell types, and because of its known high cell-specific activity and ligand specificity, the AhR presents itself as a promising drug target. Our results have implications for therapeutic optimization of flavonoids in leukaemia.

Acknowledgements

This project was funded by the Deutsche José Carreras Stiftung für Leukämieforschung (project DJCLS R 05/15).

References

1 Smith M, Barnett M, Bassan R, Gatta G, Tondini C and Kern W: Adult acute myeloid leukaemia. Crit Rev Oncol Hematol *50*: 197-222, 2004.

2 Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ and Thun MJ: Cancer statistics, 2004. CA Cancer J Clin *54*: 8-29, 2004.

3 Steriti R: Nutritional support for chronic myelogenous and other leukemias: a review of the scientific literature. Altern Med Rev *7*: 404-409, 2002.

4 Formica JV and Regelson W: Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol *33*: 1061-1080, 1995.

5 Anto RJ, George J, Babu KV, Rajasekharan KN and Kuttan R: Antimutagenic and anticarcinogenic activity of natural and synthetic curcuminoids. Mutat Res *370*: 127-131, 1996.

6 Choudhuri T, Pal S, Das T and Sa G: Curcumin selectively induces apoptosis in deregulated cyclin D1-expressing cells at G_2 phase of cell cycle in a p53-dependent manner. J Biol Chem *280*: 20059-20068, 2005.

7 Hsu CH and Cheng AL: Clinical studies with curcumin. Adv Exp Med Biol *595*: 471-480, 2007.

8 Mu C, Jia P, Yan Z, Liu X, Li X and Liu H: Quercetin induces cell cycle G_1 arrest through elevating Cdk inhibitors p21 and p27 in human hepatoma cell line (HepG2). Methods Find Exp Clin Pharmacol *29*: 179-183, 2007.

9 Aggarwal BB, Banerjee S, Bharadwaj U, Sung B, Shishodia S and Sethi G: Curcumin induces the degradation of cyclin E expression through ubiquitin-dependent pathway and up-regulates cyclin-dependent kinase inhibitors p21 and p27 in multiple human tumor cell lines. Biochem Pharmacol *73*: 1024-1032, 2007.

10 Yang CS, Landau JM, Huang MT and Newmark HL: Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr *21*: 381-406, 2001.

11 Knockaert M, Blondel M, Bach S, Leost M, Elbi C, Hager GL, Nagy SR, Han D, Denison M, Ffrench M, Ryan XP, Magiatis P, Polychronopoulos P, Greengard P, Skaltsounis L and Meijer L: Independent actions on cyclin-dependent kinases and aryl hydrocarbon receptor mediate the antiproliferative effects of indirubins. Oncogene *23*: 4400-4412, 2004.

12 Ciolino HP, Daschner PJ and Yeh GC: Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect *CYP1A1* transcription differentially. Biochem J *340(Pt 3)*: 715-722, 1999.

13 Schmidt JV and Bradfield CA: Ah receptor signaling pathways. Annu Rev Cell Dev Biol *12*: 55-89, 1996.

14 Denison MS and Nagy SR: Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol *43*: 309-334, 2003.

15 Elferink CJ: Aryl hydrocarbon receptor-mediated cell cycle control. Prog Cell Cycle Res *5*: 261-267, 2003.

16 Birnie GD: The HL60 cell line: a model system for studying human myeloid cell differentiation. Br J Cancer Suppl *9*: 41-45, 1988.

17 Martin D, Brun C, Remy E, Mouren P, Thieffry D and Jacq B: GOToolBox: functional analysis of gene datasets based on Gene Ontology. Genome Biol *5*: R101, 2004.

18 Danial NN: BCL-2 family proteins: critical checkpoints of apoptotic cell death. Clin Cancer Res *13*: 7254-7263, 2007.

19 Inoue S: Analyses of cell cycle and DNA. Rinsho Byori *49*: 835-841, 2001.

20 Fritsche E, Schafer C, Calles C, Bernsmann T, Bernshausen T, Wurm M, Hubenthal U, Cline JE, Hajimiragha H, Schroeder P, Klotz LO, Rannug A, Furst P, Hanenberg H, Abel J and Krutmann J: Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. Proc Natl Acad Sci USA *104*: 8851-8856, 2007.

