Short Communication

Evaluation of the noncovalent binding interactions between polycyclic aromatic hydrocarbon metabolites and human *p53* cDNAYin Wei ^{a,*}, Yuan Lin ^b, Ai-Qian Zhang ^{b,*}, Liang-Hong Guo ^a, Jie Cao ^a^a State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China^b State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, 210093, China

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ABSTRACT

The binding of reactive polycyclic aromatic hydrocarbon (PAH) metabolites, formed enzymatically, to DNA is a crucial step in PAH carcinogenesis *in vivo*. We investigated the noncovalent binding interactions between 11 PAH metabolites and human *p53* complementary DNA (*p53* cDNA) using the fluorescence displacement method and molecular docking analysis. All of the examined metabolites predominantly interacted with *p53* cDNA by intercalation instead of groove binding. The dissociation constants ranged from 0.02 to 12.34 μM . Of the metabolites tested, 1-hydroxypyrene and 3-hydroxybenzo[a]pyrene showed the strongest binding affinities to DNA, while 2-naphthol was the weakest DNA intercalator. The intercalation of the metabolites was stabilized by stacking the PAH phenyl rings with the DNA base pairs and the formation of hydrogen bonds between the oxide or hydroxyl groups on the metabolites, and DNA bases or backbones. The binding of the metabolites to DNA showed some sequence selectivity. The binding affinities and hydrogen bonds for 3-hydroxybenzo[a]pyrene, benzo[a]pyrene-4,5-dihydroepoxide (BPE) and benzo[a]pyrene-r-7,t-8-dihydrodiol-t-9,10-epoxide (BPDE) differed. It seems that the functional groups on the periphery of the PAH aromatic ring play crucial roles in regulating its binding affinity with DNA. Although it was difficult to determine the correlation between DNA noncovalent binding affinity and carcinogenicity for some of the PAH metabolites, the present study improved our understanding of the formation of PAH metabolite-DNA adducts.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental organic pollutants. Some PAHs have been shown to have mutagenic and carcinogenic effects both *in vivo* in rodents and *in vitro* in mammalian (including human) cell lines (WHO, 1998). While PAHs are relatively inert, the critical step in PAH carcinogenesis is the *in vivo* enzymatic generation of reactive PAH metabolites (Miller and Miller, 1981; Harvey, 1991).

The covalent binding of PAH metabolites to DNA follows a rapid interaction between the metabolites and base pairs (Meehan et al., 1982). The proven key carcinogenic product, benzo[a]pyrene-r-7,t-8-dihydrodiol-t-9,10-epoxide (BPDE), intercalates rapidly to the base pairs of DNA to form a complex, which undergoes protonation to yield an intercalated triol carbonium ion intermediate (Meehan et al., 1982; Geacintov et al., 1981, 1982a,b). About 92% of BPDE is then hydrolyzed to yield tetraols, which also physically associate with DNA, and the remaining 8% of BPDE covalently binds to DNA (Shahbaz et al., 1986). BPDE binds covalently to the exocyclic amino group of guanine in

DNA. Other planar PAH diol-epoxides that contain a bay or fjord region, such as benz[a]anthracene and chrysene, bind covalently and almost exclusively with guanine bases in DNA. By contrast, the fjord region diol-epoxides of benzo[c]phenanthrene, with a distortion from the planarity of the parent molecule, react with adenine and guanine bases in DNA (Dipple et al., 1987; Jerina et al., 1991). The poorly repaired DNA adducts can persist until replication and cause mutations (Smith et al., 2000; Hainaut and Pfeifer, 2001; Feng et al., 2002; Rodríguez et al., 2007).

Human *p53* gene is a tumour-suppressor gene (Lamb and Crawford, 1986). Mutations in the *p53* gene have been observed in more than 50% of human cancers (Hainaut et al., 1997; Levine, 1997). More than 90% of the mutations in *p53* gene cause amino acid changes on the P53 protein. Mutations of the *p53* gene may cause the loss of its tumor suppressor function and gain oncogenic function (Lane and Benichmol, 1990). Exposure to cigarette smoke is correlated with guanine-cytosine (GC) to thymine-adenine (TA) mutations in *p53* in lung cancers (Miller et al., 1992; Harris, 1993). PAHs are major carcinogens in cigarette smoke. PAH metabolites, including BPDE, can preferentially form DNA adducts at mutational hotspots in the *p53* gene, which is one of the most commonly mutated genes in smoking-related lung cancers (Smith et al., 2000; Hainaut et al., 2001; Feng et al., 2002).

