Repair of oxidative DNA damage: assessing its contribution to cancer prevention

Andrew Collins[2] and Vikki Harrington[1]

Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen
AB21 0RD, UK and [1]Robert Gordon University, Aberdeen, UK

DNA repair is a crucial factor in maintaining a low steady-state level of oxidative DNA damage and protecting us from cancer. Cancer case–control studies, using indirect assays in which chromosome breakage in lymphocytes is taken as a measure of failure to repair DNA, indicate an association between poor repair and cancer risk, but case–control studies can be misleading. Surprisingly little is known of the variations in repair capacity within healthy human populations. It is likely that differences in repair enzyme activity result from genetic polymorphisms in repair genes, which have been shown, in some cases, to be linked to cancer. There is a need for prospective studies, in which genotype is analysed (for a range of repair and related genes) and repair activity measured *before* cancer has developed. Using a new method to measure repair in an extract of lymphocytes, based on a modification of the comet assay, we are seeking answers to the following questions: what is the normal range of repair activities in healthy humans; do differences in repair capacity correlate with genetic variations; is low repair capacity associated with a high risk of cancer; how important is DNA repair rate in determining the steady-state level of damage; what is the extent of intra-individual variation; is repair modulated by environmental factors or induced by damage; are there differences in repair capacity between men and women; what is the association of DNA repair with ageing?

Oxidative DNA damage and repair

Oxidative damage to DNA is readily measured in human cells, but estimates of the background level of the most abundant (and potentially mutagenic) oxidized base, 8-oxoguanine, have varied over orders of magnitude (Collins *et al.*, 1997). It now seems clear, and is becoming widely accepted, that DNA oxidation occurs during sample preparation for analysis by HPLC or GC-MS (ESCODD, 2002; Gedik *et al.*, 2002). Recent determinations with methods free of this artefact indicate a true level of damage of ~1–5 8-oxoguanines/10^6 guanines (Gedik *et al.*, 2002; ESCODD, in press). This relatively low level of damage reflects the presence in all cells of antioxidant defences and DNA repair. Reactive oxygen species abound, as a by-product of respiration, but most are removed by antioxidant enzymes or scavengers such as glutathione (as well as by dietary antioxidants such as vitamin C, carotenoids, flavonoids, etc.). The damage measured is in a dynamic steady-state; the input (regulated by antioxidants) is balanced by the output, i.e. DNA repair.

Small alterations to bases, including oxidation and alkylation, are mainly repaired by the pathway known as base excision repair (BER). A lesion-specific glycosylase removes the base and the resulting apurinic/apyrimidinic (AP) site is converted to a break. A small gap (one or a few nucleotides) is filled in by DNA polymerase plus ligase (Evans *et al.*, 2000). BER is distinct from nucleotide excision repair (NER), which deals with bulky adducts and helix distortions and leads to removal of an oligonucleotide of ~28 bases and insertion of a correspondingly long repair patch. Defects in NER are known in humans, in the form of the rare genetic disease xeroderma pigmentosum, which is characterized by extreme sun sensitivity and skin cancer incidence (Wood, 1997). It is notable that no corresponding diseases implicating BER have been described. Reactive oxygen is so pervasive that oxidative damage is inevitable and mutations that disable the repair pathway are likely to be lethal *in embryo*. Alternatively, back-up pathways exist, so that oxidative damage is ultimately repaired and mutation has no major effect in terms of human disease.

8-Oxoguanine is removed from DNA in eukaryotes by the glycosylase OGG1 (Radicella *et al.*, 1997; Roldán-Arjona *et al.*, 1997; Rosenquist *et al.*, 1997). The enzyme has an associated lyase activity; the product of glycosylase action is held at the enzyme active site as a Schiff base which is prone to breakdown, leading to scission of the phosphodiester backbone (Dodson and Lloyd, 2002). This is relatively slow and the enzyme AP endonuclease (APE1) can perform the breakage more quickly, releasing the glycosylase for further attack on other damaged bases. Thus APE1 stimulates OGG1 activity (Hill *et al.*, 2001; Vidal *et al.*, 2001).

The *ogg1* knockout mouse lacks OGG1 activity and yet accumulates oxidative DNA damage to only a small degree. It has a normal lifespan and is not cancer prone when compared with the wild-type (Klungland *et al.*, 1999). Removal of 8-oxoguanine does occur in cells cultured from the knockout mutant, but with a delay, indicating the existence of an alternative repair pathway (Klungland *et al.*, 1999).

