Published in final edited form as:
DNA Repair (Amst). 2013 December ; 12(12): . doi:10.1016/j.dnarep.2013.08.019.

Slow accumulation of mutations in *Xpc*$^{-/-}$ mice upon induction of oxidative stress

Joost P.M. Melis[1,2], **Raoul V. Kuiper**[1,3], **Edwin Zwart**[1], **Joke Robinson**[1], **Jeroen L.A. Pennings**[1], **Conny T.M. van Oostrom**[1], **Mirjam Luijten**[1,2], and **Harry van Steeg**[1,2,*]

[1]National Institute for Public Health and the Environment, Center for Health Protection, Bilthoven, 3721 MA, the Netherlands [2]Leiden University Medical Center, Department of Toxicogenetics, Leiden, 2300 RC, the Netherlands [3]Dutch Molecular Pathology Center, Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, 3584 CL, the Netherlands

Abstract

XPC is one of the key DNA damage recognition proteins in the global genome repair route of the nucleotide excision repair (NER) pathway. Previously, we demonstrated that NER-deficient mouse models *Xpa*$^{-/-}$ and *Xpc*$^{-/-}$ exhibit a divergent spontaneous tumor spectrum and proposed that XPC might be functionally involved in the defense against oxidative DNA damage. Others have mechanistically dissected several functionalities of XPC to oxidative DNA damage sensitivity using *in vitro* studies. XPC has been linked to regulation of base excision repair (BER) activity, redox homeostasis and recruitment of ATM and ATR to damage sites, thereby possibly regulating cell cycle checkpoints and apoptosis. XPC has additionally been implicated in recognition of bulky (e.g. cyclopurines) and non-bulky DNA damage (8-oxodG). However, the ultimate contribution of the XPC functionality *in vivo* in the oxidative DNA damage response and subsequent mutagenesis process remains unclear. Our study indicates that *Xpc*$^{-/-}$ mice, in contrary to *Xpa*$^{-/-}$ and wild type mice, have an increased mutational load upon induction of oxidative stress and that mutations arise in a slowly accumulative fashion. The effect of non-functional XPC *in vivo* upon oxidative stress exposure appears to have implications in mutagenesis, which can contribute to the carcinogenesis process. The levels and rate of mutagenesis upon oxidative stress correlate with previous findings that lung tumors in *Xpc*$^{-/-}$ mice overall arise late in the lifespan and that the incidence of internal tumors in XP-C patients is relatively low in comparison to skin cancer incidence.

Keywords

Xpc; *Xpa*; Nucleotide Excision Repair; Oxidative DNA damage; Mutagenesis; Carcinogenesis

*Contact information Harry van Steeg, Center for Health Protection, National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Phone: 31-30-274-2102; Harry.van.Steeg@rivm.nl.

Conflict of interest
Authors declare that there are no conflicts of interest.

1. Introduction

In nucleotide excision repair (NER), over 30 proteins are involved in the complete removal and repair of bulky DNA lesions [1]. Two pivotal proteins in this cascade are XPA and XPC, which belong to the family of XP proteins linked to the severe autosomal recessive disorder Xeroderma pigmentosum (XP). Fewer than 40% of individuals with the disease, of which XP-A and XP-C patients are the most common worldwide, survive beyond age 20 years [2–5] due to their extreme sensitivity to (UV−)DNA damage and subsequent carcinogenic events. NER can be divided in two sub-pathways: global genome NER (GG-NER), which covers repair genome wide, and transcription coupled NER (TC-NER), which is responsible for repair of the transcribed strand of active genes [2, 6]. XP-C patients are only defective in the GG-NER pathway, while XP-A patients are hampered in both GG-NER and TC-NER sub-pathways [1]. In mice, similar characteristics to the human disorders can be mimicked by knocking out one of these XP genes. In a previous work, we presented the spontaneous phenotypes of the *Xpa*- and *Xpc*-deficient knock-out mouse models in a congenic C57BL/6J background [7]. We established that $Xpa^{-/-}$ and $Xpc^{-/-}$ mice (hereafter referred to as *Xpa* and *Xpc*, respectively) are both cancer prone but, although being part of the same DNA repair pathway, show a divergent spontaneous tumor phenotype. Compared to wild type C57BL/6J mice, both mouse models showed an increase in spontaneous hepatocellular tumors, but strikingly only *Xpc* exhibited an increased incidence of spontaneous lung tumors. Mutant frequency analyses demonstrated that the tumor phenotype could be correlated to the increase in mutational load in those tissues during aging [7]. The *Xpc* specific increase in lung tumors pointed towards a possibly increased sensitivity of *Xpc* mice to oxygen exposure and a putative role of XPC in a process, putatively besides NER, preventing or removing oxidative DNA damage. *In vitro*, XPC has been mechanistically linked to oxidative DNA damage response by others. XPC was shown to regulate base excision repair (BER) activity, disturb redox homeostasis, cell cycle progression and apoptosis [8–19]. Besides functionality outside of NER, several studies showed that NER is able to repair bulky oxidative DNA lesions caused by oxidative stress [20–22]. We now examined whether *Xpc*-deficiency leads to increased mutagenesis caused by oxidative stress in comparison to a wild type and an *Xpa*-deficient situation and how the level and rate of mutagenesis can ultimately contribute to the carcinogenesis process.