In our previous study, we used a rapid fluorescence displacement method to study the binding affinities of PAHs with DNA (Wei and

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Guo, 2009). Molecular docking analysis is an effective method for quantitative structure–activity relationship (QSAR) studies and to reveal the interaction between small molecules and proteins or DNA (Holt et al., 2008; Aranda et al., 2008). In this study, we investigated the noncovalent binding interactions between 11 PAH metabolites and *p53* complementary DNA (*p53* cDNA). The fluorescence method was used in conjunction with molecular docking analysis to reveal the binding mode, binding affinity and intermolecular interaction between these PAH metabolites and *p53* cDNA. It is anticipated that the information obtained in the present study will enhance our understanding of the formation of PAH metabolite–DNA adducts.

2. Experimental section

2.1. Reagents and preparation of *p53* cDNA

The detailed descriptions of reagents, with their abbreviations, and preparation of *p53* cDNA are available in Supplementary data.

2.2. Fluorescence measurements

Fluorescence emission spectra were recorded on a PerkinElmer LS 55 fluorescence spectrometer at 20 °C in a right-angle configuration; a 1-cm light-path quartz cuvette was used to assess the binding of fluorescent probes to DNA; a 3-mm light-path quartz cuvette was used to assess the binding of PAH metabolites to DNA by fluorescence displacement measurement, and a 10-nm slit width was set for excitation and emission. The excitation wavelengths were 504 nm and 344 nm for TO and H33258, respectively. The emission spectra were recorded between 510 nm and 560 nm for TO, and between 400 nm and 520 nm for H33258. The DNA was dissolved in 50 mM of Tris–HCl buffer (pH 7.4). Stock solutions of the tested organic compounds were prepared in methanol, and then added to the dye/DNA solution, with a methanol content of less than 5%. The method of data analysis is available in Supplementary data.

2.3. Molecular docking analysis

We performed docking analysis using Surflex-Dock in Sybyl version 7.3 (Tripos Associates), operating under Red Hat Linux 4.0 on a Dell workstation (Intel(R)Xeon(R) 5140, 2.33 GHz CPU, 2 GB memory). The binding models for PAH intercalation onto the human *p53* gene could not be obtained directly because the crystal structures of the related complexes were not available in Protein Data Bank (PDB) (<http://www.rcsb.org/pdb>). Fortunately, the PDB contained extensive information regarding the crystal structures of the intercalator–DNA complexes, and the high-resolution crystal structures of these complexes were used to establish the DNA intercalation cavity model to explore the binding interactions with the selected PAH metabolites. To avoid symmetry problems and maintain electrostatic consistence with TO, ellipticine was selected as a reference intercalator because its interaction mechanism excludes groove binding. The crystal structure for the ligand–nucleic acid complex for ellipticine was obtained from the PDB (identification number: 1z3f). The resolution of the structure was 1.5 Å. Ellipticine intercalated between the cytosine and guanine nucleotides in the sequence d(CGATCG)₂, and the PDB files were visually inspected using Jmol (11.7.46), Swiss PDB Viewer (4.0.1), and Sybyl (7.3). All water molecules were removed, and the hydrogen atoms were added as appropriate. In addition, the relevant energy optimization of the ligands was conducted using Tripos Force Field and atom charge was calculated by the Gasteiger–Hückel method; the criterion for energy gradient convergence was 0.05 kcal/mol. The molecular docking process was determined by the Surflex method in SYBYL 7.3. The score was then calculated and the contributions of the different interactive forces were analyzed. The flexibility of the related base and DNA backbone

around the intercalation site was taken into account by small-scale molecular dynamic simulations.