The molecular epidemiological approach

The molecular epidemiological approach measures molecular biomarkers, usually in samples of blood, rather than simply looking at disease incidence. The biomarkers might represent exposure to disease-causing or protective agents, individual susceptibility or early stages in development of disease. Exposure to carcinogens can be assessed by measuring DNA damage, adducts covalently bound to albumin or urinary metabolites. In certain cases, the link between exposure and cancer may be strong enough that measurement of these biomarkers gives an indication of cancer risk; aflatoxin, a causative agent in liver cancer, is a notable example (Montesano *et al.*, 1997). However, in general, too many other steps intervene between DNA damage and carcinogenesis for there

---

[2]To whom correspondence should be addressed.

to be a clear link between markers of individual exposure and cancer risk. One of these steps, obviously, is DNA repair.

It is likely that in a human population there will be variations in BER, resulting from genetic polymorphisms and even gene deletions (if the alternative pathway theory is correct). Such genetic heterogeneity might be expected to lead to differences in the steady-state level of DNA damage, variations in cancer risk and even differential ageing, since one theory of ageing proposes the accumulation of oxidatively damaged biological molecules, including DNA, as the underlying cause.

Berwick and Vineis (2000) have reviewed the many reports linking inadequate repair of oxidative DNA damage to an increased risk of cancer in man. These mostly take the form of case–control studies, i.e. they compare the repair capacity in lymphocytes from cancer patients with control cells from subjects not suffering from the disease. The matching of cases to controls is crucial, but frequently is not carefully performed. The assay for DNA repair in most common use is a very indirect one, based on the sensitivity of cells to a DNA-damaging agent, usually the radiomimetic chemical bleomycin; the end-point is generally chromosome breakage, which clearly could reflect factors other than DNA repair, including antioxidant protection and cell cycle kinetics.

In some studies, a more direct assay for DNA repair was employed. Jałoszyński et al. (1997) exposed lymphocytes from breast cancer patients and controls to bleomycin and assessed the ability to repair strand breaks on incubation for 0.5–1 h. Cancer patients typically showed lower repair rates. Schmezer et al. (2001) examined repair in stimulated lymphocytes from lung cancer patients and controls, treated with bleomycin. The rate of removal of damage in a 15 min incubation was significantly lower in lung cancer patients than in controls. A similar result was reported by Leprat et al. (1998), who compared the capacity to repair radiation-induced DNA breaks in lymphocytes from patients who developed thyroid cancer as a consequence of radiotherapy for a previous cancer and from healthy controls.

An assay was developed in which the cells under study do not receive the damage directly, but are exposed to a damaged plasmid, introduced by transfection. The plasmid contains a reporter gene; if the damage is satisfactorily repaired, the reporter gene product is synthesized and its intensity or reactivity is taken to indicate repair capacity (Athas et al., 1991). This approach has been applied principally to the examination of variations in NER, using plasmids damaged by UV light or a bulky adduct. Comparing basal cell carcinoma (skin cancer) patients and controls, risk of the disease was associated with reduced DNA repair capacity, although with borderline statistical significance (Wei et al., 1993).

There is a potential problem with case–control studies, since, however good the matching, the cases are inevitably different from the controls because they have the disease and the parameter measured may be an effect rather than a cause of this condition. It is not unlikely that DNA repair, antioxidant capacity and cell proliferation may be altered as a result of cancer. In addition, as pointed out by Hemminki et al. (2000), extrapolation from results obtained with lymphocytes to other tissues that are the real cancer target can be risky. Notwithstanding the serious reservations, these studies do consistently indicate a positive association between sensitivity to mutagens and cancer occurrence.

Genetic polymorphisms in human *OGG1* (*hOGG1*) have been investigated for links with cancer. A polymorphism at codon 326 gives rise to alternative Ser and Cys in the protein, and the Cys-containing protein was less able to suppress mutation in a bacterial complementation assay (Kohno et al., 1998). The same group investigated the distribution of this polymorphism in gastric cancer cases and controls in Japan and found no difference (Shinmura et al., 1998). Further polymorphisms, located in the 5′-non-coding region, were identified (Ishida et al., 1999); a G→T transition at position –18 was significantly associated with risk of adenocarcinoma of the lung. Chevillard et al. (1998) found homozygous mutations in *hOGG1* in one out of 15 human kidney carcinomas and in two of 25 small cell lung cancer samples. Normal tissue, available for the kidney sample, had a homozygous wild-type genotype. Probably the inactivation of *OGG1* occurs during carcinogenesis by mutation in one allele and deletion of the other. Blons et al. (1999) found no evidence for an involvement of *hOGG1* polymorphisms in head and neck carcinogenesis.