2. Material & Methods

2.1 Cell culture

Primary mouse embryonic fibroblasts (MEFs) were isolated from E13.5 day embryos of wild type, *Xpa* and *Xpc* mice (all in C57BL/6J background), genotyped and cultured as described previously [23, 24]. MEFs were cultured for 3 days per passage at 3% or 20% O_2. The first passage for all groups was performed at 3% O_2, subsequently they were split to either 3% or 20% O_2 for two additional passages. A minimum of three different embryos, all from different mothers, was used per genotype.

2.2 Mice

Generation and characterization of *Xpa* and *Xpc* mice has been described before [25, 26]. The mice were crossed with pUR288-*LacZ* C57BL/6J transgenic mice line 30, homozygous for *LacZ* integration on chromosome 11 and PCR-genotyped as described previously [23, 24]. The experimental *in vivo* setup was approved by the institute's Ethical Committee on Experimental Animals, according to national legislation. The health state of the mice was checked daily. During exposure, mice were weighed weekly for the first eight weeks of exposure and biweekly for the remainder of the exposure study to monitor health conditions of the animals. Feed uptake was measured in the first 5 weeks.

Animals were kept in a stringently controlled environment, fed *ad libitum* and kept under a normal day/night rhythm. Animals were removed from the study when found dead or moribund. Autopsy was performed on animals of all cohorts; tissues were stored for further histopathological analyses. In addition, a selective set of tissues was snap frozen in liquid N_2 for *LacZ* mutant frequency analysis.

2.3 In vivo experimental design

Ten-week-old wild type (C57BL/6J), *Xpa* and *Xpc* mice were marked, randomized in cohorts and sacrificed 12 or 39 weeks after start of the exposure. All genotypes (n=20/group) were exposed to either control feed or the compounds di(2-ethylhexyl) phthalate (DEHP (CAS 117-81-7, Sigma-Aldrich, Zwijndrecht, the Netherlands), 6,000 ppm, female mice) or methyl viologen (paraquat (CAS 1910-42-5, Sigma-Aldrich), 100 ppm, male mice). The concentrations of DEHP (6,000 ppm) and paraquat (100 ppm) were chosen based on results of two-year bioassay carcinogenicity testing in rodents leading to only a slightly toxic effect [27, 28, pp.23–24 EPA 738-F-96-018 (August 1997)].

2.4 Histopathology

Tissue samples of each animal were preserved in a neutral aqueous phosphate-buffered 4% formaldehyde solution. Tissues required for microscopic examination were embedded in paraffin wax, sectioned at 4μm and stained with haematoxylin and eosin. Detailed microscopic examination was performed on liver, lung and kidney of all mice and on all gross lesions suspected of being tumors or representing major pathological conditions. Non-neoplastic findings were either scored using 1) an ordinal scale or 2) based on incidence. Unstained slides were mounted with Cytoseal-XYL (Richard-Allan Scientific, Thermo Fisher Scientific, MI, USA) and viewed under UV light for semi-quantitative assessment of lipofuscin deposition. Fisher exact test statistics have been used to test whether a parameter was significantly regulated over treatment and genotype (p<0.05).

2.5 LacZ mutant frequency analyses

DNA was extracted from MEFs, frozen liver or lung and analyzed for mutant frequency as described previously [7]. Two-way ANOVA analyses and post-hoc t-tests, using a Bonferroni correction for multiple testing to compare mutant frequencies between all groups, were performed and were considered significant if p<0.05.

3. Results

3.1 Increased mutational load in Xpc MEFs upon atmospheric oxygen exposure

In our previous study we have shown that *Xpc*-deficient mice are more prone to lung cancer than *Xpa* and wild type mice, possibly due to oxidative DNA damage induced by chronic atmospheric oxygen exposure [7]. In the present study, we first verified if different oxygen conditions in *Xpc* mouse embryonic fibroblasts (MEFs) could be correlated to the amount of oxidative DNA damage that has been fixed as mutations. We cultured wild type, *Xpa* and *Xpc* MEFs at either normoxic (3%) or atmospheric (20%) oxygen levels for three passages under the same conditions as described in the past [7]. *LacZ* mutant frequency analyses revealed that in MEFs cultured at 3% oxygen pressure, the mutant frequency in both *Xpa* and *Xpc* cells was already slightly, but not significantly, increased compared to wild type cells (Figure 1). However at 20% oxygen pressure, *LacZ* mutant frequencies were only increased significantly in *Xpc* MEFs compared to their normoxic treated controls (p<0.05).

