Carcinogenic polycyclic aromatic hydrocarbons induce CYP1A1 in human cells via a p53-dependent mechanism

Laura E. Wohak · Annette M. Krais · Jill E. Kucab · Julia Stertmann · Steinar Øvrebø · Albrecht Seidel · David H. Phillips · Volker M. Arlt

Received: 29 August 2014 / Accepted: 4 November 2014 / Published online: 15 November 2014 © The Author(s) 2014.

Abstract The tumour suppressor gene TP53 is mutated in more than 50% of human tumours, making it one of the most important cancer genes. We have investigated the role of TP53 in cytochrome P450 (CYP)-mediated metabolic activation of three polycyclic aromatic hydrocarbons (PAHs) in a panel of isogenic colorectal HCT116 cells with differing TP53 status. Cells that were TP53(+/+), TP53(+/−), TP53(−/−), TP53(R248W+/+) or TP53(R248W−/) were treated with benzo[a]pyrene (BaP), dibenz[a,h]anthracene and dibenzo[a,l]pyrene, and the formation of DNA adducts was measured by 32P-postlabeling analysis. Each PAH formed significantly higher DNA adduct levels in TP53(+/+) cells than in the other cell lines. Bypass of the need for metabolic activation by treating cells with the corresponding reactive PAH-diol-epoxide metabolites resulted in similar adduct levels in all cell lines, which confirms that the influence of p53 is on the metabolism of the parent PAHs. Western blotting showed that CYP1A1 protein expression was induced to much greater extent in TP53(+/+) cells than in the other cell lines. CYP1A1 is inducible via the aryl hydrocarbon receptor (AHR), but we did not find that expression of AHR was dependent on p53; rather, we found that BaP-induced CYP1A1 expression was regulated through p53 binding to a p53 response element in the CYP1A1 promoter region, thereby enhancing its transcription. This study demonstrates a new pathway for CYP1A1 induction by environmental PAHs and reveals an emerging role for p53 in xenobiotic metabolism.

Keywords Benzo[a]pyrene · Tumour suppressor p53 · Cytochrome P450 · Carcinogen metabolism · DNA adducts

Introduction

The TP53 tumour suppressor gene, which encodes the protein p53, is mutated in over 50% of human tumours and is one of the most important cancer genes (Olivier et al. 2010). p53, often described as the guardian of the genome, is involved in multiple cellular functions; amongst these is the response to cellular stress induced by various types of DNA damage, thereby delaying DNA synthesis or cell division to allow DNA repair or inducing apoptosis. In normal, unstressed cells, p53 protein expression is kept low via ubiquitin-mediated proteolysis that is regulated by the E3 ubiquitin ligase MDM2. Disruption of the normal p53 response by TP53 mutation leads to the development of tumours. Besides acquired somatic mutations in the TP53
gene being a common feature of the cancer genotype, germline mutations can cause predisposition to a wide spectrum of early onset cancers associated with the Li–Fraumeni and Li–Fraumeni-like syndromes (Olivier et al. 2010). Furthermore, some TP53 polymorphisms in coding and noncoding regions have been shown to increase cancer susceptibility and to modify cancer phenotypes in TP53 mutation carriers (Whibley et al. 2009). In addition to its role in the DNA damage response, p53 has also been found to regulate metabolic pathways such as glycolysis and oxidative phosphorylation thereby linking p53 not only to cancer, but also to other diseases such as diabetes and obesity, and to other physiological processes such as ageing (Maddocks and Vouladen 2011). Thus, the repertoire of genes subject to p53 control as a master regulatory transcription factor extends across a diverse group of biological activities (Menendez et al. 2009). It has also been observed that abrogation of p53 activity by knockout or knockdown of TP53 in human cells in vitro affects carcinogen activation (Hockley et al. 2008; Simoes et al. 2008) and drug metabolism (Goldstein et al. 2013), but as yet little is known about the mechanism of this phenomenon.

Polycyclic aromatic hydrocarbons (PAHs) are formed by the incomplete combustion of organic matter (Baird et al. 2005; IARC 2010) and are widely distributed in the environment. Several of them are highly carcinogenic, including benzo[a]pyrene (BaP), the most widely studied, as well as dibenzo[a,h]anthracene (DB[a,h]A) and dibenz[a,l]pyrene (DB[a,l]P) (Chang et al. 2013; Crowell et al. 2014; Lemieux et al. 2011; Siddens et al. 2012). PAHs require metabolic activation in order to exert their genotoxic effects (Baird et al. 2005; Luch and Baird 2005). Metabolic activation is catalysed predominantly by cytochrome P450-dependent monooxygenases (CYPs) resulting in highly reactive diol-epoxides capable of forming covalent DNA adducts that can lead to mutations through errors in DNA replication (Phillips 2005).

In previous studies, we observed that many of the gene expression changes induced by BaP in human cells in vitro were in genes linked to pathways of p53 function (Hockley et al. 2006, 2008). Furthermore, we found that DNA adduct formation by BaP was significantly diminished in cells in which TP53 was knocked out or silenced (by siRNA inhibition) relative to cells with normal p53 function (Hockley et al. 2008).

In order to evaluate the impact of the cellular TP53 status on the metabolic activation of a variety of different PAHs, a panel of isogenic human HCT116 cell lines that differ only with respect to their endogenous TP53 status, expressing either wild-type (WT) p53 [TP53(+/+)], heterozygous p53 [TP53(+/−)] or mutant p53 [TP53(R248W+/+) or TP53(R248W−/−)], or with a complete knockout of p53 [TP53(−/−)] (Sur et al. 2009) was treated with BaP, DB[a,h]A or DB[a,l]P. PAH-DNA adduct formation was determined by 32P-postlabelling. Expression of xenobiotic metabolising enzymes (XMEs) involved in PAH metabolism (e.g. CYP1A1) was determined by Western blotting and qRT-PCR. Cells were also treated with the reactive PAH-diol-epoxides in order to bypass the need for metabolic activation. We investigated whether p53 impacts on the regulation of XME expression such as CYP1A1 via the aryl hydrocarbon receptor (AHR) or whether the expression of XMEs such as CYP1A1 may be transcriptionally regulated by direct binding of p53 to the regulatory regions of the XME genes (Goldstein et al. 2013).

### Materials and methods

#### Carcinogens

Benzo[a]pyrene (BaP; CAS no. 50-32-8; purity ≥96 %) was obtained from Sigma-Aldrich and (±)-anti-benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide (BPDE) was synthesized as reported previously (Hockley et al. 2008). Dibenzo[a,h]anthracene (DB[a,h]A; CAS no. 53-70-3; purity ≥99.9 %), dibenz[a,l]pyrene (DB[a,l]P; CAS no. 191-30-0; purity ≥99.9 %), (±)-anti-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydro-DB[a,h]A (DB[a,h]ADE) and (±)-anti-11,12-dihydroxy-13,14-epoxy-11,12,13,14-tetrahydro-DB[a,l]P (DB[a,l]PDE) were synthesized at the Biochemical Institute for Environmental Carcinogens, Prof. Dr. Gernot Grimmer-Foundation, Germany, according to the literature methods (Platt and Oesch 1981; Lee and Harvey 1980; Karle et al. 1977; Luch et al. 1994). The BaP metabolites (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (BaP-r,7,8-dihydrodiol) and (±)-r,7,8,9-c,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BaP-tetrol-1-1) were also synthesized at the Biochemical Institute for Environmental Carcinogens using earlier published methods (Platt and Oesch 1983; Yagi et al. 1977). Mass spectrometry data and high field 1H-NMR spectra (400 MHz) were in essential agreement to those published previously.