With the information from the human genome and, in particular, the analysis of single nucleotide polymorphisms (SNPs), there is ever-increasing interest in the genetic determinants of susceptibility to cancer and other diseases. The simplest approach to identifying links between genotype and cancer occurrence is to carry out case–control studies, but the problem of matching the control group to the cancer patients remains. A prospective study would be ideal. In this, the genetic data are collected from a suitable cohort (or, more simply, DNA samples are stored); at some time in the future, patients with disease are identified and matched with healthy controls from within the same cohort; a 'nested case–control' design. Genotypes in the two groups can then be compared without danger of selection bias. Polymorphisms are, by definition, genetic variants occurring in 1% or more of the population. Although some may occur at frequencies of ~50%, they are typically much rarer, which means that large numbers of subjects must be screened to achieve statistically sound conclusions. Interactions between two (or more) gene products are often important, and investigating these at the level of genetic polymorphisms necessitates a further increase in numbers.

As Berwick and Vineis (2000) state at the end of their survey of 'repair' and cancer; 'It is not clear that conducting these [genotyping] studies without concomitant studies of expression and/or function will be fruitful'. Ideally, in a prospective study with nested case–control design at a later stage, the relevant phenotypic biomarkers should be measured at the time of sample collection, as it is unlikely that samples stored frozen for many years will be amenable to analysis. Simple, rapid and robust methods for biomarker measurement are therefore required. Given good functional assays for DNA damage and repair, an alliance with genotype analysis would allow us to answer crucial questions.

1. What is the normal range of DNA repair activities in healthy humans?
2. Do differences in repair capacity correlate with any genetic variations?
3. How constant is individual repair capacity?
4. Is low repair capacity associated with high risk of cancer?
5. How important are different repair pathways and variations in repair rate in determining the steady-state level of damage?
6. Is repair modulated by environmental factors? Is it induced by damage or enhanced by dietary factors?

7. Does repair capacity decline with age? Do particular repair phenotypes or genotypes lead to accelerated ageing?

DNA repair assays

If we are to measure DNA repair function, what is the choice of assays? As well as being simple, robust and rapid, the assay should of course be relevant (it should measure repair of the lesion of interest, in this case oxidative base damage) and it should be well-characterized in terms of sensitivity, experimental reproducibility and inter- and intra-individual variation.

Unscheduled DNA synthesis (UDS)

UDS is a traditional assay for DNA repair (Lambert *et al.*, 1979); incorporation of [³H]thymidine into the DNA of cells treated with a genotoxic agent is measured in the absence of replication (either because cells are in a non-proliferating stage of the cell cycle or because replication is artificially inhibited). UDS is easily demonstrated after UV damage, but incorporation is very low during BER, since the gaps to be filled are far smaller. Even when testing for NER, the doses that must be used are close to the level that saturates the assay and above those that kill most cells (Hu *et al.*, 1996). If possible, for a valid DNA repair assay test doses of damage should be well below lethal doses and preferably close to the level likely to be encountered in life. UDS is clearly not suitable for assessing BER of oxidative damage.

Cellular repair

In the standard bleomycin sensitivity assay described earlier, the effect of repair is assessed by the number of chromatid breaks present. A more direct approach is to measure the DNA damage and monitor its removal. Probably the best, and certainly the most popular, method for measuring DNA breaks is the comet assay (single cell alkaline gel electrophoresis). The cells are embedded in agarose on a microscope slide and lysed in Triton X-100 and 2.5 M NaCl. This removes membranes, cytoplasm and most nuclear proteins, leaving the DNA as tightly packed supercoiled loops attached to the residual nuclear matrix in a nucleoid. The presence of breaks relaxes the supercoiling and on subsequent alkaline electrophoresis the relaxed loops are drawn out to form a 'comet' as viewed by fluorescence microscopy. The higher the break frequency, the more DNA is in the tail of the comet. This approach was used by Jałoszyński *et al.* (1997), Leprat *et al.* (1998) and Schmezer *et al.* (2001) to follow the kinetics of rejoining of breaks. [Leprat *et al.* (1998) modified the normal procedure by embedding the cells in agarose and then incubating them for repair, rather than embedding them *after* repair incubation.]