3.2 Neoplastic and non-neoplastic lesions upon in vivo pro-oxidant exposures

To verify whether *Xpc*-deficiency also results in increased mutagenesis upon induced oxidative stress *in vivo*, wild type, *Xpa* and *Xpc* mice (all harboring the *LacZ* gene for mutational analysis) were exposed to pro-oxidative substances for either 12 or 39 weeks. Female mice were exposed to DEHP (6,000 ppm, via feed), while male mice were exposed to paraquat (100 ppm, via feed).

Body weight measurements over the duration of exposures demonstrated a decrease in all genotypes upon DEHP exposure, while paraquat exposure only had a minor effect on body weight (SI 1). Dietary uptake in both exposure groups was comparable to the uptake of the control diet group (SI 2).

Histopathological analyses were performed on 39 weeks exposed mice (detailed results are shown in SI 3). In total, few neoplastic lesions were found: in the livers of DEHP-exposed wild type and *Xpa* mice one benign tumor (adenoma) for each genotype, but no malignant tumors, were observed. One benign tumor (adenoma) was identified in the group of untreated *Xpa* mice. The only malignant liver tumors (two carcinomas) in the study were observed in the DEHP-treated *Xpc* animals. Also, one malignant tumor (lymphoma) in lung was found in the untreated *Xpc* group. None of the animals (male mice) exposed to paraquat, nor any of their concurrent controls, carried a tumor.

Several notable non-neoplastic lesions were observed in animals treated with pro-oxidants for 39 weeks. Several of these lesions confirmed the *in vivo* exposure to DEHP and paraquat (SI 3). A suitable and generally accepted marker to assess oxidative stress levels *in vivo* is lipofuscin accumulation. This specific pathological marker confirmed increased oxidative stress levels upon both exposures in liver (depicted in Figure 2A for DEHP). Lipofuscin accumulation was less severe in paraquat compared to the DEHP exposure (SI 3). For DEHP, ductal response and eosinophylic cytoplasmic inclusions in liver were significantly increased for all genotypes due to exposure ($p<0.05$). The incidence of cholestasis was significantly increased in wild type and *Xpa* liver ($p<0.05$), but not in *Xpc* liver. In kidney, cortical scarring is significantly increased in all genotypes upon DEHP-exposure ($p<0.05$). Tubular dilatation is increased significantly in *Xpa* and *Xpc* mice and hydronephrosis incidence was markedly increased for wild type and *Xpc* ($p<0.05$). No significant exposure-related changes in lesions were found in lung tissue. Paraquat exposure resulted in an increase in severity of lymphoid hyperplasia and a strong increase in pulmonary edema in *Xpc* lung tissue only ($p<0.05$) (Figure 2B). Analyses of kidney tissue did not show any notable changes in histopathological lesions in the paraquat treated animals compared to controls.

3.3 Elevated mutational load in Xpc mice due to pro-oxidant exposure

To assess potential differences in sensitivity for oxidative DNA damage between the genotypes we analyzed the mutational load in the *LacZ* gene, as an early tumor risk marker. This analysis was performed on tissues, in which pathological evidence for exposure and carcinogenesis was found in the current and previous studies (liver and lung) [7]. We also included mutant frequency analyses after 12 weeks of exposure to the pro-oxidants to investigate the rate of mutation induction *in vivo*.

The 12-week exposures to DEHP or paraquat did not result in any significant changes in mutational load in liver or lung of all genotypes compared to the untreated corresponding genotype (Figure 3A). However, for both DEHP and paraquat the long-term exposure of 39 weeks resulted in an exposure-related two-fold increase ($p<0.05$) in mutant frequency in livers of *Xpc* mice only (Figure 3B). The long-term pro-oxidant exposures through feed did

not result in a significantly enhanced mutational load in lung compared to their untreated controls. Independent of pro-oxidant exposure however, mutational loads in the lungs of *Xpc* mice tended to increase faster over time when compared to wild type or *Xpa* (Figure 3B). This accelerated increase in mutational load during aging in *Xpc* compared to wild type and *Xpa* mice was also observed in our previous cross-sectional studies and levels of mutant frequency in lung are similar to those found previously at comparable age [7]. These results support the hypothesis that XPC-deficiency contributes to persistent oxidative DNA damage *in vivo* and, as such, can contribute to carcinogenesis.

4. Discussion

We, and others, hypothesized that XPC is involved, among others, in the prevention or removal of oxidative DNA damage, possibly by functionality in- and outside of NER [7–19, 29, 30]. *Xpc*-deficiency can therefore possibly lead to more DNA damage as a consequence of reduced capability to cope with NER-substrate lesions as well as oxidative stress-induced lesions that are no substrate for NER. The main question addressed in this study is if and to what extent persistent DNA damage induced by oxidative stress can contribute to the process of mutagenesis and carcinogenesis.