#### Cell culture and chemical treatment

Through targeted homologous recombination, a panel of isogenic HCT116 human colorectal carcinoma cell lines has been developed that differ only with respect to their endogenous TP53 status. Cells expressing either WT p53 [i.e. TP53(+/+)], heterozygous p53 [i.e. TP53(+/-)], mutant p53 [i.e. TP53(R248W+/+) or TP53(R248W−/−)] or with a complete knockout of p53 [i.e. TP53(−/−)] (Sur et al. 2009) were kindly provided by Prof. Bert Vogelstein, John Hopkins University School of Medicine, Baltimore, MD. The R248 W mutation is found in some patients with...
the Li–Fraumeni syndrome and leads to substitution of arginine for tryptophan, which results in modulated DNA binding capacity of the corresponding p53 protein product.

HCT116 cells were grown as adherent monolayers in complete growth medium: Dulbecco’s modified Eagle’s medium (#21885-025, Invitrogen) with 10 % foetal bovine serum (#10106, Invitrogen), supplemented with 100 units/mL penicillin–streptomycin. Cells were cultured at 37 °C in 5 % CO₂ and passaged before the cells surpassed 80 % confluence. For treatment, cells were seeded at 3 × 10⁴ cells/mL, (Pierce, Thermo Scientific, UK) according to the manufacturer’s instructions.

Using the BCA Protein Assay (Pierce, Thermo Scientific) protein concentration of the supernatant was determined by centrifuging for 5 min at 5,000 rpm. Subsequently, proteins were sonicated in the test compounds or solvent dimethyl sulfoxide (DMSO) as a control for up to 48 h. The DMSO concentration was always kept at ≤0.5 % of the total culture medium volume. Based on previous experiments (Hockley et al. 2008), cells were treated with 2.5 µM BaP, DB[a,h]A or DB[a,l]P. Incubations with PAH-diol-epoxides were as follows: 0.5 µM BPDE, 0.5 µM DB[a,h]ADE or 0.0025 µM DB[a,l]PDE. Cells were harvested by trypsinization and washed with phosphate-buffered saline (PBS).

In experiments using nutlin-3a (Cayman Chemicals; 18585), cells were pretreated with 5 µM nutlin-3a for 6 h prior to exposure to BaP for 24 h. Nutlin-3a remained for the whole incubation period.

Cell viability and DNA adduct analysis

Cell viability (% control) was measured using the CASY Model TT Electronic Cell Analyser (Innovatis AG, Germany). DNA was isolated from PAH-treated cells using a standard phenol/chloroform extraction method. The nucle-ase P1 digestion enrichment version of the thin-layer chromatography (TLC) ³²P-postlabelling assay was used to measure DNA adduct formation. The procedure was essentially carried out as described previously (Arlt et al. 2008; Malik et al. 2013; Phillips and Arlt 2007; Siddens et al. 2012). After chromatography, TLC sheets were scanned using a Packard Instant Imager (Dowers Grove, IL, USA) and DNA adduct levels (RAL, relative adduct labelling) were calculated from the adduct cpm, the specific activity of [γ-³²P]ATP and the amount of DNA (pmol of DNA-P) used.

Western blot analysis

After treatment cells were lysed [62.5 mM Tris (pH 6.8), 1 mM EDTA (pH 8.0), 2 % sodium dodecyl sulphate (SDS), 10 % glycerol], cells were sonicated and centrifuged for 5 min at 5,000 rpm. Subsequently, protein concentration of the supernatant was determined using the BCA Protein Assay (Pierce, Thermo Scientific, UK) according to the manufacturer’s instructions. β-Mercaptoethanol (0.1 % final; in a solution containing the loading dye bromophenol blue) was added to reduce disulphide bonds and lysates were denatured at 95 °C for 5 min. Equal amounts of protein (10–20 µg) were separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) using 4–12 % Bis–Tris gradient or 10 % Bis–Tris gels, and Western blotted as previously reported (Kucab et al. 2012). After blocking in 3 % nonfat milk (dissolved in PBS with 0.2 % Tween-20), blots were incubated overnight at 4 °C with primary antibodies or anti-serum diluted in blocking solution. The following primary antibodies and dilutions were used: anti-p53 1:2,000 (Ab-6, Calbiochem), anti-p21 (CDKN1A) 1:2,000 (556431, BD Pharmingen), anti-NQO1 1:10,000 (ab34173, Abcam) and anti-AHR 1:1,000 (ab2770, Abcam). Anti-CYP1A1 raised in rabbits against purified human recombinant CYP1A1 was a generous gift from Prof. F. Peter Guengerich (Vanderbilt University, USA) and was diluted 1:4000. The antibody to detect β-actin 1:20,000 (ab6276, Abcam) was used as loading control. The secondary horseradish peroxidase-linked antibodies were as follows: anti-mouse (#170-5047; 1:10,000) and anti-rabbit (#170-5046; 1:10,000) from BioRad.

Measurement of nucleotide excision repair (NER) capacity

To assess NER capacity in HCT116 cells, a modified comet assay was applied (Langie et al. 2006). This assay measures the ability of NER-related enzymes that are present in the cell extract to incise substrate DNA containing BPDE-DNA adducts. The substrate nucleoids were prepared from untreated HCT116 TP53(+/+) cells as reported (Langie et al. 2006). They were then exposed to BPDE (1 µM) or vehicle control (DMSO, 0.5 % in PBS) for 30 min. Preparation of the protein extracts of the HCT116 TP53(+/+) TP53(+/−), TP53(−/−), TP53(R248W+/+) and TP53(R248W−) cells, ex vivo repair incubation and electrophoresis were performed according to the published protocol (Langie et al. 2006). Dried slides were stained with ethidium bromide (10 µg/mL), and comets were analysed using a Leica fluorescence microscope (Leica DMLB 020-519-010 LB30T). DNA damage was scored using the Comet IV capture system (version 4.11; Perceptive Instruments, UK). Fifty nucleoids were assessed per slide and each sample was analysed in duplicate. All samples were measured blindly. The results from both replicates were combined for further analysis. The tail intensity (% tail DNA), defined as the percentage of DNA migrated from the head of the comet into the tail, was used as an indicative measure of the repair capacity of the cell extracts. After subtracting background levels from all data, the final DNA repair capacity was calculated as previously reported (Langie et al. 2006).
High-performance liquid chromatography (HPLC) analysis of BaP metabolites