Repair of oxidized bases can also be followed with the comet assay. An additional step is introduced; after lysis, the nucleoids are incubated with a lesion-specific endonuclease which converts oxidized bases to breaks. Endonuclease III is specific for oxidized pyrimidines, while formamidopyrimidine DNA glycosylase (FPG, the bacterial counterpart of OGG1) recognizes altered purines, viz formamidopyrimidines and 8-oxoguanine. Lymphocytes repair 8-oxoguanine, introduced by treating the cells with the photosensitizer Ro 19-8022 and visible light, relatively quickly (Figure 1).

This approach to assessing repair has the advantage of sensitivity, so that the cellular response to low levels of damage can be assessed. However, the need to carry out prolonged incubations is a drawback.
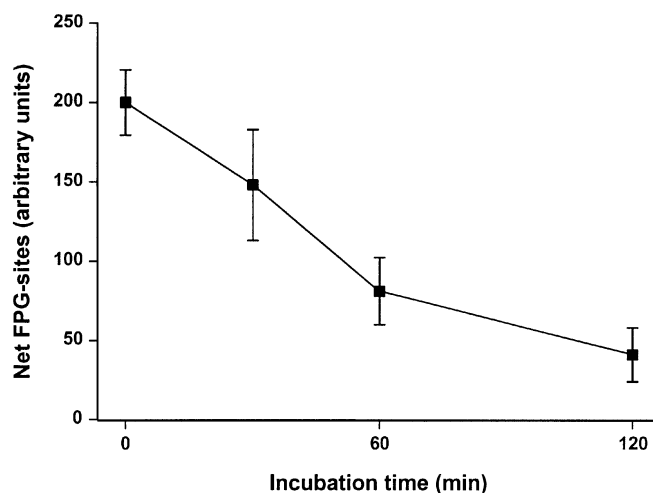

Fig. 1. Cellular repair of 8-oxoguanine. Lymphocytes were treated with 0.1 μM photosensitizer Ro 19-8022 and 2 min irradiation (33 cm from a 1000 W halogen lamp, on ice) to induce 8-oxoguanine in DNA. The cells were incubated at 37°C to allow repair to occur and the remaining 8-oxoguanines were estimated, as FPG-sensitive sites, using the comet assay. Mean values from lymphocytes from six subjects are shown, with SD.

Host cell reactivation

Lymphocytes are transfected with a plasmid containing a reporter gene [e.g. the chloramphenical acetyltransferase (*cat*) gene or the gene for luciferase] which has been damaged, for instance with UV light or benzo[*a*]pyrene diol epoxide (both repaired by NER). The ability of the transfected cells to repair the gene is assessed by the restoration of activity of the reporter gene product. However, results may be influenced by the efficiency of transfection, and a wide inter-individual variation in DNA repair capacity is seen. The assay is cumbersome, and is mainly used by one group.