3. Results and Discussion

3.1. Binding studies of PAH metabolites to *p53* cDNA

After studying the binding affinities of fluorescent probes to *p53* cDNA (detailed information available in Supplementary data), we investigated the binding interactions between PAH metabolites and DNA. In competitive binding experiments, the intercalative or minor groove binding probe was treated as a reporter and mixed with the DNA at a stoichiometric ratio, and PAH metabolites were treated as competitors. For this study, the PAH metabolites included an epoxide, four diol–epoxides and six hydroxides (Scheme 1). These compounds contain two to five fused rings and different peripheral functional groups.

In competitive binding experiments with the intercalative probe TO, all PAH metabolites bound to DNA with good affinities. For BPDE, BADE, CDE, BPE and 2-hydroxyfluorene, the fluorescence signal decreased by 60–70% because of their limited solubility in aqueous solutions. By contrast, TO fluorescence was reduced to near the background level by the other metabolites. The displacement curves are shown in Fig. 1a and Supplementary data Fig. S4A and S4B. The calculated [IC₅₀] and *K_d* values for these metabolites on *p53* cDNA are listed in Table 1. The *K_d* values of the examined compounds ranged from 0.02 to 12.34 μM. The values for 1-hydroxypyrene and 3-hydroxybenzo[a]pyrene were the lowest; thus, these two compounds bound to DNA more strongly than did the other metabolites. 2-naphthol showed the weakest binding affinity to DNA, and the other tested metabolites had intermediate binding affinity. Comparing the three metabolites of benzo[a]pyrene, 3-hydroxybenzo[a]pyrene, BPE and BPDE, 3-hydroxybenzo[a]pyrene showed the strongest binding affinity to *p53* cDNA, whereas BPDE showed the weakest affinity. It was postulated that the functional group on the periphery of the benzo[a]pyrene aromatic ring plays a crucial role in influencing its binding affinity to DNA.

The carcinogenicity of various PAHs has been previously reported. Of the tested PAHs, benzo[a]pyrene shows the greatest carcinogenic effect in humans. Naphthalene, benzo[a]anthracene, benzo[c]phenanthrene and chrysene are possibly carcinogenic in humans. Pyrene, phenanthrene, dibenz [a, c] anthracene and fluorene are currently unclassified in terms of their carcinogenic effects in humans (IARC, 2009). In our study, of the tested PAH hydroxides, 3-hydroxybenzo[a]pyrene showed the greatest binding affinity for *p53* cDNA. However, for the PAH hydroxides and diol–epoxides, the correlation between binding affinity and carcinogenicity could not be established, which may be because the genotoxicity and carcinogenicity of PAHs are closely related to covalent PAH metabolite–DNA adducts. The rapid noncovalent interactions with the base pairs represents an earlier step in the formation of covalent adducts (Meehan et al., 1982). In our rapid fluorescence displacement assay, the time to measure each PAH metabolite did not exceed five minutes. Therefore, the buffer solution was not suitable and the reaction time was not sufficient to form stable covalent adducts, although covalent adducts could form during the incubation of PAH metabolites and DNA in an aqueous solution at room temperature (Geacintov et al., 1991; Urano et al., 1991; Pirogov et al., 1998). Measurements performed under this situation were done to investigate the predominant noncovalent interaction between the metabolites and DNA.

Moreover, competitive binding experiments for the PAH metabolites were performed using the minor groove binding agent H33258 as the probe. At the excitation wavelength for H33258, 344 nm, three metabolites, including 1-hydroxypyrene, BPDE and 3-hydroxybenzo[a]pyrene, showed higher fluorescence signals at 440 nm than the probe did at the same concentration. Therefore, these compounds

Scheme 1. Structure of the 11 PAH metabolites used in this study.

could not be tested in the displacement experiment. The remaining eight metabolites could not reduce the fluorescence of H33258, indicating that they did not compete with H33258 for binding to *p53* cDNA or CT-DNA (Fig. 1b and Supplementary data Fig. S4C, S5C and S6B). The results of the displacement measurements with H33258 indicated that these PAH metabolites did not bind to the minor groove of DNA.