We first assessed *in vitro* whether *Xpc* MEFs were more prone to mutation accumulation than *Xpa* and WT MEFs due to oxidative DNA damage using atmospheric and normoxic oxygen levels during culturing. Our results demonstrate that specifically *Xpc* cells are more sensitive towards oxidative DNA damage when compared to wild type and *Xpa* cells. These results support the work of others showing that XPC is functionally involved in defending cells against persistent oxidative DNA damage. NER can be involved in the removal of bulky oxidative DNA lesions (e.g. cyclopurines) [31, 32], but a multitude of *in vitro* studies have implicated XPC to have an additional function outside of NER, involving a response towards oxidative DNA damage [8–19]. Very recent findings verified recruitment of XPC to 8-oxoG lesions, while the downstream NER proteins, XPA and XPB, did not accumulate at these lesions, thereby supporting a function of XPC outside of NER that is involved in response to oxidative stress [13]. Functional relationships between XPC and activity of BER proteins, redox homeostasis and cell cycle regulation have all been shown *in vitro* [8–13, 16–19].

It was however still unclear if, how and at what rate XPC-deficiency ultimately contributes to the level of fixated oxidative DNA damage *in vivo.* Also the possible correlation to the tumor spectrum in *Xpc*-deficient mice [7, 33] and the low incidence of internal tumors and neuropathology found in several XP-C patients [34, 35] remained unclear. To assess these questions *in vivo*, we exposed *Xpa*, *Xpc* and wild type mice to the pro-oxidants DEHP and paraquat through the diet and measured several parameters upon exposure to these substances. Paraquat is considered to be non-carcinogenic in rodents (EPA database, http://www.epa.gov/iris/subst/0183.htm, 2013) and exposure to paraquat in the present study did indeed not result in any (additional) tumor formation. The only malignant tumors observed in liver in the current study were found in DEHP-exposed *Xpc* mice. DEHP is considered a hepatic non-genotoxic rodent carcinogen in the classical two-year rodent bioassay at a dose of 6,000 ppm[27]. Apparently, the shorter exposure times (12 and 39 weeks) in comparison to the two-year bioassay less do not lead to a significant tumor response in either of the genotypes. This result is, however, in line with our previous results in wild type and *Xpa*, *Xpa***p53* and *Xpc***p53* mice [28, 36]. DEHP did induce oxidative stress in liver since increased levels of lipofuscin and cholestasis [37–39] were apparent. Although in previous studies a significant spontaneous increase was apparent at the end of life pathology in two NER-deficient mouse models (*Ercc1*$^{-/d7}$ and *Xpd*^{TTD}) [40, 41], no significant differences between the *Xpa* and *Xpc* genotypes and the wild type were found for lipofuscin increase

upon 9 month DEHP exposure. The effect of paraquat on histopathology in the liver was only marginal. However, the significant increase in lymphoid hyperplasia and the large increase in edema incidence in lungs of *Xpc* mice only demonstrated that there is a genotypical difference upon paraquat exposure. These *Xpc*-specific lesions upon oxidative stress apparently did not affect the mutational load in this tissue. It is tempting to speculate that XPC-deficiency, without affecting DNA damage levels, might be causally related to the elevated toxicity response observed in lung tissue upon paraquat exposure.

Since histopathological analyses confirmed the induction of oxidative stress in liver, we expected that pro-oxidant exposure could enhance oxidative DNA damaging events in this tissue. *LacZ* mutant frequency analyses demonstrated a significant increase in mutational load after pro-oxidant exposure in livers of *Xpc* mice only. This increase was only detected after 39 weeks of exposure. At 12 weeks of pro-oxidant exposure no significant increase in the mutational load in any of the genotypes was observed, suggesting a slow accumulation of mutations upon induced oxidative stress in *Xpc* mice. This could be due to either the relatively slow cell division in adult livers or to an additional function of XPC that involves a mechanism that prevents persistent oxidative DNA damage accumulation. In lung, *LacZ* mutant frequency analyses did not show any DEHP or paraquat exposure related effect. However, *LacZ* mutant frequencies were higher in lung of all exposed and unexposed female and male *Xpc* mice, most likely due to oxygen exposure. The mutational load of the untreated mice in the current studies after 39 weeks is comparable to the levels found in our previous studies at that age [7].