In vitro repair

A more biochemical approach involves measurement of the repair capacity of a whole cell extract. In one version of this, the extract is incubated with an oligonucleotide containing one 8-oxoguanine residue and a terminal ³²P label (Roldán-Arjona *et al.*, 1997). The alteration in size on cleavage of the phosphodiester bond at the 8-oxoguanine is detected by conventional gel electrophoresis and visualization of the ³²P-labelled fragments. Alternatively, the extract is incubated with closed circular plasmid DNA containing specific damage (~1 lesion/circle), and nicking of circles is detected by the change in electrophoretic migration. This method was developed by Redaelli *et al.* (1998) to estimate APE activity on a substrate with AP sites. In a further variation, Elliott *et al.* (2000) incubated plasmid DNA containing oxidative damage (introduced via hydroxyl radicals or singlet oxygen) with a cell-free extract of lymphocytes in the presence of deoxyribonucleoside triphosphates, one of which was labelled with ³²P. Thus they were able to measure the overall repair reaction, from incision to polymerization. The comet assay has also been adapted to measure *in vitro* repair (Collins *et al.*, 2001). The damaged substrate comprises gel-embedded nucleoids from cells treated with Ro 19-8022 and light to induce 8-oxoguanine; they are incubated with extract from the cells of interest and the rate of incision is ascertained. This assay was validated using cell extract from an *ogg1* knockout mouse cell line, which, in contrast to extract from wild-type cells, had no activity.
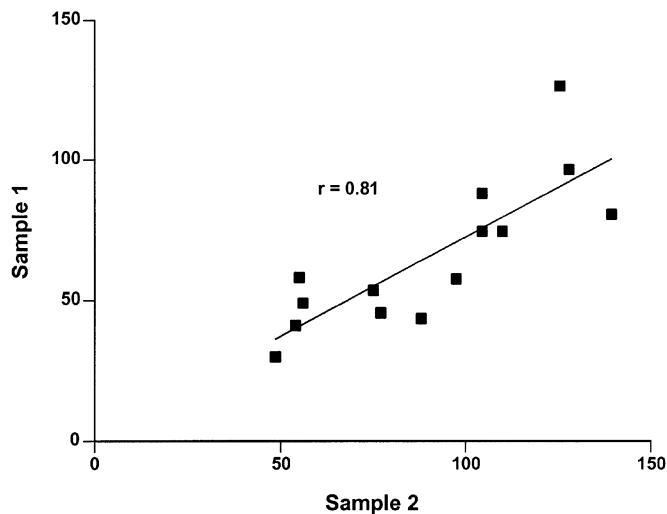
Fig. 2. DNA repair capacity of human lymphocytes measured *in vitro* with the comet assay. Lymphocyte samples were taken from 14 volunteers on two occasions 5 weeks apart. Repair capacity was estimated as the incision activity during 10 min incubation with a substrate containing 8-oxoguanine. The figure shows the extent of variation in repair capacity (arbitrary units) in a normal population and the intra-individual reproducibility.

Gene expression

Changes in the level of *OGG1* mRNA are determined by semi-quantitative RT–PCR, real time PCR or by hybridization with a cDNA or oligonucleotide microarray.

Addressing some of the questions

What is the normal range of repair activities in healthy humans?

Janssen *et al.* (2001) measured OGG1 activity in lymphocytes from 34 healthy individuals, using the 8-oxodG-oligonucleotide cleavage assay. Activities varied over a 2-fold range. We find a range of ~3-fold using the *in vitro* test based on the comet assay (Figure 2 and unpublished results). APE activity was assessed by Redaelli *et al.* (1998); the range in a group of 10 normal subjects was 2.5-fold. Variation of this order, whether it arises from genetic differences or is the result of environmental influences, is sufficient to have potential consequences for health. However, the variation could simply represent experimental variation or ill-defined fluctuations.

How stable is individual repair capacity?

Figure 2 shows the OGG1 activity measured in lymphocyte samples taken from our 14 volunteers on two occasions, 5 weeks apart. The strong correlation indicates that, at least in the short term, the potential for repair is stable. Intra-individual variation is far less than inter-individual variation.

Do differences in repair capacity correlate with any genetic variations?

The relative inefficiency of the OGG1 protein with Cys326 compared with Ser326 in the bacterial complementation assay (Kohno *et al.*, 1998) has been mentioned already. In Europeans, the polymorphism occurs with allele frequencies of 75% for Ser326 and 25% for Cys326, but it does not result in altered OGG1 activity in the plasmid cleavage assay (Janssen *et al.*, 2001).

Does repair capacity decline with age? Are there differences between men and women?

Janssen *et al.* (2001) found no difference between men and women in lymphocyte OGG1 activity and no influence of

smoking habit. An early study of NER (Lambert *et al.*, 1979) found a decrease of 30% in UV-induced repair synthesis between the ages of 20 and 90 and a decline of 0.6% per year was reported by Moriwaki *et al.* (1996) using the plasmid host cell reactivation assay. Barnett and King (1995) used the comet assay to measure DNA strand breaks in lymphocytes immediately after H_2O_2 treatment and after incubation for 90 min. Lymphocytes from 65–69 year olds had significantly more residual breaks at that time than did lymphocytes from 35–39 year olds. However, in a recent investigation of lymphocytes from 226 subjects aged between 21 and 88 we saw no effect of age on the ability to nick 8-oxoguanine-containing nucleoids in the *in vitro* comet assay (Harrington *et al.*, unpublished results). Animal studies may be informative, although ageing in rodents (lifespan ~2 years) is arguably not comparable with ageing in man. In an *in vitro* plasmid-nicking assay, nuclear OGG1 activity from mouse liver decreased slightly with age, while mitochondrial OGG1 actually increased significantly (de Souza-Pinto *et al.*, 2001)

How important is DNA repair rate in determining the steady-state level of damage?