For the analysis of BaP metabolites, culture medium (2 mL) from exposed cells was collected and stored at −20 °C until further processing. These samples were analysed at the National Institute of Occupational Health, Norway, as described (Dendele et al. 2014). Briefly, the medium was diluted to 10 mL with water, applied to a pre-conditioned (5 mL methanol and 10 mL water) Sep-Pak C18 cartridge (Millipore Corporation, Milford, MA, USA), followed by a wash with water (10 mL), and eluted with 100 % methanol (5 mL). The methanol eluate was evaporated to dryness at 45 °C under a nitrogen stream and suspended in 100 mL (5 mL methanol and 10 mL water) Sep-Pak C18 cartridge diluted to 10 mL with water, applied to a preconditioned the National Institute of Occupational Health, Norway, as described. These samples were analysed at King’s College London. Briefly, 5 mL of ethyl acetate. Extracts were evaporated and taken up in 200 μL methanol, of which 20-μL aliquots were injected. HPLC analysis was performed using a HPLC Agilent 1100 System (Agilent Technologies) with a SunFireTM C18 reverse phase column (250 × 4.6 mm, 5 μm; Waters) using a methanol/water gradient at a flow rate of 0.2 mL/min. A linear gradient of 20–100 % methanol over 10 min was followed by isocratic elution for 20 min. This was succeeded by a linear gradient of 100–20 % methanol for 10 min, and then isocratic elution of 20 % methanol for 5 min. Total run time was 60 min. The metabolites were analysed by fluorescence detection (excitation wavelength 375 nm, emission wavelength 450 nm). The two BaP metabolites analysed, BaP-7,8-dihydrodiol and BaP-tetrol-I-1, were identified using authentic standards.

Cell cycle

After treatment with 2.5 μM BaP for 24 and 48 h, HCT116 cells were harvested and fixed in ice cold 70 % ethanol. For staining with propidium iodide (Invitrogen, UK), cells were pelleted, washed with PBS and resuspended in staining buffer containing 40 μg/mL propidium iodide, 100 μg/mL RNase (Qiagen, UK) in PBS. Samples were incubated at 37 °C for 1 h in the dark and subsequently stored at 4 °C overnight before analysis. The DNA content of 10,000 cells per sample was analysed using a BD FACSCanto II (BD Biosciences, UK) at 488 nm.

Gene expression analysis

HCT116 cells were treated with 2.5 μM BaP for 24 h. RNA was isolated using RNeasy Mini kit (Qiagen, UK) and reverse-transcribed into cDNA using the SuperScript III reverse transcriptase kit (Invitrogen, UK). Relative quantification of CYP1A1 and CYP1B1 mRNA expression was performed using fluorescent qRT-PCR with the ABI PRISM 7500HT Fast Sequence Detection System (Applied Biosystems, UK) as described previously (Hockley et al. 2006, 2008). CYP1A1 and CYP1B1 expression was detected using TaqMan® gene expression primers and probes (CYP1A1-Hs00153120_m1 and CYP1B1-Hs00164383_m1). Relative gene expression was calculated using the comparative threshold cycle (C_T) method.

Chromatin immunoprecipitation (ChIP)

HCT116 TP53(+/+) and TP53(−/−) cells were treated with 2.5 μM BaP for 24 h. In order to specifically induce p53 and its target p21, cells were also treated with 10 μM nutlin-3a. Cells underwent cross-linking (in 0.75 % formaldehyde for 10 min at room temperature) followed by quenching with glycine (in 0.125 M glycine for 5 min at room temperature). Cells were rinsed twice with 10 mL cold PBS, scraped and centrifuged (5 min, 1,000×g). Subsequently, cells were lysed (50 mM HEPES–KO H pH 7.5, 140 mM sodium chloride, 1 mM EDTA, 0.1 % SDS, 1 % Triton X-100, 0.1 % sodium deoxycholate, and 1 % protease inhibitors), sonicated to an average DNA fragment length of 500 base pairs (6 × 15 s) and then centrifuged (15 min, 8 000×g, 4 °C). The chromatin solution was precleared by adding protein A beads (sc-2001, Santa Cruz Biotechnology) that were pre-absorbed with BSA and salmon sperm DNA for 1 h at 4 °C. Immunoprecipitation of chromatin was performed with 5 μL of monoclonal α-p53 antibody (CM1, Cambridge Bioscience) or with nonrelevant rabbit anti-IgG (Abcam, ab46540) overnight at 4 °C. Immunoprecipitates were washed three times with washing buffer (20 mM Tris–HCl pH 8.0, 500 mM sodium chloride, 1 % Triton X-100, 2 mM EDTA pH 8.0, 0.1 % SDS, and 1 % protease inhibitors). Samples were treated with 2 μL RNase A (0.5 mg/mL) for 30 min (37 °C), followed by 30 μL proteinase K (20 mg/mL) for 2 h, (37 °C). DNA was isolated.
by phenol/chloroform extraction. qRT-PCR was performed as described above with each sample containing 5 µL of immunoprecipitated DNA; values were normalised for 1 % input values. Five independent experiments were performed.

ChIP primer sequences for the **CDKN1A** promoter (5′ p53 response element) were adapted from previous studies (Kaeser and Iggo 2002; Laptenko et al. 2011). ChIP primer sequences for the **CYP1A1** promoter (5′ p53 response element) were adapted from Rockefeller University (http://linkage.rockefeller.edu/p53; 16 October 2012) and verified by bioinformatic analysis. Primers are in italics, TaqMan probes are in bold, and p53-binding sites (**CDKN1A**, **CYP1A1**) are underlined:

**CDKN1A** promoter:

CTGGACTGGGCACCTTTGGCTCCCCACGCTGAGC
CTCCTCGCATTGGCAGGCTGTCCTCCACAG
GAACATCTTGGGCAGCAGCTGTGGCTCT
GATTGTGGTTTCGCGCTAGAAGCTGTC
CAACACTGTGGAGCTCGTGGCATAGAAGA
GGCTTGTTGGCTATTTCTGCTGGTCTC
GTTTTGAGTTGAGGAAGG
CYP1A1 promoter:

AGCCTCAAGCTACTGCAAGGAACAAACCAAG
CTGAAGTCCAGCTGGCAACCTGCTTGTG
CAGCGGGCGCCGCGGGGATGAGAAATTTG
TGCTCAATCATTTCTTTGATATGGTTATTATTTTCT
ACTTTAACACTAAACTTTGGCATATATCT
AACAAAGCATTGGAAGCATCCTACGTGAAAGAG
CCAGACACAC

Results

Cell viability after PAH exposure

In the previous work, treatment with 2.5 µM BaP and 0.5 µM BPDE was found to induce DNA adduct formation in HCT116 **TP53(+/+)** cells within a linear dose–response range (Hockley et al. 2008). The optimal concentrations of DB[a,h]A and DB[a,l]P were determined in initial experiments to be similar to that of BaP (data not shown). Thus, HCT116 cells were treated with 2.5 µM BaP, DB[a,h]A or DB[a,l]P, which allowed direct comparison of the potency of each PAH to induce DNA adduct formation. Whereas DB[a,h]ADE could be tested at the same concentration as BPDE, the high cytotoxicity of DB[a,l]PDE meant that the selected concentration for treatment was 200-fold lower, at 0.0025 µM. Under these treatment conditions, no impact on cell viability (>80 % relative to controls) was observed in any of the isogenic HCT116 cell lines after PAH exposure for 24 and 48 h (Supporting Figure 2), which was in line with previous findings (Hockley et al. 2008).

