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TP53 mutations induced by BPDE in Xpa-WT and Xpa-Null human *TP53* knock-in (Hupki) mouse embryo fibroblasts

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ABSTRACT

Somatic mutations in the tumour suppressor gene *TP53* occur in more than 50% of human tumours; in some instances exposure to environmental carcinogens can be linked to characteristic mutational signatures. The Hupki (human *TP53* knock-in) mouse embryo fibroblast (HUF) immortalization assay (HIMA) is a useful model for studying the impact of environmental carcinogens on *TP53* mutagenesis. In an effort to increase the frequency of *TP53*-mutated clones achievable in the HIMA, we generated nucleotide excision repair (NER)-deficient HUFs by crossing the Hupki mouse with an *Xpa*-knockout (Xpa-Null) mouse. We hypothesized that carcinogen-induced DNA adducts would persist in the *TP53* sequence of Xpa-Null HUFs leading to an increased propensity for mismatched base pairing and mutation during replication of adducted DNA. We found that Xpa-Null Hupki mice, and HUFs derived from them, were more sensitive to the environmental carcinogen benzo[*a*]pyrene (BaP) than their wild-type (Xpa-WT) counterparts. Following treatment with the reactive metabolite of BaP, benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE), Xpa-WT and Xpa-Null HUF cultures were subjected to the HIMA. A significant increase in *TP53* mutations on the transcribed strand was detected in Xpa-Null HUFs compared to Xpa-WT HUFs, but the *TP53*-mutant frequency overall was not significantly different between the two genotypes. BPDE induced mutations primarily at G:C base pairs, with approximately half occurring at CpG sites, and the predominant mutation type was G:C > T:A in both Xpa-WT and Xpa-Null cells. Further, several of the *TP53* mutation hotspots identified in smokers' lung cancer were mutated by BPDE in HUFs (codons 157, 158, 245, 248, 249, 273). Therefore, the pattern and spectrum of BPDE-induced *TP53* mutations in the HIMA are consistent with *TP53* mutations detected in lung tumours of smokers. While Xpa-Null HUFs exhibited increased sensitivity to BPDE-induced damage on the transcribed strand, NER-deficiency did not enhance *TP53* mutagenesis resulting from damage on the non-transcribed strand in this model.

1. Introduction

The tumour suppressor p53 plays a crucial role in the DNA damage response, garnering the title 'guardian of the genome' [1]. A paramount function of p53 is to prevent DNA synthesis and cell division, or to promote apoptosis, following DNA damage, which it performs primarily by regulating a large network of transcriptional targets [2,3]. Disruption of the normal p53 response by *TP53* mutation contributes to transformation by eliminating a key pathway of cellular growth control, enabling the survival and proliferation of stressed or damaged cells. Somatic mutations in *TP53* occur in more than 50% of human cancers [4,5]. The majority of *TP53* mutations are missense and occur between codons 125 and 300, corresponding to the coding region for the DNA binding domain [6]. Over 28,000 *TP53* mutations from human tumours have been catalogued in the International Agency for Research on Cancer (IARC) TP53 mutation database, providing a key resource for studying the patterns and frequencies of these mutations in cancer [7]. Interestingly, exposure to some environmental carcinogens can be linked to characteristic signatures of mutations in *TP53*, which provide molecular clues to the aetiology of human tumours [8].

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A useful model for studying human *TP53* mutagenesis is the partial human *TP53* knock-in (Hupki) mouse, in which exons 4-9 of human *TP53* replace the corresponding mouse exons [9]. The Hupki mouse and Hupki mouse embryo fibroblasts (HUFs) have been used for both *in vivo* and *in vitro* studies of *TP53* mutations induced by environmental carcinogens [10,11]. *TP53* mutagenesis can be studied in cell culture using the HUF immortalization assay (HIMA). In this assay primary HUFs are first treated with a mutagen to induce mutations. The treated cultures, along with untreated control cultures, are then serially passaged under standard culture conditions, whereby the majority of HUFs will undergo p53-dependent senescent growth arrest, due to the sensitivity of mouse cells to atmospheric oxygen levels (20%). HUFs that have accumulated mutagen-induced or spontaneous mutations (*e.g.* in *TP53*) that enable bypass of senescence continue to proliferate and ultimately become established into immortalized cell lines. DNA from immortalized HUF clones is then sequenced to identify *TP53* mutations. Environmental carcinogens that have been examined using the HIMA include ultraviolet (UV) radiation [12], benzo[*a*]pyrene (BaP) [13,14] and aristolochic acid I (AAI) [12,15]; in all cases the induced *TP53* mutation pattern corresponded to the pattern found in human tumours from patients exposed to these mutagens.

To protect the genome from mutation, several efficient mechanisms exist in cells to repair damage to DNA. One key repair system responsible for removing damage induced by certain environmental carcinogens is the nucleotide excision repair (NER) pathway. NER removes several types of structurally distinct DNA lesions including UV-induced photolesions, intrastrand crosslinks and chemically-induced bulky DNA adducts, such as those formed after exposure to polycyclic aromatic hydrocarbons (PAHs) [16]. NER operates in two distinct subpathways: global genomic NER (GG-NER) that recognizes lesions that cause local structural distortions in the genome, and transcription-coupled NER (TC-NER) that responds to lesions that block the progression of RNA polymerase II (RNAPII) on the transcribed strand of transcriptionally active genes. Following damage recognition, a common set of factors are recruited that ultimately incise the DNA 5′ and 3′ to the lesion to remove a 24-32 nucleotide fragment. The undamaged strand serves as a template for replicative DNA polymerases to fill the gap, which is finally sealed by ligation [17].