It may seem intuitively obvious that a high repair rate will lead to a decrease in the steady-state level of damage. However, we do not know enough about the regulation of repair. An alternative model is that the more reactive oxygen there is, the more repair is induced and, in consequence, the steady-state level of damage is kept constant, in which case no correlation between damage and repair would be expected. Analysis of repair rates and damage levels would help to decide between these possibilities, but the data are not yet available.

Is repair modulated by environmental factors? Is it induced by damage or enhanced by dietary factors?

There are some relevant reports of studies with animals and cultured cells. Kim *et al.* (2001) exposed pulmonary type II-like epithelial cells to crocidolite asbestos; repair activity (on an oligonucleotide containing 8-oxoguanine) and *hOGG1* mRNA expression increased significantly. A similar effect of asbestos on OGG1 was seen in hamster and rat lungs (Yamaguchi *et al.*, 1999) and in rats exposed by intra-tracheal instillation to diesel exhaust particles, the level of 8-oxoguanine in DNA showed an increase, followed by an increase in repair activity and induction of *OGG1* mRNA synthesis (Tsurudome *et al.*, 1999). Cherng *et al.* (2002) found an induction of *OGG1* mRNA expression in lung adenocarcinoma CL3 cells by H_2O_2 and cooking oil fumes, and in a group of cooks and housewives exposed to these fumes *hOGG1* mRNA expression was also higher relative to an unexposed control group. Janssen *et al.* (2001) quote unpublished data showing a significant decrease in OGG1 activity in workers exposed to metal dust. Turning to presumably beneficial effects related to nutrition, Tomasetti *et al.* (2001) found that supplementation with coenzyme Q_{10} enhanced *in vitro* repair of 8-oxoguanine by lymphocyte extract and we have recently demonstrated an enhancement of repair by consumption of fruit (Collins *et al.*, in preparation).

Conclusions

Is low repair capacity associated with high risk of cancer? In our view, with the limitations of the case–control studies so far undertaken, this is still an open question, which can be answered only by a large-scale prospective study. Repair rates

should be measured and genotypes analysed and then over the ensuing years cancer incidence recorded. To our knowledge, no such study has yet been undertaken. Even with this approach, there is an underlying assumption that lymphocytes are a representative cell type to tell us about repair capacity in the body as a whole, and this assumption is hard to test in humans.

Acknowledgements

References

Athas,W.F., Hedayati,M.A., Matanoski,G.M., Farmer,E.R. and Grossman,L. (1991) Development and field-test validation of an assay for DNA repair in circulating human lymphocytes. *Cancer Res.*, **51**, 5786–5793.

Barnett,Y.A. and King,C.M. (1995) An investigation of antioxidant status, DNA repair capacity and mutation as a function of age in humans. *Mutat. Res.*, **338**, 115–128.

Berwick,M. and Vineis,P. (2000) Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J. Natl Cancer Inst.*, **92**, 874–897.

Blons,H., Radicella,J.P., Laccourreye,O., Brasnu,D., Beaune,P., Boiteux,S. and Laurent-Puig,P. (1999) Frequent allelic loss at chromosome 3p distinct from genetic alterations of the 8-oxoguanine DNA glycosylase 1 gene in head and neck cancer. *Mol. Carcinog.*, **26**, 254–260.

Cherng,S.-H., Huang,K.H., Yang,S.-C., Wu,T.-C., Yang,J.-L. and Lee,H. (2002) Human 8-oxoguanine DNA glycosylase 1 mRNA expression as an oxidative stress exposure biomarker of cooking oil fumes. *J. Toxicol. Environ. Health*, **65A**, 265–278.

Chevillard,S., Radicella,J.P., Levalois,C., Lebeau,J., Poupon,M.-F., Oudard,S., Dutrillaux,B. and Boiteux,S. (1998) Mutations in *OGG1*, a gene involved in the repair of oxidative DNA damage, are found in human lung and kidney tumours. *Oncogene*, **16**, 3083–3086.

Collins,A., Cadet,J., Epe,B. and Gedik,C. (1997) Problems in the measurement of 8-oxoguanine in human DNA. Report of a workshop, DNA Oxidation, held in Aberdeen, UK, 19–21 January, 1997. *Carcinogenesis*, **18**, 1833–1836.

Collins,A.R., Dušinská,M., Horváthová,E., Munro,E., Savio,M. and Štětina,R. (2001) Inter-individual differences in DNA base excision repair activity measured *in vitro* with the comet assay. *Mutagenesis*, **16**, 297–301.