Expression of DNA damage response proteins and cell cycle alteration after PAH exposure

Expression of DNA damage response proteins p53 and p21 was assessed by Western blot analysis after BaP exposure for 24 and 48 h (Fig. 1). Each cell line displayed a time-independent and treatment-independent level of p53 expression, except for **TP53(−/−)** cells which did not express p53 as expected. Equal expression levels were detectable in **TP53(+/+)**, **TP53(R248W/+)** and **TP53(R248W−/)** cells (Fig. 1b), whereas p53 levels decreased in **TP53(+/−)** relative to **TP53(+/+)** cells (Fig. 1a). The p53 target p21 was expressed in all cell lines, but no induction was found after BaP treatment compared with control (untreated) samples (Fig. 1). The highest p21 levels were observed in **TP53(+/+)** cells. Expression levels were reduced in **TP53(+/−)** cells and further reduced in **TP53(−/−) cells** (Fig. 1a). **TP53(R248W+)** cells showed similar levels of p21 expression to **TP53(+/−) cells**, whereas hardly any p21 expression was seen in **TP53(R248W−/) cells** (Fig. 1b).

As shown in Supporting Figure 3A, cell cycle parameters as measured by flow cytometry were modestly altered after 24 h in **TP53(R248W+/+) and TP53(R248W−/) relative to TP53(+/+) cells**. After 48 h, BaP treatment had a marginal effect on cell cycle in **TP53(+/+) cells** relative to controls (Supporting Figure 3B), consistent with previous results (Hockley et al. 2008).

Formation of PAH-DNA adducts

PAH-DNA adduct formation after 24 and 48 h was determined by the 32P-postlabelling method (Fig. 2). After BaP and BPDE treatment (Fig. 2a, b), the DNA adduct pattern on TLC was qualitatively similar in all isogenic HCT116 cells, consisting of one single adduct spot, previously identified as 10-(deoxyguanosin-2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (dG-N2-BPDE) (Arlt et al. 2008). DB[a,h]A treatment also resulted in the formation of one major adduct spot which corresponded to the adduct spot obtained after treatment with DB[a,h]ADE (Fig. 2c, d). Exposure to DB[a,l]P induced a pattern consisting of four major adduct spots (Fig. 2e), of which three were found after treatment with DB[a,l]PDE (i.e. spots 1–3; Fig. 2f). No DNA adducts were detected in controls (data not shown).

Quantitative analysis obtained by 32P-postlabelling revealed a time-dependent increase in DNA adduct formation from 24 to 48 h after treatment with the parent PAH (Fig. 2a, c, e). In **TP53(+/+) cells**, adduct levels were ~twofold higher and up to 1.5-fold higher for the other cell lines at 48 h than at 24 h. Amongst the PAHs, the genotoxic potencies increased in the order DB[a,h]A ≪ BaP < DB[a,l]P. Strikingly, PAH-induced DNA adduct levels were up to eightfold lower in **TP53(−/−) cells**.
A or 2.5 μM DB[A] and ~fourfold for DB[a,l]P.

Fig. 1 Western blot analysis of p53, p21 (CDKN1A), CYP1A1, AHR and NQO1 protein expression in isogenic HCT116 cells after exposure to various PAHs or BPDE. Cells were exposed to 2.5 μM BaP, 0.5 μM BPDE, 2.5 μM DB[a,h]A or 2.5 μM DB[a,l]P and harvested after the times indicated. a HCT116 TP53(+/+) cells compared to TP53(+/−) and TP53(−/−) cells. b HCT116 TP53(+/+) cells compared to TP53(R248W+/+) and TP53(R248W−/−) cells. β-Actin protein expression was used as a loading control. Representative images of the Western blotting are shown; at least duplicate analysis was performed from independent experiments.

than in TP53(+/+) cells; the difference was ~eightfold for BaP, ~sixfold for DB[a,h]A and ~fourfold for DB[a,l]P. Interestingly, adduct levels in TP53(+/−) cells were almost the same as those found in TP53(−/−) cells. It is also noteworthy that adduct levels in both mutant TP53 cell lines, TP53(R248W+/+) and TP53(R248W−/−), were manifoldly lower compared with TP53(+/−) cells, but generally ~1.5 times higher than in TP53(+/−) cells.

In contrast, similar adduct levels in TP53(+/+) cells relative to all other cell lines were observed after treatment with BPDE (Fig. 2b). In line with this observation, adduct levels were also similar after DB[a,h]ADE or DB[a,l]PDE exposure in all cell lines (Fig. 2d, f). For all three PAH-diol-epoxides, DNA adduct formation was lower after 48 h relative to 24-h treatment. It is also noteworthy that adduct levels after shorter diol-epoxide exposure, namely 2 h, were similar in all cell lines (data not shown). More importantly, as PAH-diol-epoxides do not require metabolic activation to bind to DNA, these findings suggest that the differences in DNA adduct formation observed with the parent PAHs are the consequence of the different capacities of the cells to metabolically activate the PAHs. In other words, these results suggest that the cellular TP53 status impacts on the metabolic bioactivation of the parent PAHs.

CYP1A1 protein expression after PAH exposure

Bioactivation of many PAHs including BaP, DB[a,h]A and DB[a,l]P is catalysed by CYP1A1 (Baird et al. 2005; Luch and Baird 2005). To determine the impact of the cellular TP53 status in HCT116 cells on the CYP1A1-mediated metabolic activation of the PAHs tested, CYP1A1 protein levels were determined by Western blotting after 24 and 48 h. As shown in Fig. 1, basal levels of CYP1A1 were low in all cell lines, but CYP1A1 was induced after PAH exposure. A striking finding was that CYP1A1 induction correlated with the PAH-induced DNA adduct levels in each cell line (compare Fig. 2). In contrast, after BPDE exposure, for example, no CYP1A1 induction was observed (Fig. 1). The lack of CYP1A1 induction after BPDE correlated with the finding that BPDE exposure of the cells resulted in similar DNA adduct levels regardless of the TP53 status (compare Fig. 2b).

PAHs such as BaP, DB[a,h]A and DB[a,l]P can bind to and activate AHR (Hockley et al. 2007). Thus, the
activated receptor is capable of modulating the expression of its target genes such as\textit{CYP1A1} (Hockley et al. 2007). As shown in Fig. 1, AHR expression in whole cell lysates decreased in all cell lines to the same extent after PAH exposure.