Taken together, our *in vitro* and *in vivo* results show a deviant response of *Xpc* mice upon oxidative stressors compared to wild type and *Xpa* mice. Upon paraquat exposure, our results in lung tissue imply a role of XPC functioning in preventing toxicity on a different level than (detectable) genotoxicity. This finding leaves us with an interesting question to be answered in future research. Results found in liver suggest that XPC is involved in the defense against persistent oxidative DNA damage. Very recently, XPC was shown to be specifically recruited to oxidative DNA lesions without a subsequent NER response (also XPA was not recruited), demonstrating functionality outside of NER concerning oxidative DNA damage. XPC therefore is not only involved in preventing carcinogenesis by repairing (oxidative) NER substrate lesions, but most likely also by involvement in repairing non-bulky oxidative DNA lesions. Even though it has now been shown that XPC is involved in oxidative DNA damage repair without subsequent recruitment of NER proteins *in vitro*, involvement of other NER factors in the removal of oxidative DNA damage cannot be excluded altogether in our *in vivo* study: *Xpa* cells accumulating oxidative DNA damage over time may undergo TC-NER-associated apoptosis, thereby possibly underestimating the mutational load in *Xpa* liver. The rate of total oxidative induced DNA damage *in vivo* appears to be a relatively slow process over time. Nevertheless, oxidative stress-induced mutations appear to contribute to the carcinogenic events, which were apparent in XPC-deficient mice [7, 33] and probably also in XP-C patients [34, 35].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Animal Facilities of the Netherlands Vaccine Institute (NVI). We also thank Prof. Dr. Errol Friedberg for providing us with the *Xpc* mouse model. The work presented here was in part financially supported by NIH/NIEHS (Comparative Mouse Genomics Centers Consortium) Grant 1UO1 ES11044 and by STW Grant STW-LGC.6935.

List of abbreviations

BER	base excision repair
DEHP	di(2-ethylhexyl)phthalate
GG-NER	global genome nucleotide excision repair
MEF	mouse embryonic fibroblast
TC-NER	transcription coupled nucleotide excision repair
XP	xeroderma pigmentosum
Xpa	xeroderma pigmentosum group A
Xpc	xeroderma pigmentosum group C

References

1. Friedberg, EC.; Walker, GC.; Siede, W.; Wood, RD.; Schultz, RA.; Ellenberger, T. DNA Repair and Mutagenesis. ASM Press; 2006.
2. Cleaver JE, Lam ET, Revet I. Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. Nat Rev Genet. 2009; 10:756–768. [PubMed: 19809470]
3. Kraemer KH, Lee MM, Scotto J. DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. Carcinogenesis. 1984; 5:511–514. [PubMed: 6705149]
4. Kraemer KH, Lee MM, Scotto J. Xeroderma pigmentosum. Cutaneous, ocular, and neurologic abnormalities in 830 published cases. Arch Dermatol. 1987; 123:241–250. [PubMed: 3545087]
5. Kraemer KH. Sunlight and skin cancer: another link revealed. Proc Natl Acad Sci U S A. 1997; 94:11–14. [PubMed: 8990152]
6. Hanawalt PC, Ford JM, Lloyd DR. Functional characterization of global genomic DNA repair and its implications for cancer. Mutat Res. 2003; 544:107–114. [PubMed: 14644313]
7. Melis JP, Wijnhoven SW, Beems RB, Roodbergen M, van den BJ, Moon H, Friedberg E, van der Horst GT, Hoeijmakers JH, Vijg J, van Steeg H. Mouse models for xeroderma pigmentosum group A and group C show divergent cancer phenotypes. Cancer Res. 2008; 68:1347–1353. [PubMed: 18316597]
8. Abbasi R, Efferth T, Kuhmann C, Opatz T, Hao X, Popanda O, Schmezer P. The endoperoxide ascaridol shows strong differential cytotoxicity in nucleotide excision repair-deficient cells. Toxicol Appl Pharmacol. 2012; 259:302–310. [PubMed: 22280988]
9. D'Errico M, Parlanti E, Teson M, de Jesus BM, Degan P, Calcagnile A, Jaruga P, Bjoras M, Crescenzi M, Pedrini AM, Egly JM, Zambruno G, Stefanini M, Dizdaroglu M, Dogliotti E. New functions of XPC in the protection of human skin cells from oxidative damage. EMBO J. 2006; 25:4305–4315. [PubMed: 16957781]
10. Han W, Ming M, Zhao R, Pi J, Wu C, He YY. The Nrf1 CNC-bZIP protein promotes cell survival and nucleotide excision repair through maintaining glutathione homeostasis. J Biol Chem. 2012
11. Kassam SN, Rainbow AJ. Deficient base excision repair of oxidative DNA damage induced by methylene blue plus visible light in xeroderma pigmentosum group C fibroblasts. Biochem Biophys Res Commun. 2007; 359:1004–1009. [PubMed: 17573042]
12. Liu SY, Wen CY, Lee YJ, Lee TC. XPC silencing sensitizes glioma cells to arsenic trioxide via increased oxidative damage. Toxicol Sci. 2010; 116:183–193. [PubMed: 20403967]
13. Menoni H, Hoeijmakers JH, Vermeulen W. Nucleotide excision repair-initiating proteins bind to oxidative DNA lesions in vivo. J Cell Biol. 2012; 199:1037–1046. [PubMed: 23253478]
14. Okamoto Y, Chou PH, Kim SY, Suzuki N, Laxmi YR, Okamoto K, Liu X, Matsuda T, Shibutani S. Oxidative DNA damage in XPC-knockout and its wild mice treated with equine estrogen. Chem Res Toxicol. 2008; 21:1120–1124. [PubMed: 18447394]