Based on the binding studies of PAH metabolites to DNA, we observed that these PAH metabolites predominantly bind to DNA by intercalation. The dissociation constants of the tested metabolites ranged from 0.02 to 12.34 μM for *p53* cDNA and from 0.12 to 97.28 μM for CT-DNA (Table 1). The K_d values of the metabolites were smaller for *p53* cDNA than for CT-DNA, with one exception, 2-hydroxybenzo[c]phenanthrene. Therefore, the PAH metabolites showed stronger binding affinity for *p53* cDNA. The data revealed that the binding affinities of the PAH metabolites to DNA showed some sequence selectivity. Thus, it was necessary to investigate the intermolecular interaction between DNA and the PAH metabolites, and determine the preferred base pairs involved in intercalation. This study was conducted using molecular docking analysis.

3.2. Molecular docking analysis of noncovalent intercalation of PAH metabolites to DNA base pairs

Compared with the rigid lock-and-key mechanism of groove binding, intercalation follows an induced-fit mechanism. In the present study, we focused on the noncovalent molecular interactions

between the intercalator and DNA, which might stabilize the resulting complex. The influence of cavity formation and the hydrophobic transfer of the intercalator from solution to the interaction site was considered to be constant for all PAH metabolites. In fact, the cavity production for the intercalator induced a marked DNA conformational change (detailed information available in Supplementary data).

For PAH metabolites, the overwhelming driving force for TO replacement might stem from hydrogen bond formation and cavity complementarities to the non-polar phenyl ring. Specifically, BPDE, a representative bay-region diol-epoxide, exhibited a variety of binding modes because the hydroxyl group and epoxy structure provide several hydrogen bond donors and acceptors (Fig. 2a, b and c). As shown in Fig. 2a, there was one prevailing conformation in which the 8-hydroxyl of BPDE formed strong direct hydrogen bonds to the nitrogen atom on guanine ($\text{N}^3\text{-dG}$) and to the oxygen atom on the deoxyribose ring. The hydrogen bonds linked the 7-hydroxyl of BPDE to the hydrogen atoms on guanine ($\text{N}^2\text{-dG}$ and $\text{N}^1\text{-dG}$) and to the oxygen atom on cytosine. Instead, the higher energy conformation shown in Fig. 2b and c indicated a different binding mode. Other PAH diol-epoxides such as BADE could also establish a hydrogen bond network with guanine and cytosine (Fig. 2d). However, because of the missing phenyl ring, the hydrophobic interaction decreased significantly; therefore, the binding affinity of BADE was relatively weak, compared with that of BPDE.

Although bay-region diol-epoxides are believed to be the ultimate metabolic carcinogens for several classes of PAHs, studies have shown that other region adducts are also formed (Fields et al., 2004). BPE is a

Fig. 1. Fluorescence displacement curves of PAH metabolites, BPDE (○), DADE (●), BADE (*), CDE (▲) and BPE (△), which were titrated into 0.75 μg/mL of *p53* cDNA + 0.75 μM of TO (a), or 1 μg/mL of *p53* cDNA + 0.36 μM of H33258 (b). Each concentration was repeated three times.

K-region epoxide and does not contain a free hydroxyl group. Hydrogen bonds only formed between the 4,5-oxide group on BPE and N²-dG (Fig. 2e). This intercalation process might also be accompanied by epoxide hydrolysis and subsequent *in vivo* oxidation (Harvey, 1991). For the mono-hydroxylated metabolites tested in the present study, the hydrogen bond linked the hydroxyl group on PAH ring to guanine (data not shown). In addition, the complex stabilization required strong stacking of the PAH phenyl ring with an adjacent purine and pyrimidine. One more phenyl ring in the molecular structure helped 9-phenanthrol to intercalate into the base pairs more easily than 2-naphthol.

Table 1

IC₅₀ values and dissociation constants (*K_d*) of PAH metabolites with *p53* cDNA and CT-DNA were obtained by fluorescence displacement with TO as the probe.

PAH metabolite	<i>p53</i> cDNA		CT-DNA	
	[IC ₅₀] (μM)	<i>K_d</i> (μM)	[IC ₅₀] (μM)	<i>K_d</i> (μM)
2-Naphthol	359	12.34	795	97.28
2-Hydroxyfluorene	272	9.36	142	17.38
9-Phenanthrol	12	0.41	58	7.10
1-Hydroxypyrene	1.7	0.06	5.1 ^a	0.62 ^a
2-Hydroxybenzo [c]phenanthrene	50	1.72	3.7	0.45
3-Hydroxybenzo [a]pyrene	0.7	0.02	1.0	0.12
BPDE	253	8.70	118	14.44
DADE	29	0.99	11	1.35
BADE	128	4.38	143	17.50
CDE	90	3.08	69	8.44
BPE	44	1.51	58	7.10

^a Data from Wei and Guo (2009).