\textbf{CYP1A1 and CYP1B1 mRNA expression after BaP treatment}

RT-PCR is a sensitive and specific measure of gene expression (Hamouchene et al. 2011; Hockley et al. 2006) and was used to evaluate \textit{CYP1A1} and \textit{CYP1B1} expression changes in HCT116 cells treated with BaP for 24 h. Similar to the results obtained by Western blotting, \textit{CYP1A1} mRNA induction after BaP treatment was lower in all cells relative to TP53(+/-) cells (Fig. 3a) and correlated with BaP-DNA adduct formation in each cell line (compare Fig. 1a). Small alterations in \textit{CYP1B1} mRNA induction were found in some cell lines (Fig. 3b); again \textit{CYP1B1} expression was lower relative to TP53(+/-) cells in all other cell lines. However, the fold differences appeared to be subtle, suggesting that any change in \textit{CYP1B1} expression may not significantly influence the differences in BaP-DNA adduct levels observed in these cell lines.

**Fig. 2** DNA adduct levels (RAL, relative adduct labelling) detected by \textsuperscript{32}P-postlabelling in isogenic HCT116 cells after exposure to various PAHs and their corresponding diol-epoxides. Cells were exposed to a 2.5 μM BaP, b 0.5 μM BPDE, c 2.5 μM DB\textsubscript{a,h}A, d 0.5 μM DB\textsubscript{a,h}ADE, e 2.5 μM DB\textsubscript{a,l}P or f 0.0025 μM DB\textsubscript{a,l}PDE and harvested after the times indicated. The values are the mean ± range of duplicate cell incubations; each DNA sample was analysed in duplicate in separate experiments. Statistical analysis was performed by one-way ANOVA followed by the Tukey post hoc test \([***p < 0.005, \text{ different from HCT116 TP53(+/-)}]\). *Insets* Autoradiographic profiles of DNA adducts formed in HCT116 cells after exposure; the origins, at the bottom left-hand corners, were cut off before exposure.
NQO1 protein expression after BaP treatment

As BaP-derivatives can also be metabolised by NAD(P)H:quinone oxidoreductase (NQO1) (Luch and Baird 2005), we also determined NQO1 protein expression in HCT116 cells after BaP exposure. It appears that a marginal induction of NQO1 protein levels was observed upon BaP treatment after 48 h (Fig. 1). However, this induction was very small and independent of the cellular TP53 status.

Impact of cellular TP53 status on BaP metabolism

In order to obtain further insights into the effects of TP53 status on BaP metabolism, BaP metabolites were analysed in the cell culture medium by HPLC analysis. A scheme showing the structures of BaP metabolites analysed is given in Supplementary Figure 4.

Impact of cellular TP53 status on BaP metabolism

In order to obtain further insights into the effects of TP53 status on BaP metabolism, BaP metabolites were analysed in the cell culture medium by HPLC analysis. A scheme showing the structures of BaP metabolites analysed is given in Supplementary Figure 4. As shown in Table 1, the levels of all BaP metabolites analysed in the TP53(+/+) cells were significantly higher than in all other cultures. For example, the level of BaP-t-7,8-dihydrodiol, a precursor of BPDE, was ~fivefold higher in TP53(+/+) than in TP53(−/−) cell cultures. Overall, the levels of BaP metabolites formed by each cell line corresponded to the BaP-DNA adduct levels observed in these cells.

Impact of p53 induction on BaP metabolism and CYP1A1 expression

Nutlin-3a, an inhibitor of MDM2, was used to induce p53 in TP53(+/+) cells. Using Western blot analysis, it was found that p53 was induced upon nutlin-3a pre-treatment which subsequently led to an increase in CYP1A1 levels in cells co-exposed to BaP for 24 h (Fig. 4a). CYP1A1 protein levels were slightly induced in cells treated solely with nutlin-3a for up to 30 h. Similar experiments carried out in TP53(−/−) cells showed a marginal induction of CYP1A1 levels after treatment with BaP alone, but pre-treatment with nutlin-3a had no effect on CYP1A1 expression levels (Fig. 4a).

The p53/nutlin-3a-mediated induction of CYP1A1 in TP53(+/+) cells after BaP exposure also altered the formation of BaP metabolites. For these experiments, the formation of BaP-t-7,8-dihydrodiol and BaP-tetrol-II-1 was determined in the cell culture medium using HPLC analysis. These metabolites were chosen as they are, in the first case, precursors of BPDE and, in the second case, one of the hydrolysis products of BPDE. As shown in Fig. 4b, c, the levels of both BaP metabolites increased in TP53(+/+) cells and co-exposed to nutlin-3a and BaP relative to cells treated with BaP alone, indicating that p53 induction by nutlin-3a increases CYP1A1-mediated BaP metabolism. As seen before, the levels of both BaP metabolites were lower in TP53(−/−) compared with TP53(+/+) cells (Fig. 4b, c; compare Table 1). In TP53(−/−) cells, no change was observed for BaP-t-7,8-dihydrodiol, whereas BaP-tetrol-I-1 was significantly higher in TP53(−/−) cells treated with nutlin-3a and BaP relative to cells treated with BaP alone, but the change was small.

Binding of p53 to the DNA-regulatory elements of p53 (p53REs) in the promoter region of CYP1A1 after BaP exposure

After demonstrating that alterations in the cellular p53 levels can impact on the CYP1A1-mediated metabolism of BaP and subsequent formation of BaP-DNA adducts, we wanted to explore the potential mechanism behind this phenomenon. It is well known that p53 induces gene expression of its target genes by binding to p53REs and thereby inducing their transcription (Menendez et al. 2009). Recent studies have indicated that p53 can induce gene expression of CYP3A4 by binding to p53REs (Goldstein et al. 2013). Therefore,
we hypothesised that CYPIA1 expression may also be regulated by p53. After in silico analysis of the regulatory region upstream of the CYPIA1 gene, a putative p53RE was identified. We used ChIP to investigate whether p53 can bind to the p53RE identified in the regulatory region of CYPIA1. In order to validate the efficiency of the ChIP assay, we confirmed that p53 binds to a known p53RE of CDKN1A (p21) (Kaeser and Iggo 2002; Laptenko et al. 2011), a downstream target of p53, p53 binds to a known p53RE of CDKN1A (p21) (Kaeser and Iggo 2002; Laptenko et al. 2011), a downstream target of p53, to determine whether the cellular TP53 status impacted on NER in HCT116 cells, we phenotypically assessed NER capacity. Cell extracts from HCT116 cells were examined for their ability to repair BPDE-induced DNA adducts using a modified comet assay (Langie et al. 2006). Utilising this assay, we found that all HCT116 cell lines used in the present study had the same NER capacity (Supporting Figure 5).