15. Parlanti E, D'Errico M, Degan P, Calcagnile A, Zijno A, van dP I, van der Horst GT, Biard DS, Dogliotti E. The cross talk between pathways in the repair of 8-oxo-7,8-dihydroguanine in mouse and human cells. Free Radic Biol Med. 2012; 53:2171–2177. [PubMed: 23010470]
16. Rezvani HR, Rossignol R, Ali N, Benard G, Tang X, Yang HS, Jouary T, de Verneuil H, Taieb A, Kim AL, Mazurier F. XPC silencing in normal human keratinocytes triggers metabolic alterations through NOX-1 activation-mediated reactive oxygen species. Biochim Biophys Acta. 2011; 1807:609–619. [PubMed: 21167810]
17. Rezvani HR, Kim AL, Rossignol R, Ali N, Daly M, Mahfouf W, Bellance N, Taieb A, de Verneuil H, Mazurier F, Bickers DR. XPC silencing in normal human keratinocytes triggers metabolic alterations that drive the formation of squamous cell carcinomas. J Clin Invest. 2011; 121:195–211. [PubMed: 21123941]
18. Shimizu Y, Iwai S, Hanaoka F, Sugasawa K. Xeroderma pigmentosum group C protein interacts physically and functionally with thymine DNA glycosylase. EMBO J. 2003; 22:164–173. [PubMed: 12505994]
19. Shimizu Y, Uchimura Y, Dohmae N, Saitoh H, Hanaoka F, Sugasawa K. Stimulation of DNA Glycosylase Activities by XPC Protein Complex: Roles of Protein-Protein Interactions. J Nucleic Acids. 2010
20. Brooks PJ, Wise DS, Berry DA, Kosmoski JV, Smerdon MJ, Somers RL, Mackie H, Spoonde AY, Ackerman EJ, Coleman K, Tarone RE, Robbins JH. The oxidative DNA lesion 8,5′-(S)-cyclo-2′-deoxyadenosine is repaired by the nucleotide excision repair pathway and blocks gene expression in mammalian cells. J Biol Chem. 2000; 275:22355–22362. [PubMed: 10801836]
21. Brooks PJ. The case for 8,5′-cyclopurine-2′-deoxynucleosides as endogenous DNA lesions that cause neurodegeneration in xeroderma pigmentosum. Neuroscience. 2007; 145:1407–1417. [PubMed: 17184928]
22. Brooks PJ. The 8,5′-cyclopurine-2′-deoxynucleosides: candidate neurodegenerative DNA lesions in xeroderma pigmentosum, and unique probes of transcription and nucleotide excision repair. DNA Repair (Amst). 2008; 7:1168–1179. [PubMed: 18495558]
23. Dolle ME, Giese H, Hopkins CL, Martus HJ, Hausdorff JM, Vijg J. Rapid accumulation of genome rearrangements in liver but not in brain of old mice. Nat Genet. 1997; 17:431–434. [PubMed: 9398844]
24. Wijnhoven SW, Kool HJ, Mullenders LH, Slater R, van Zeeland AA, Vrieling H. DMBA-induced toxic and mutagenic responses vary dramatically between NER-deficient Xpa, Xpc and Csb mice. Carcinogenesis. 2001; 22:1099–1106. [PubMed: 11408355]
25. Cheo DL, Ruven HJ, Meira LB, Hammer RE, Burns DK, Tappe NJ, van Zeeland AA, Mullenders LH, Friedberg EC. Characterization of defective nucleotide excision repair in XPC mutant mice. Mutat Res. 1997; 374:1–9. [PubMed: 9067411]
26. de Vries A, van Steeg H. Xpa knockout mice. Semin Cancer Biol. 1996; 7:229–240. [PubMed: 9110400]
27. David RM, Moore MR, Finney DC, Guest D. Chronic toxicity of di(2-ethylhexyl)phthalate in mice. Toxicol Sci. 2000; 58:377–385. [PubMed: 11099649]
28. Mortensen A, Bertram M, Aarup V, Sorensen IK. Assessment of carcinogenicity of di(2-ethylhexyl)phthalate in a short-term assay using Xpa-/- and Xpa-/-/p53+/- mice. Toxicol Pathol. 2002; 30:188–199. [PubMed: 11950162]
29. Melis JP, Luijten M, Mullenders LH, van Steeg H. The role of XPC: implications in cancer and oxidative DNA damage. Mutat Res. 2011; 728:107–117. [PubMed: 21763452]
30. Melis JP, van Steeg H, Luijten M. Oxidative DNA Damage and Nucleotide Excision Repair. Antioxid Redox Signal. 2012
31. Randerath K, Randerath E, Smith CV, Chang J. Structural origins of bulky oxidative DNA adducts (type II I-compounds) as deduced by oxidation of oligonucleotides of known sequence. Chem Res Toxicol. 1996; 9:247–254. [PubMed: 8924599]
32. Wang Y. Bulky DNA lesions induced by reactive oxygen species. Chem Res Toxicol. 2008; 21:276–281. [PubMed: 18189366]