Although the complicated context of *p53* cDNA and the limited number of simulation studies precluded any firm conclusion regarding the specific binding modes of the complexes, the result of the molecular docking analysis still provided useful information regarding the non-covalent interactions of the PAH metabolites with *p53* cDNA. Similar binding capacity for the ligand/thymine-adenine (TA) complexes was shown by replacing cytosine-guanine (CG) with TA. The molecular docking scores for the interactions between the tested metabolites and base pairs stacking CG or TA are summarized in Table 2. The score values ranged from 4.11 to 7.83 for CG (Score_{CG}) and from 3.96 to 6.50 for TA (Score_{TA}). Larger scores indicated stronger binding affinity of the small molecules to the DNA base pairs. Therefore, the metabolites with larger score values should have smaller *K_d* values. As expected, 1-hydroxypyrene and 3-hydroxybenzo[a]pyrene bound more strongly to *p53* cDNA than did the other metabolites, and they had the highest docking score values. However, the smallest docking score value for 2-naphthol was related to its weakest binding affinity. Significant correlations were revealed between docking score and log-normalized *K_d* values for all of the tested metabolites (Fig. 3). P-values were less than 0.01 for both kinds of base-pair stacking, and the *R*² values were 0.8417 and 0.5977 for CG and TA, respectively. The significant negative relationship suggested that the molecular interaction provided a large contribution to the intercalation replacement.

For each PAH metabolite, differences in docking score values were also observed between CG and TA (Table 2). The data indicated that, although the intercalation of the metabolites between CG was similar to that of TA, the interaction showed preference for CG, but 2-hydroxyfluorene was the only exception to this preference. Although interactions between the metabolites and all kinds of base stacking were not simulated, we expect that the binding affinities of PAH metabolites to DNA showed sequence selectivity. Therefore, it was not surprising that the PAH metabolites exhibited different binding affinities to *p53* cDNA and CT-DNA in the fluorescence displacement measurements.

4. Conclusion

Here, we investigated the binding mode and binding affinity of 11 PAH metabolites to cancer-related DNA, human *p53* cDNA, using fluorescence displacement and molecular docking analysis. The data revealed that these metabolites predominantly interacted with DNA by intercalation, not by groove binding. In the competitive binding experiments, TO was used as the probe, and all tested metabolites bound to DNA with good affinity. Of the metabolites tested, 1-hydroxypyrene and 3-hydroxybenzo[a]pyrene were the strongest DNA intercalators. 2-naphthol was the weakest DNA binder. When the DNA groove binding agent H33258 was used as the probe, none of the PAH metabolites exhibited any significant competition with the probe for DNA. The molecular docking analysis identified different hydrogen bonds and stacking interactions that stabilized the metabolized PAH-DNA complexes. The formation of hydrogen bonds between oxide or hydroxyl groups on the PAH metabolites and DNA bases or backbones stabilized the intercalation of the metabolites between the base pairs. The DNA binding affinities of the PAH metabolites and DNA sequence selectivity were also confirmed by molecular docking analysis. Moreover, three metabolites of benzo[a]pyrene, 3-hydroxybenzo[a]pyrene, BPE and BPDE, showed different binding affinities to *p53* cDNA. It was postulated that the functional group on the periphery of the benzo[a]pyrene aromatic ring played a crucial role in mediating its interaction and binding affinity to DNA. In the present study, it proved difficult to determine the correlation between the DNA noncovalent binding affinity and the carcinogenicity of PAHs. We suggest that the carcinogenesis of PAHs should be investigated further by examining the covalent and noncovalent binding interactions between more PAH metabolites and the human *p53* gene, which is now underway in our laboratory.