**Discussion**

The repertoire of genes subject to p53 control extends across a diverse group of biological activities that include apoptosis, cell cycle regulation, senescence, energy metabolism, angiogenesis, cell differentiation and immune response. In this study, we show that carcinogenic PAHs can induce CYPIA1 in human cells via a p53-dependent mechanism, indicating a novel role for p53 in CYPIA1-mediated carcinogen metabolism. CYPIA1 is one of the key enzymes involved in PAH metabolism, and we found that alterations of the cellular TP53 status as well as nutlin-3a-induced p53 activation had dramatic effects on the metabolism of a variety of PAHs, namely BaP, DB[a,h]A and DB[a,l]P.

Using a panel of isogenic colorectal HCT116 cells differing only with respect to their endogenous TP53 status, we found that complete loss of p53 function resulted
in considerably lower PAH-DNA adduct levels compared with cells having wild-type p53 (Fig. 1). This is in line with a previous observation using the HCT116 TP53(+/+) and TP53(−/−) cells exposed to BaP and where results were confirmed by knocking down TP53 in WT cells by siRNA (Hockley et al. 2008). Previous gene expression data suggested that off-target effects of the TP53 knockout procedure by homologous recombination in HCT116 cells were minimal at the gene expression level (Hockley et al. 2008). Furthermore, the same phenomenon was observed in other cell lines (e.g. lung A549 cells) with stably knocked down p53 expression, although the difference in adduct levels in cells with no p53 expression was greater (Hockley et al. 2008). As this phenomenon was not observed after exposure to the PAH-diol-epoxides (compare Fig. 2), these
results indicate that the levels of p53 expression impact on the metabolic activation of the parent PAH.

In the present study, we also showed that BaP metabolites measured in the culture media were higher with HCT116 TP53(+/+) cells than with TP53(−/−) cells (Table 1). The measured metabolites included BaP-tetrols, BaP-dihydrodiols and hydroxylated-BaP metabolites demonstrating that CYP-mediated metabolism was impaired in TP53(−/−) cells, which correlated with lower BaP-DNA adduct formation in these cells. Higher induction of CYP1A1 expression (Fig. 3) and an increase in CYP1A1 protein levels (Fig. 1) in HCT116 cells with WT p53 explains the difference in BaP metabolism observed in TP53(−/−) cells. Furthermore, pre-treatment of HCT116 TP53(+/+) cells with nutlin-3a led to p53 induction and subsequent stimulation of CYP1A1 expression after co-exposure to BaP (Fig. 4a). Consequently, p53 induction by nutlin-3a enhanced CYP1A1-mediated BaP metabolism (Fig. 4b).

Many studies have demonstrated the induction of p53 in response to PAH exposure and this can be of significance for the molecular phenomenon (i.e. impact of TP53 status on PAH-induced DNA adduct formation) described above. We and others previously identified p53 as an important component of PAH-induced transcriptional responses in human cell lines and in vivo (Hamouchene et al. 2011; Hockley et al. 2006, 2008; Labib et al. 2012; Malik et al. 2012, 2013). Thus, these gene–environment interactions need to be taken into account with regards to PAH metabolism. Furthermore, as many anti-cancer treatment regimens are composed of several drugs of which at least one is a p53 activating drug, our molecular observation may also be of clinical importance (Goldstein et al. 2013). Therefore, these potential drug-environmental interactions may also be carefully considered for human risk assessment.

A well-established molecular mechanism for the induction of CYP1A1 is via AHR with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) being one of the best characterised ligands for this orphan nuclear receptor. (Wang et al. 2011) PAHs such as BaP can also bind to and activate AHR thereby enhancing their own metabolic activation (Hockley et al. 2007). However, a recent study has indicated that p53 can induce gene expression of certain CYPs by binding to p53REs in the promoter region of these CYPs. It was shown that p53 induces the activity of CYP3A4, one of the major drug-metabolising enzymes in the liver, via its binding to p53REs and the subsequent transcriptional enhancement of CYP3A4 (Goldstein et al. 2013). In the present study, we propose a new mechanism by which CYP1A1 is induced by p53. Using ChIP analysis, we showed that p53 can bind to a p53RE in the regulatory region upstream of the CYP1A1 gene in HCT116 TP53(+/+) cells after BaP exposure (Fig. 5). Consequently, these results indicate that BaP is able to induce CYP1A1 not only by binding as a ligand to AHR promoting CYP1A1 transcriptional activation, but also by induction of p53 which can subsequently trigger CYP1A1 transcription via its binding to p53REs.

Both complete and partial inactivation of tumour suppressors such as TP53 can play a critical role in the pathogenesis of cancer (Berger et al. 2011). It has been shown that reductions in p53 dosage and function can impact on a cell’s ability to respond to DNA damage (Berger et al. 2011; Bunz et al. 1999). Deletions of the TP53 locus are frequently found in human cancer, and loss of heterozygosity (LOH) has been shown to occur in a fraction of tumours harbouring a TP53 mutation (Berger and Pandolfi 2011). TP53 is a powerful example of how haploinsufficiency promotes tumourigenesis, whereby loss of one copy of TP53 gives rise to a phenotype intermediate to that occurring after complete loss of the gene (Berger and Pandolfi 2011). As such, survival of Trp53(+/−) mice show an intermediate survival to that Trp53(−/−) and Trp53(+/+) mice, and tumours that develop in Trp53(−/−) mice do not always display loss of the remaining WT allele (Berger et al. 2011; Venkatachalam et al. 1998). Therefore, it was expected that HCT116 TP53(+/+) cells generate an intermediate response to those observed in TP53(−/−) and TP53(+/+) cells. Interestingly, HCT116 TP53(+/+) cells behaved more similar to TP53(−/−) cells than TP53(+/+) cells with regards to PAH-DNA adduct formation, while adduct formation induced by the corresponding PAH-diol-epoxides again did not differ (Fig. 2). This observation was in accordance with the fact that HCT116 TP53(+/+) and TP53(−/−) cells formed similar amounts of BaP metabolites (Table 1). Expression of CYP1A1, at both the gene and protein level (Figs. 1, 3), was also similar in both cell lines. These results suggest that in HCT116 TP53(+/−) cells, the remaining WT allele is not sufficient for normal cellular function. This possibility has been indicated in another study using these cells where loss of one WT allele resulted in a ~fourfold reduction in TP53 mRNA and protein levels compared with TP53(+/+) cells before and after p53 stabilization resulting from UV radiation (Lynch and Milner 2006). One reason for this phenomenon could be that p53 function is dependent on its tetrameric structure and reduction in dosage of the WT protein by 50 % may result in disproportionate reduction in active tetramer concentrations (Santarosa and Ashworth 2004).
158, 175, 245, 248 and 273) characteristic of PAHs present in tobacco smoke. (Denissenko et al. 1996) Of note, hotspot mutations found at codons 175, 248, 249 and 273 together account for over 25 % of all missense mutations identified in human cancers (Olivier et al. 2010). Mutant p53 expressed in preneoplastic and/or neoplastic cells can severely limit or abolish the capacity of p53 to regulate its target genes (Freed-Pastor and Prives 2012). One TP53 hotspot mutation found in human tumours, R248 W, has been identified as a contact mutation that induces conformational changes and abolishes the tumour suppressive activity of p53 (Song et al. 2007). Both in HCT116 cells where one WT allele was replaced with mutant (R248 W) allele, and in cells where one WT allele was inactivated by homologous recombination and the other was replaced with a mutant (R248 W) allele, PAH metabolism and PAH-DNA adduct formation was altered relative to TP53(+/+) cells (Table 1; Figs. 1, 2, 3). The response in HCT116 cells with mutant p53 was similar to that in cells having a complete knockout of p53. However, it is possible that cells carrying a different TP53 mutation may respond differently. A more comprehensive approach to studying the impact of distinct TP53 mutations in cells with a closely matched genetic background for comparative functional analysis of p53 and carcinogen metabolism in the future has been published recently (Odell et al. 2013; Wei et al. 2011).