De Souza-Pinto,N.C., Hogue,B.A. and Bohr,V.A. (2001) DNA repair and aging in mouse liver: 8-oxodG glycosylase activity increase in mitochondrial but not in nuclear extracts. *Free Radic. Biol. Med.*, **30**, 916–923.

Dodson,M.L. and Lloyd,R.S. (2002) Mechanistic comparisons among base excision repair glycosylases. *Free Radic. Biol. Med.*, **32**, 678–682.

Elliott,R.M., Astley,S., Southon,S. and Archer,D.B. (2000) Measurement of cellular repair activities for oxidative DNA damage. *Free Radic. Biol. Med.*, **28**, 1438–1446.

ESCODD (2002) Inter-laboratory validation of procedures for measuring 8-oxo-7,8-dihydroguanine/8-oxo-7,8-dihydro-2′-deoxyguanosine in DNA. *Free Radic. Res.*, **36**, 239–245.

ESCODD. Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus. *Carcinogenesis*, in press.

Evans,A.R., Limp-Foster,M. and Kelley,M.R. (2000) Going APE over ref-1. *Mutat. Res.*, **461**, 83–108.

Gedik,C.M., Boyle,S.P., Wood,S.G., Vaughan,N.J. and Collins,A.R. (2002) Oxidative stress in humans; validation of biomarkers of DNA damage. *Carcinogenesis*, **23**, 1441–1446.

Hemminki,K., Xu,G. and Le Curieux,F. (2000) Re: Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J. Natl Cancer Inst.*, **92**, 1536–1537.

Hill,J.W., Hazra,T.K., Izumi,T. and Mitra,S. (2001) Stimulation of human 8-oxoguanine-DNA glycosylase by AP-endonuclease: potential coordination of the initial steps in base excision repair. *Nucleic Acids Res.*, **29**, 430–438.

Hu,J.J., Roush,G.C., Berwick,M., Dubin,N., Mahabir,S., Chandiramani,M. and Boorstein,R. (1996) Effects of dietary supplementation of α-tocopherol on plasma glutathione and DNA repair activities. *Cancer Epidemiol. Biomarkers Prev.*, **5**, 263–270.

Ishida,T., Takashima,R., Fukayama,M. *et al.* (1999) New DNA polymorphisms of human *MMH/OGG1* gene: prevalence of one polymorphism among lung-adenocarcinoma patients in Japanese. *Int. J. Cancer*, **80**, 18–21.

Jałoszyński,P., Kujawski,M., Czub-Swierczek,M., Markowska,J. and

Szyfter,K. (1997) Bleomycin-induced DNA damage and its removal in lymphocytes of breast cancer patients studied by comet assay. *Mutat. Res.*, **385**, 223–233.

Janssen,K., Schlink,K., Götte,W., Hippler,B., Kaina,B. and Oesch,F. (2001) DNA repair activity of 8-oxoguanine DNA glycosylase 1 (OGG1) in human lymphocytes is not dependent on genetic polymorphism Ser^{326}/Cys^{326}. *Mutat. Res.*, **486**, 207–216.

Kim,H.-N., Morimoto,Y., Tsuda,T., Ootsuyama,Y., Hirohashi,M., Hirano,T., Tanaka,I., Lim,Y., Yun,I.-G. and Kasai,H. (2001) Changes in DNA 8-hydroxyguanine levels, 8-hydroxyguanine repair activity and hOGG1 and hMTH1 mRNA expression in human lung alveolar epithelial cells induced by crocidolite asbestos. *Carcinogenesis*, **22**, 265–269.

Klungland,A., Rosewell,I., Hollenbach,S., Larsen,E., Daly,G., Epe,B., Seeberg,E., Lindahl,T. and Barnes,D.E. (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc. Natl Acad. Sci. USA*, **96**, 13300–13305.

Kohno,T., Shinmura,K., Tosaka,M., Tani,M., Kim,S.-R., Sugimura,H., Nohmi,T., Kasai,H. and Yokota,J. (1998) Genetic polymorphisms and alternative splicing of the *hOGG1* gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. *Oncogene*, **16**, 3219–3225.

Lambert,B., Ringborg,U. and Skoog,L. (1979) Age-related decreases of ultraviolet light-induced DNA repair synthesis in human peripheral leukocytes. *Cancer Res.*, **39**, 2792–2795.