21 Frericks M, Burgoon LD, Zacharewski TR and Esser C: Promoter analysis of TCDD-inducible genes in a thymic epithelial cell line indicates the potential for cell-specific transcription factor crosstalk in the AhR response. Toxicol Appl Pharmacol *232*: 268-279, 2008.

22 Kanli A and Savli H: Differential expression of 16 genes coding for cell cycle- and apoptosis-related proteins in vitamin D-induced differentiation of HL-60 cells. Exp Oncol *29*: 314-316, 2007.

23 Efferth T, Fu YJ, Zu YG, Schwarz G, Konkimalla VS and Wink M: Molecular target-guided tumor therapy with natural products derived from traditional Chinese medicine. Curr Med Chem *14*: 2024-2032, 2007.

24 Syng-Ai C, Kumari AL and Khar A: Effect of curcumin on normal and tumor cells: role of glutathione and bcl-2. Mol Cancer Ther *3*: 1101-1108, 2004.

25 Gasiewicz TA, Henry EC and Collins LL: Expression and activity of aryl hydrocarbon receptors in development and cancer. Crit Rev Eukaryot Gene Expr *18*: 279-321, 2008.

26 Aggarwal BB and Shishodia S: Suppression of the nuclear factor-kappaB activation pathway by spice-derived phytochemicals: reasoning for seasoning. Ann NY Acad Sci *1030*: 434-441, 2004.

27 Pang PH, Lin YH, Lee YH, Hou HH, Hsu SP, and Juan SH: Molecular mechanisms of *p21* and *p27* induction by 3-methylcholanthrene, an aryl-hydrocarbon receptor agonist, involved in antiproliferation of human umbilical vascular endothelial cells. J Cell Physiol *215*: 161-171, 2008.

28 Matikainen T, Perez GI, Jurisicova A, Pru JK, Schlezinger JJ, Ryu HY, Laine J, Sakai T, Korsmeyer SJ, Casper RF, Sherr DH, and Tilly JL: Aromatic hydrocarbon receptor-driven *Bax* gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. Nat Genet *28*: 355-360, 2001.

29 Shishodia S, Amin HM, Lai R and Aggarwal BB: Curcumin (diferuloylmethane) inhibits constitutive NF-kappaB activation, induces G_1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma. Biochem Pharmacol *70*: 700-713, 2005.

30 Frericks M, Meissner M, and Esser C: Microarray analysis of the AHR system: tissue-specific flexibility in signal and target genes. Toxicol Appl Pharmacol *220*: 320-332, 2007.

31 Suzuki T and Nohara K: Regulatory factors involved in species-specific modulation of arylhydrocarbon receptor (AhR)-dependent gene expression in humans and mice. J Biochem *142*: 443-452, 2007.

32 Nagy SR, Liu G, Lam KS and Denison MS: Identification of novel Ah receptor agonists using a high-throughput green fluorescent protein-based recombinant cell bioassay. Biochemistry *41*: 861-868, 2002.

33 Adachi J, Mori Y, Matsui S and Matsuda T: Comparison of gene expression patterns between 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and a natural arylhydrocarbon receptor ligand, indirubin. Toxicol Sci *80*: 161-169, 2004.

34 Adachi J, Mori Y, Matsui S, Takigami H, Fujino J, Kitagawa H, Miller CA, III, Kato T, Saeki K and Matsuda T: Indirubin and indigo are potent aryl hydrocarbon receptor ligands present in human urine. J Biol Chem *276*: 31475-31478, 2001.

35 Sinal CJ, Webb CD and Bend JR: Differential *in vivo* effects of alpha-naphthoflavone and beta-naphthoflavone on CYP1A1 and CYP2E1 in rat liver, lung, heart, and kidney. J Biochem Mol Toxicol *13*: 29-40, 1999.

36 Lee JC, Kim J, Park JK, Chung GH and Jang YS: The antioxidant, rather than prooxidant, activities of quercetin on normal cells: quercetin protects mouse thymocytes from glucose oxidase-mediated apoptosis. Exp Cell Res *291*: 386-397, 2003.

37 Aggarwal BB and Shishodia S: Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol *71*: 1397-1421, 2006.

38 Safe S, Qin C, and McDougal A: Development of selective aryl hydrocarbon receptor modulators for treatment of breast cancer. Expert Opin Investig Drugs *8*: 1385-1396, 1999.

Received March 26, 2009
Revised October 6, 2009
Accepted October 12, 2009