The responses seen after PAH exposure in HCT116 cells with altered p53 expression have been similar for different compounds in this study, but the response to other environmental carcinogens could be different. Another environmental carcinogen which has been tested in HCT116 TP53(+/+) and TP53(−/−) cells is aristolochic acid I (AAI), a herbal drug derived from the Aristolochia species which is a potent human carcinogen involved in the development of aristolochic acid nephropathy and linked to urothelial cancer (Gokmen et al. 2013; Schmeiser et al. 2014). The major activation pathway for AAI is nitroreduction, NQO1 being the most efficient activating enzyme, while CYP1A-mediated demethylation contributes to AAI detoxification (Stiborova et al. 2014). AAI-DNA adduct formation was significantly lower in TP53(−/−) relative to TP53(+/+) cells (Simoes et al. 2008). Interestingly, CYP1A1 gene expression measured by qRT-PCR analysis was higher in TP53(−/−) compared with TP53(+/+) cells after AAI exposure (Simoes et al. 2008), suggesting that cellular TP53 status may impact on CYP1A1-mediated AAI detoxification. However, the mechanism by which TP53 status influences AAI metabolism and AAI-DNA adduct formation in human cells remains to be investigated further.

In contrast, DNA adduct formation by a nitroarene, 3-nitrobenzantrone (3-NBA), was similar in HCT116 TP53(+/+) and TP53(−/−) cell lines (Hockley et al. 2008; Simoes et al. 2008). This observation indicates that the cellular impact of p53 on carcinogen metabolism depends on the agent studied and/or that only certain XMEs depend on p53 function. The most efficient enzyme to activate 3-NBA to DNA adducts is NQO1 (Arlt et al. 2005, 2006; Stiborova et al. 2010). Thus, NQO1 seems to be an enzyme not influenced by p53, which is in line with the fact that NQO1 protein expression was not altered in the HCT116 cell lines after BaP exposure (Fig. 2). Another recent study showed that induction of CYP2E1 gene expression is under the transcriptional control of p53 (Leung et al. 2013). As CYP2E1 overexpression inhibited migration of highly invasive MDA-MB-231 breast cancer cells expressing mutant p53, the authors concluded that manipulation of CYP2E1 protein expression could be potentially exploited in breast cancer therapy (Leung et al. 2013). However, in the context of our investigations, this study indicates that CYP2E1-mediated carcinogen metabolism may be altered by p53 expression and seems to be a promising target for future investigations. One typical substrate of CYP2E1 is ethanol which is the main cause of various liver diseases (Beier and McClain 2010). Another recent study demonstrated that UDP-glucuronosyltransferase 2B7 (UGT2B7) expression is regulated via the p53 pathway (Hu et al. 2014). Epirubicin, an anticancer drug, stimulated UGT2B7 promoter activity via p53RE in human hepatocarcinoma HepG2 cells and similarly nutlin-3a enhanced UGT2B7 expression and recruited p53 protein to the UGT2B7 p53RE in these cells (Hu et al. 2014). Collectively, these findings suggest a novel role for p53 in the regulation of both phase I and phase II XMEs.

In summary, we found that carcinogenic PAHs induce CYP1A1 expression in human cells via a p53-dependent mechanism. Thus, our identification of CYP1A1 as novel p53 target gene provides new insights into the mechanism controlling CYP1A1 expression. These results also provide new fundamental insights into the mechanism(s) of p53 in PAH-induced carcinogenesis. These results indicate that p53 may impact on the efficacy of chemotherapeutic drugs. However, they also indicate that gene–environment interactions need to be taken into account with regards to carcinogen metabolism. Future investigations will need to clarify which other XMEs depend on p53 function and which other environmental carcinogens depend on p53 for their metabolic activation.

**Acknowledgments** The study was funded, in part, by Cancer Research UK (Grant C313/A14329) and the Wellcome Trust (Grants 101126/Z/13/Z and 101126/B/13/Z). Laura Wohak was supported by a PhD studentship from the Institute of Cancer Research, London, United Kingdom. Annette Krais was supported by a fellowship from the German Research Foundation (DFG). Julia Stertmann received a Summer Student Placement Award from the United Kingdom Environmental Mutagen Society (UKEMS). We thank Dr. Phil Cunningham from King’s College London for advice and help with the bioinformatic analysis for the primer design.

© Springer
Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

Arlt VM, Stiborova M, Henderson CJ, Osborne MR, Bieler CA, Frei E, Martinke V, Sopko B, Wolf CR, Schmeiser HH, Phillips DH (2005) Environmental pollutant and potent mutagen 3-nitrobenzanthrone forms DNA adducts after reduction by NAD(P)H: quinone oxidoreductase and conjugation by acetylttransferases and sulfotransferases in human hepatic cytosols. Cancer Res 65:2644–2652

Arlt VM, Schmeiser HH, Osborne MR et al (2006) Identification of three major DNA adducts formed by the carcinogenic air pollutant 3-nitrobenzanthrone in rat lung at the C8 and N2 position of guanine and at the N6 position of adenine. Int J Cancer 118:2139–2146

Arlt VM, Stiborova M, vom Brocke J, Simoes ML, Lord GM, Nortier JL, Hollstein M, Phillips DH, Schmeiser HH (2007) Aristolochic acid mutagenesis: molecular clues to the etiology of Balkan endemic nephropathy-associated urothelial cancer. Carcinogenesis 28:2253–2261

Arlt VM, Stiborova M, Henderson CJ, Thiemann M, Frei E, Aimova D, Singh R, Gamboa da Costa G, Schmitz OJ, Farmer PB, Wolf CR, Phillips DH (2008) Metabolic activation of benzo[a]pyrene in vitro by hepatic cytochrome P450 contrasts with detoxification in vivo: experiments with hepatic cytochrome P450 reductase null mice. Carcinogenesis 29:656–665

Baird WM, Hooven LA, Mahadevan B (2005) Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environ Mol Mutagen 45:106–114

Beier JJ, McClain CJ (2010) Mechanisms and cell signaling in alcoholic liver disease. Biol Chem 391:1249–1264

Berger AH, Pandolfi PP (2011) Haplo-insufficiency: a driving force in cancer. J Pathol 223(2):137–146

Berger AH, Knudson AG, Pandolfi PP (2011) A continuum model for tumour suppression. Nature 476:163–169