Fig. 2. Stereo views of three selected PAH metabolites binding to the CG base stacking of d(CGATCG)₂. The prevailing conformations of BPDE (a), BADE (d) and BPE (e), higher energy conformations of BPDE (b and c).

Table 2

Log-normalized K_d values (pK_d) for PAH metabolites binding to p53 cDNA, and molecular docking scores of the intercalation of PAH metabolites to CG ($Score_{CG}$) and TA ($Score_{TA}$) base stacking.

PAH metabolite	pK_d	$Score_{CG}$	$Score_{TA}$
2-Naphthol	4.909	4.11	3.96
2-Hydroxyfluorene	5.029	5.18	5.59
9-Phenanthrol	6.384	5.90	5.53
1-Hydroxypyrene	7.237	6.73	5.99
2-Hydroxybenzo [c]phenanthrene	5.764	5.79	5.49
3-Hydroxybenzo [a]pyrene	7.620	7.83	6.50
BPDE	5.060	5.26	5.12
DADE	6.004	6.12	5.65
BADE	5.359	5.17	5.06
CDE	5.511	5.97	5.67
BPE	5.821	5.63	5.35

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2010.09.024.

References

- Aranda R, Villalba K, Ravina E, Masaguer CF, Brea J, Areias F, et al. Synthesis, binding affinity, and molecular docking analysis of new benzofuranone derivatives as potential antipsychotics. *J Med Chem* 2008;51:6085–94.
- Dipple A, Pigott MA, Agarwal SK, Yagi H, Sayer JM, Jerina DM. Optically active benzo[c]phenanthrene diol-epoxides bind extensively to adenine in DNA. *Nature* 1987;327:535–6.
- Feng Z, Hu W, Chen JX, Pao A, Li H, Rom W, et al. Preferential DNA damage and poor repair determine ras gene mutational hotspot in human cancer. *J Natl Cancer Inst* 2002;94:1527–36.
- Fields WR, Desiderio JG, Leonard RM, Burger EE, Brown BG, Doolittle DJ. Differential c-myc expression profiles in normal human bronchial epithelial cells following treatment with benzo[a]pyrene, benzo[a]pyrene-4, 5 epoxide, and benzo[a]pyrene-7, 8–9, 10 diol epoxide. *Mol Carcinog* 2004;40:79–89.
- Geacintov NE, Yoshida H, Ibanez V, Harvey RG. Non-covalent intercalative binding of 7, 8-dihydroxy-9, 10-epoxybenzo[a]pyrene to DNA. *Biochem Biophys Res Commun* 1981;100:1569–77.
- Geacintov NE, Gagliano AG, Ibanez V, Harvey RG. Spectroscopic characterizations and comparisons of the structures of the covalent adducts derived from the reactions of 7, 8-dihydroxy-7, 8, 9, 10-tetrahydrobenzo[a]pyrene-9, 10-oxide, and the 9, 10-epoxides of 7, 8, 9, 10-tetrahydrobenzo[a]pyrene and 9, 10, 11, 12-tetrahydrobenzo[e]pyrene with DNA. *Carcinogenesis* 1982a;3:247–53.
- Geacintov NE, Yoshida H, Ibanez V, Harvey RG. Noncovalent binding of 7β, 8α-dihydroxy-9α, 10α-epoxytetrahydrobenzo[a]pyrene to DNA and its catalytic effect on the hydrolysis of the diol epoxide to tetrol. *Biochemistry* 1982b;21:1864–9.
- Geacintov NE, Cosman M, Mao B, Alfano A, Ibanez V, Harvey RG. Spectroscopic characteristics and site I/site II classification of *cis* and *trans* benzo[a]pyrene diol-epoxide enantiomer-guanosine adducts in oligonucleotides and polynucleotides. *Carcinogenesis* 1991;12:2099–108.
- Hainaut P, Pfeifer GP. Patterns of p53 GT transversions in lung cancers reflect the primary mutagenic signature of DNA-damage by tobacco smoke. *Carcinogenesis* 2001;22:367–74.
- Hainaut P, Soussi T, Shomer B, Hollstein M, Greenblatt M, Hovig E, et al. Database of p53 gene somatic mutations in human tumors and cell lines: updated compilation and future prospects. *Nucleic Acids Res* 1997;25:151–7.
- Hainaut P, Olivier M, Pfeifer GP. TP53 mutational spectrum in lung cancers and mutagenic signature of components of tobacco smoke: lessons from the IARC TP53 mutation database. *Mutagenesis* 2001;16:551–3.