Mouse models deficient in various NER components have been generated not only to study the role of NER in the repair of different types of damage and ascertain how this relates to cancer risk [18,19], but also to increase the sensitivity of carcinogenicity studies [20]. For example, *Xpa*-knockout (Xpa-Null) mice, or cells derived from them, are deficient in both GG-NER and TC-NER. Xpa-Null mice are highly sensitive to environmental carcinogens [18,21] and exhibit accelerated and enhanced tumour formation after treatment with carcinogens such as UV and PAHs like BaP, compared with wild-type (Xpa-WT) mice [19,22,23]. Increased mutation frequencies of a *lacZ* reporter gene have been measured in tissues from Xpa-Null mice treated with the aforementioned carcinogens, and an increased rate of p53-mutated foci was detected on the skin of Xpa-Null *Trp53(+/−)* mice exposed to UVB [21,22,24]. Further, in *in vitro* studies, cells with reduced or deficient repair capacity were also more sensitive to the lethal or mutagenic effects of DNA damage [18,25,26].

Here we have generated an *Xpa*-deficient Hupki mouse strain with the aim of increasing *TP53* mutation frequency in the HIMA. As Xpa-Null cells are completely deficient in NER, we hypothesized that carcinogen-induced DNA adducts would persist in the *TP53* sequence of Xpa-Null HUFs, leading to an increased propensity for mismatched base pairing and mutation during replication of adducted DNA [24,27]. In the present study primary Xpa-WT and Xpa-Null HUFs were treated with benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE), the activated metabolite of the human carcinogen BaP [28,29], which forms pre-mutagenic BPDE-DNA adducts (*i.e.* 10-(deoxyguanosin-N^2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene [BPDE-N^2-dG]) that can be removed by NER (Fig. S1) [30]. BPDE-treated HUFs were subjected to the HIMA and *TP53* mutations in immortalized clones were identified by direct dideoxy sequencing of exons 4-9. The induced *TP53* mutation patterns and spectra were compared between the two *Xpa* genotypes and to mutations found in human tumours.

2. Materials and methods

2.1. Carcinogens

BaP (#B1760) was purchased from Sigma-Aldrich. For *in vitro* treatments, BaP was dissolved in DMSO (Sigma #D2650) to a stock concentration of 1 mM and stored at −20 °C. For *in vivo* treatments, BaP was dissolved in corn oil at a concentration of 12.5 mg/mL. BPDE was synthesized at the Institute of Cancer Research (London, UK) using a previously published method [31]. BPDE was dissolved in DMSO to a stock concentration of 2 mM under argon gas and stored at −80 °C in single-use aliquots.

2.2. Details of mouse strains and crossbreeding

Hupki mice (Trp53$^{tm1/Holl}$ (Arg/Arg codon 72), homozygous for a knock-in *TP53* allele harbouring the wild-type human *TP53* DNA sequence spanning exons 4–9) in the 129/Sv background [9] were kindly provided by Monica Hollstein (German Cancer Research Center; Heidelberg, Germany). Transgenic *Xpa*$^{+/-}$ mice, heterozygous for the *Xpa*-knockout allele on a C57Bl/6 background [18,22] were obtained from the National Institute for Public Health and the Environment (Bilthoven, The Netherlands). In the *Xpa*-knockout allele, exon 3, intron 3 and exon 4 have been replaced by a neomycin resistance cassette with a PGK2 promoter. To generate Hupki mice carrying an *Xpa*-knockout allele, *Hupki*$^{+/+}$ mice were first crossed with *Xpa*$^{+/-}$ mice. Progeny with the *Hupki*$^{+/-}$;*Xpa*$^{+/-}$ genotype were then backcrossed to *Hupki*$^{+/+}$ stock to generate *Hupki*$^{+/+}$ animals that were *Xpa*$^{+/+}$ or *Xpa*$^{+/-}$. *Hupki*$^{+/+}$;*Xpa*$^{+/-}$ and *Hupki*$^{+/+}$;*Xpa*$^{+/+}$ offspring were thereafter intercrossed to maintain the colony and produce *Xpa*$^{+/+}$ and *Xpa*$^{-/-}$ (referred to here as Xpa-WT and Xpa-Null, respectively) mice and embryos for experiments. Animals were bred at the Institute of Cancer Research in Sutton, UK and kept under standard conditions with food and water *ad libitum*. All animal procedures were carried out under license in accordance with the law and following local ethical review.

2.3. Genotyping

The *Hupki* and *Xpa* genotype was determined in mouse pups or embryos by PCR prior to experiments. To extract DNA for genotyping, ear clips or cells were suspended in 400 μL of 50 mM NaOH and heated to 95 °C for 15 min. Next, 35 μL of 1 M Tris-HCl (pH 8.0) was added to each sample, followed by centrifugation for 20 min at 13,000 rpm. The supernatant was used for genotyping. Primers and PCR reaction conditions for the *Hupki*, mouse *Trp53* or *Xpa* alleles are described in Table S1.

2.4. In-vivo carcinogen treatment

Female Xpa-WT and Xpa-Null Hupki mice (~3 months old) were treated with BaP as indicated below and sacrificed either 24 h or 5 days after the last administration following treatment regimens published previously [28,32]. Several organs (liver, lung, small