Bunz F, Hwang PM, Torrance C, Waldman T, Zhang Y, Dillehay L, Berger AH, Pandolfi PP (2011) Haplo-insufficiency: a driving force in human cancer cells alters the responses to therapeutic agents. J Clin Invest 129:213–224

Chang RL, Wood AW, Huang MT, Xie JG, Cui XX, Reuhl KR, Boyd DR, Lin Y, Shih WJ, Balani SK, Yagi H, Jerina DM, Conney AH (2013) Mutagenicity and tumorigenicity of the four enantiopure bay-region 3,4-diol-1,2-epoxide isomers of dibenz[a,h]anthracene. Carcinogenesis 34:2184–2191

Crowell SR, Hanson-Drury S, Williams DE, Corley RA (2014) In vitro metabolism of benzo[a]pyrene and dibenzo[def,p]chrysene in rodent and human hepatic microsomes. Toxicol Lett 228:48–55

Dendele B, Tekpli X, Hardonnier K, Holme JA, Debare L, Catheline D, Arlt VM, Nagy E, Phillips DH, Ovrebo S, Mollerup S, Poet M, Chevannes M, Rioux V, Dimanche-Boitrel MT, Sergent O, Hockley SL, Arlt VM, Brewer D, Giddings I, Phillips DH (2007) AHR- and DNA-damage-mediated gene expression responses induced by benzo[a]pyrene in human cell lines. Chem Biol Interact 170:10385–10390

Dokmen O, Beckerman R, Freulich E, Prives C (2011) p53 binding to nucleosomes within the p21 promoter in vivo leads to nucleosome remodeling-dependent NHE1 pathway. Chem Biol Interact 207:41–51

Denissenko MF, Pao A, Tang M, Pfeifer GP (1996) Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. Science 274:430–432

Ford JM (2005) Regulation of DNA damage recognition and nucleotide excision repair: another role for p53. Mutat Res 577:195–202
Leung T, Rajendran R, Singh S, Garva R, Krstic-Demonacos M, Demonacos C (2013) Cytochrome P450 2E1 (CYP2E1) regulates the response to oxidative stress and migration of breast cancer cells. Breast Cancer Res 15:R107

Luch A, Baird WM (2005) Metabolic activation and detoxification of polycyclic aromatic hydrocarbons. Imperial College Press, London, pp p19–p96

Luch A, Glatt HR, Platt KL, Oesch F, Seidel A (1994) Synthesis and mutagenicity of diastereomeric fjord-region 11,12-dihydridrodiol 13,14-epoxides of dibenz[a,j]pyrene. Carcinogenesis 15:2507–2516

Lynch CJ, Milner J (2006) Loss of one p53 allele results in four-fold reduction of p53 mRNA and protein: a basis for p53 haplo-insufficiency. Oncogene 25:3463–3470

Maddocks OD, Vousden KH (2011) Metabolic regulation by p53. J Mol Med 89:237–245

Malik AI, Williams A, Lemieux CL, White PA, Yauk CL (2012) Hepatic mRNA, microRNA, and miR-34a-target responses in mice after 28 days exposure to doses of benzo[a]pyrene that elicit DNA damage and mutation. Environ Mol Mutagen 53:10–21

Malik AI, Rowan-Carroll A, Williams A, Lemieux CL, Long AS, Arlt VM, Phillips DH, White PA, Yauk CL (2013) Hepatic genotoxicity and toxicogenomic responses in MutaMouse males treated with dibenz[a,j]anthracene. Mutagenesis 28:543–554

Menendez D, Inga A, Resnick MA (2009) The expanding universe of p53 targets. Nat Rev 9:724–737

Odell AF, Odell LR, Askham JM, Alogheli H, Ponnambalam S, Hollstein M (2013) A novel p53 mutant found in iatrogenic urothelial cancers is dysfunctional and can be rescued by a second-site global suppressor mutation. J Biol Chem 288:16704–16714

Olivier M, Hollstein M, Hainaut P (2010) TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol 2:a001008

Phillips DH (2005) Macromolecular adducts as biomarkers of human exposure to polycyclic aromatic hydrocarbons. Imperial College Press, London, pp p137–p169

Phillips DH, Arlt VM (2007) The 32P-postlabeling assay for DNA repair and recombination. Nat Rev Mol Cell Biol 6:44–55

Siddens LK, Larkin A, Krueger SK, Bradfield CA, Waters KM, Tilton SC, Pereira CB, Lohr CV, Arlt VM, Phillips DH, Williams DE, Baird WM (2012) Polycyclic aromatic hydrocarbons as skin carcinogens: comparison of benzo[a]pyrene, dibenzo[def,p] chrysene and three environmental mixtures in the FVB/N mouse. Toxicol Appl Pharmacol 264:377–386

Song H, Hollstein M, Xu Y (2007) p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. Nat Cell Biol 9:573–580

Stiborova M, Martinek V, Svobodova M, Sistkova J, Dvorak Z, Ulrichova J, Simanek V, Frei E, Schmeiser HH, Phillips DH, Arlt VM (2010) Mechanisms of the different DNA adduct forming potentials of the urban air pollutants 2-nitrobenzanilide and carcinogenic 3-nitrobenzanilide. Chem Res Toxicol 23:1192–1201

Stiborova M, Frei E, Arlt VM, Schmeiser HH (2014) Knockout and humanized mice as suitable tools to identify enzymes metabolizing the human carcinogen aristolochic acid. Xenobiotica 44:135–145

Sur S, Pagliarini R, Bunz F, Rago C, Diaz LA Jr, Kinzler KW, Vogelstein B, Papadopoulos N (2009) A panel of isogenic human cancer cells suggests a therapeutic approach for cancers with inactivated p53. Proc Natl Acad Sci USA 106:3964–3969

Venkataram S, Shi YP, Jones SN, Vogel H, Bradley A, Pinkel D, Donehower LA (1998) Retention of wild-type p53 in tumors from p53 heterozygous mice: reduction of p53 dosage can promote cancer formation. EMBO J 17:4657–4667

Wang T, Gavin HM, Arlt VM, Lawrence BP, Fenton SE, Medina D, Vorderstrasse BA (2011) Aryl hydrocarbon receptor activation during pregnancy, and in adult nulliparous mice, delays the subsequent development of DMBA-induced mammary tumors. Int J Cancer 128:1509–1523

Wei QX, Odell AF, van der Hoeven F, Hollstein M (2011) Rapid derivation of genetically related mutants from embryonic cells harboring a recombinase-specific Trp53 platform. Cell Cycle 10:1261–1270

Whibley C, Pharoah PD, Hollstein M (2009) p53 polymorphisms: cancer implications. Nat Rev 9:95–107

Yagi H, Thakker DR, Hernandez O, Koreeda M, Jerina DM (1977) Synthesis and reactions of the highly mutagenic 7,8-diol 9,10-epoxides of the carcinogenic benzo[a]pyrene. J Am Chem Soc 99:1604–1611