Leprat,F., Alapetite,C., Rosselli,F., Ridet,A., Schlumberger,M., Sarasin,A., Suarez,H.G. and Moustacchi,E. (1998) Impaired DNA repair as assessed by the 'comet' assay in patients with thyroid tumors after a history of radiation therapy: a preliminary study. *Int. J. Radiat. Oncol. Biol. Phys.*, **40**, 1019–1026.

Montesano,R., Hainaut,P. and Wild,C.P. (1997) Hepatocellular carcinoma: from gene to public health. *J. Natl Cancer Inst.*, **89**, 1844–1851.

Moriwaki,S.-I., Ray,S., Tarone,R.E., Kraemer,K.H. and Grossman,L. (1996) The effect of donor age on the processing of UV-damaged DNA by cultured human cells: reduced DNA repair capacity and increased DNA mutability. *Mutat. Res.*, **364**, 117–123.

Radicella,J.P., Dhérin,C., Desmaze,C., Fox,M.S. and Boiteux,S. (1997) Cloning and characterization of *hOGG1*, a human homolog of the *OGG1* gene of *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **94**, 8010–8015.

Redaelli,A., Magrassi,R., Bonassi,S., Abbondandolo,A. and Frosina,G. (1998) AP endonuclease activity in humans: development of a simple assay and analysis of ten normal individuals. *Teratog. Carcinog. Mutagen.*, **18**, 17–26.

Roldán-Arjona,T., Wei,Y.-F., Carter,K.C., Klungland,A., Anselmino,C., Wang,R.-P., Augustus,M. and Lindahl,T. (1997) Molecular cloning and functional expression of a human cDNA encoding the antimutator enzyme 8-hydroxyguanine-DNA glycosylase. *Proc. Natl Acad. Sci. USA*, **94**, 8016–8020.

Rosenquist,T.A., Zharkov,D.O. and Grollman,A.P. (1997) Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proc. Natl Acad. Sci. USA*, **94**, 7429–7434.

Schmezer,P., Rajaee-Behbahani,N., Risch,A., Thiel,S., Rittgen,W., Drings,P., Dienemann,H., Kayser,K.W., Schulz,V. and Bartsch,H. (2001) Rapid screening assay for mutagen sensitivity and DNA repair capacity in human peripheral blood lymphocytes. *Mutagenesis*, **16**, 25–30.

Shinmura,K., Kohno,T., Kasai,H., Sugimura,H. and Yokota,J. (1998) Infrequent mutations of the *hOGG1* gene, that is involved in the excision of 8-hydroxyguanine in damaged DNA, in human gastric cancer. *Jap. J. Cancer Res.*, **89**, 825–828.

Tomasetti,M., Alleva,R., Borghi,B. and Collins,A.R. (2001) *In vivo* supplementation with coenzyme Q10 enhances the recovery of human lymphocytes from oxidative DNA damage. *FASEB J.*, **15**, 1425–1427.

Tsurudome,Y., Hirano,T., Yamato,H., Tanaka,I., Sagai,M., Hirano,H., Nagata,N., Itoh,H. and Kasai,H. (1999) Changes in levels of 8-hydroxyguanine in DNA, its repair and *OGG1* mRNA in rat lungs after intratracheal administration of diesel exhaust particles. *Carcinogenesis*, **20**, 1573–1576.

Vidal,A.E., Hickson,I.D., Boiteux,S. and Radicella,J.P. (2001) Mechanism of stimulation of the DNA glycosylase activity of hOGG1 by the major human AP endonuclease: bypass of the AP lyase activity step. *Nucleic Acids Res.*, **29**, 1285–1292.

Wei,Q., Matanoski,G.M., Farmer,E.R., Hedayati,M.A. and Grossman,L. (1993) DNA repair and aging in basal cell carcinoma: a molecular epidemiology study. *Proc. Natl Acad. Sci. USA*, **90**, 1614–1618.

Wood,R.D. (1997) Nucleotide excision repair in mammalian cells. *J. Biol. Chem.*, **272**, 23465–23468.

Yamaguchi,R., Hirano,T., Ootsuyama,Y., Asami,S., Tsurudome,Y., Fukada,S., Yamato,H., Tsuda,T., Tanaka,I. and Kasai,H. (1999) Increased 8-hydroxyguanine in DNA and its repair activity in hamster and rat lung after intratracheal instillation of crocidolite asbestos. *Jap. J. Cancer Res.*, **90**, 505–509.