33. Hollander MC, Philburn RT, Patterson AD, Velasco-Miguel S, Friedberg EC, Linnoila RI, Fornace AJ Jr. Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. Proc Natl Acad Sci U S A. 2005; 102:13200–13205. [PubMed: 16141330]
34. Giglia G, Dumaz N, Drougard C, Avril MF, Daya-Grosjean L, Sarasin A. p53 mutations in skin and internal tumors of xeroderma pigmentosum patients belonging to the complementation group C. Cancer Res. 1998; 58:4402–4409. [PubMed: 9766670]
35. Bernardes de Jesus BM, Bjoras M, Coin F, Egly JM. Dissection of the molecular defects caused by pathogenic mutations in the DNA repair factor XPC. Mol Cell Biol. 2008; 28:7225–7235. [PubMed: 18809580]
36. Melis JP, Speksnijder EN, Kuiper RV, Salvatori DC, Schaap MM, Maas S, Robinson J, Verhoef A, van Benthem J, Luijten M, van Steeg H. Detection of genotoxic and non-genotoxic carcinogens in Xpc(-/-)p53(+/-) mice. Toxicol Appl Pharmacol. 2012
37. Sohal RS, Brunk UT. Lipofuscin as an indicator of oxidative stress and aging. Adv Exp Med Biol. 1989; 266:17–26. [PubMed: 2486150]
38. Jung T, Hohn A, Grune T. Lipofuscin: detection and quantification by microscopic techniques. Methods Mol Biol. 2010; 594:173–193. [PubMed: 20072918]
39. Tiao MM, Lin TK, Wang PW, Chen JB, Liou CW. The role of mitochondria in cholestatic liver injury. Chang Gung Med J. 2009; 32:346–353. [PubMed: 19664341]
40. Dolle ME, Kuiper RV, Roodbergen M, Robinson J, de VS, Wijnhoven SW, Beems RB, de la Fonteyne L, de WP, van dP I, Niedernhofer LJ, Hasty P, Vijg J, Hoeijmakers JH, van SH. Broad segmental progeroid changes in short-lived Ercc1(-/Delta7) mice, Pathobiol. Aging Age Relat Dis. 2011; 1
41. Wijnhoven SW, Beems RB, Roodbergen M, van den Berg J, Lohman PH, Diderich K, van der Horst GT, Vijg J, Hoeijmakers JH, van SH. Accelerated aging pathology in ad libitum fed Xpd(TTD) mice is accompanied by features suggestive of caloric restriction. DNA Repair (Amst). 2005; 4:1314–1324. [PubMed: 16115803]

Highlights

- Oxidative stress results in increased mutational load in *Xpc*$^{-/-}$ mice and cells
- Oxidative-stress induced mutagenesis in *Xpc*$^{-/-}$ liver is a slow process
- Oxidative stress results in a different mutational load in *Xpc*$^{-/-}$ and *Xpa*$^{-/-}$ mice

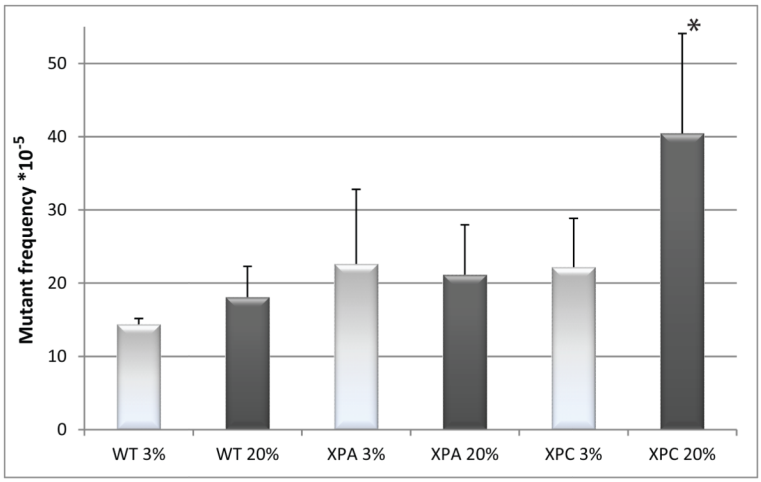

Figure 1.