Fig. 3. Correlations between molecular docking scores of the PAH metabolites intercalating to the CG (•) and TA (○) base stacking, and log-normalized K_d values (pK_d) of the metabolites binding to p53 cDNA.

- Harris CC. p53: at the crossroads of molecular carcinogenesis and risk assessment. *Science* 1993;262:1980–1.
- Harvey RG. Polycyclic aromatic hydrocarbons, chemistry and carcinogenicity. Cambridge, UK: Cambridge Univ. Press; 1991. p. 11–87.
- Holt PA, Chaires JB, Trent JO. Molecular docking of intercalators and groove-binders to nucleic acids using autodock and surflex. *J Chem Inf Model* 2008;48:1602–15.
- IARC. IARC monographs on the evaluation of carcinogenic risks to humans; 2009. Lyon, France.
- Jerina DM, Chadha A, Cheh AM, Schurdak ME, Wood AW, Sayer JM. Covalent bonding of bay region diol epoxides to nucleic acids. In: Witmer CM, Snyder R, Jollow DJ, Kalf GF, Koscis JJ, Sipes IC, editors. *Biological Reactive Intermediates IV*. New York: Plenum Press; 1991. p. 533–53.
- Lamb P, Crawford L. Characterization of the human p53 gene. *Mol Cell Biol* 1986;6:1379–85.
- Lane DP, Benchimol S. p53: oncogene or anti-oncogene? *Genes Dev* 1990;4:1–8.
- Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell* 1997;88:323–31.
- Meehan T, Gamper H, Becker JF. Characterization of reversible, physical binding of benzo[a]pyrene derivatives to DNA. *J Biol Chem* 1982;257:10479–85.
- Miller EC, Miller JA. Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. *Cancer* 1981;47:2327–45.
- Miller CW, Simon K, Aslo A, Kok K, Yokota J, Buys CHCM, et al. p53 mutations in human lung tumors. *Cancer Res* 1992;52:1695–8.
- Pirogov N, Shafirovich V, Kolbanovskiy A, Solntsev K, Courtney SA, Amin S, et al. Role of hydrophobic effects in the reaction of a polynuclear aromatic diol epoxide with oligodeoxynucleotides in aqueous solutions. *Chem Res Toxicol* 1998;11:381–8.
- Rodríguez FA, Cai Y, Lin C, Tang Y, Kolbanovskiy A, Amin S, et al. Exocyclic amino groups of flanking guanines govern sequence-dependent adduct conformations and local structural distortions for minor groove-aligned benzo[a]pyrenyl-guanine lesions in a GG mutation hotspot context. *Nucleic Acids Res* 2007;35:1555–68.
- Shahbaz M, Geacintov NE, Harvey RG. Noncovalent intercalative complex formation and kinetic flow linear dichroism of racemic syn- and anti-benzo[a]pyrenediol epoxide-DNA solutions. *Biochemistry* 1986;25:3290–6.
- Smith LE, Denissenko MF, Bennett WP, Li H, Amin S, Tang M, et al. Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. *J Natl Cancer Inst* 2000;92:803–11.
- Urano S, Price HL, Fetzter SM, Briedis AV, Milliman A, LeBreton PR. Photoemission probes of hydrocarbon-DNA interactions: A comparison of DNA influences on the reactivities of (±)-trans-7, 8-dihydroxy-anti-9, 10-epoxy-7, 8, 9, 10-tetrahydrobenzo[a]pyrene, benzo[a]pyrene 4, 5-oxide, and benz[a]anthracene 5, 6-Oxide. *J Am Chem Soc* 1991;113:3881–93.
- Wei Y, Guo LH. Binding interaction between polycyclic aromatic compounds and DNA by fluorescence displacement method. *Environ Toxicol Chem* 2009;28:940–5.
- WHO. Selected non-heterocyclic polycyclic aromatic hydrocarbons; Environmental Health Criteria 202, Geneva; 1998.