LacZ mutant frequencies in MEFs cultured at normoxic (3% O_2) or atmospheric (20% O_2) conditions. MEFs were cultured at 3% O_2 pressure the first passage and for two subsequent passages at either 3% or 20% oxygen pressure. *p<0.05 20% O_2 versus 3% O_2 level (Anova, post-hoc t-test with Bonferroni correction).

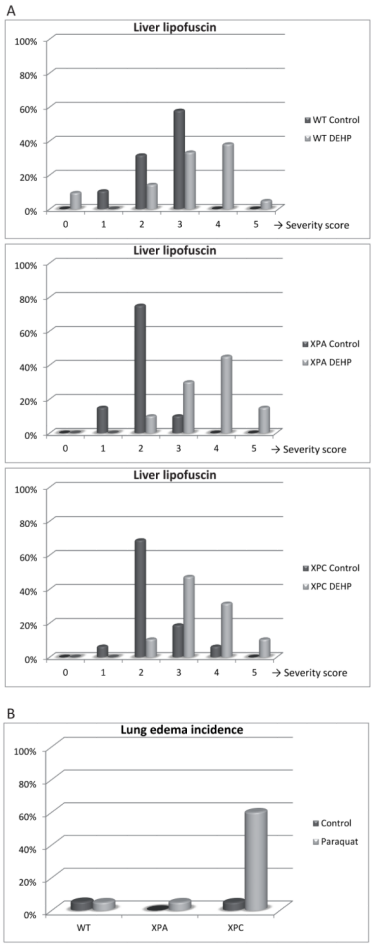

Figure 2.

Representative examples of exposure-related pathological findings in liver and lung. (**A**) Lipofuscin levels (*in vivo* marker for oxidative stress levels) in liver significantly increased in severity for all genotypes upon 39 weeks exposure of pro-oxidants DEHP when compared to their subsequent untreated controls (Fisher Exact test, $p<0.05$). Lipofuscin was assessed on an ordinal scale ranging from 0 (none) − 5 (high). (**B**) Incidence of peribronchiolar edema increased significantly (Fisher Exact test, $p<0.05$) in *Xpc* mice only upon 39 weeks of exposure to paraquat.

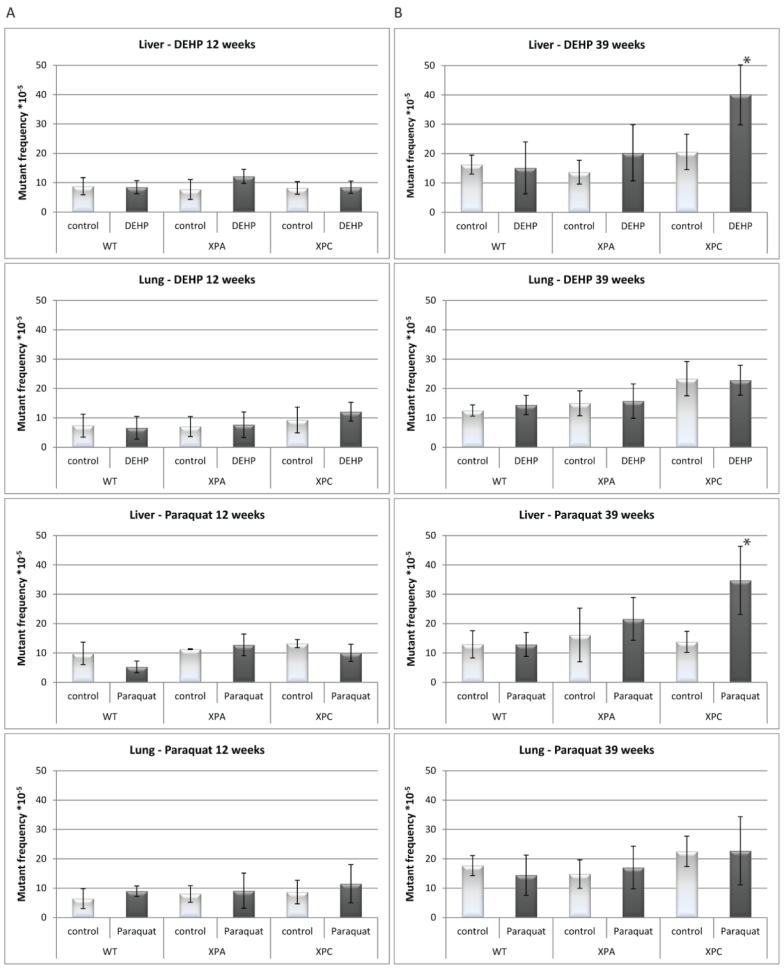

Figure 3.

LacZ mutant frequencies in liver and lung after pro-oxidant exposure of 12 (**A**, left column) and 39 weeks (**B**, right column). Mice were exposed to either: DEHP (female mice), paraquat (male mice) or control feed (male and female mice) for 39 weeks. *$p<0.05$ treatment versus control (ANOVA, post-hoc t-test with Bonferroni correction)