Chapter 16

Cigarette Smoke and Oxidative DNA Modification

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16.1. Introduction

In this chapter, we give an overview of the nature of oxidative DNA modification, which factors are of importance for oxidation and repair of DNA, how to analyze the lesions and avoid pitfalls of oxidation, how to set up experiments, and how to interpret such experiments. We end up with an evaluation about the degree to which we can associate oxidative DNA modifications with cancer, particularly in relation to cigarette smoking.

In 1992, Leandersson and Tagesson (1992) showed that cigarette smoke increased DNA damage in cultured human lung cells, and at the same time our group showed that cigarette smoking induced increased DNA modification in humans (Loft et al. 1992), measured by urinary excretion of 8-hydroxy-2'-deoxyguanosine (8oxodG); this lesion is the most examined prototype of oxidative DNA modification. Later we showed that smoking cessation reduced DNA modification. Hiroshi Kasai's group (1997) showed that cigarette smoking induces an increase in 8-hydroxydeoxyguanosine in a central site of the lung. Taken together, these findings provide strong evidence that cigarette smoking induces oxidative stress to DNA in the form of increased oxidative base modification.

The area of oxidative DNA modification has particularly been promoted by the pioneers in this area: Hiroshi Kasai, who was the first to report the 8-hydroxy-2'-deoxyguanosine modification in 1984 (Kasai and Nishimura 1984), based on studies on mutagens in heated glucose, and Robert Floyd, who reported that this lesion could be measured by high-performance liquid chromatography (HPLC) with electrochemical detection (Floyd et al. 1986). In an excellent review in 1997, Hiroshi Kasai listed most of the reports about findings in different organs and diseases (Kasai 1997).

Bruce Ames promoted the relationship between oxidative DNA modification and aging, and also the relationship to the antioxidant intake (Ames 1989a, b; Ames et al. 1993), and hypothesized that micronutrient deficiency was a major cause of cancer (Ames 2001).

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16.2 Nature and Extent of Oxidative DNA Modifications in DNA

The elucidation of the chemical nature of DNA oxidation was done by the pioneering work of Miral Dizdaroglu (Dizdaroglu 1985, 1991, 1993, 1994; Dizdaroglu and Bergtold 1986; Dizdaroglu et al. 2002) and Jean Cadet (Cadet and Treoule 1978; Cadet et al. 1986, 2003, 2005), preceded by the first report of the 8oxodG modification in 1984 (Kasai and Nishimura 1984).

Oxidation can modify DNA in several positions, in the purine/pyrimidine moiety as well as in the sugar moiety in almost any part of the molecule. After the initial oxidative modification, further rearrangements/changes can occur. For example, the initial modification of guanine can form the C8-OH radical of guanine, which can undergo ring opening, forming the Fapy (2,6-diamino-4-hydroxy-5-formamidopyrimidine) or forming the 8-oxo form dependent on the redox conditions in the reaction mixture or in cells. In total, the reported number of different possible modifications to DNA from oxidation gets close to 100; however, only few of these have been demonstrated in the in vivo situation (Cadet et al. 1997, 2003; Dizdaroglu 1991,1994; Guetens et al. 2002; Schram 1998).

With regard to oxidative modifications because of cigarette smoke, there are no reports of specific oxidative adducts, rather the reports unanimously indicate an increase is preexisting modifications. On the other hand, the literature is mainly focused on the 8oxodG lesion, so it is not for certain known if tobacco smoke gives a specific pattern of oxidation. The reported levels of 8oxodG in nuclear DNA vary over a vide range but may be about 1–10 per million dGs, as expected because 8oxodG is repaired very fast (Asami et al. 1996, 1997; Halliwell 2002; Spencer et al. 1996). In comparison, the levels of polycyclic aromatic hydrocarbon-derived adducts are about 1–10 per 100 million nucleotides (Farmer and Shuker 1999; Godschalk et al. 2002), i.e., about 100 times less frequent. However, as pointed out in an excellent review of smoking-related DNA adducts in a variety of human tissues (Phillips 2002), the half-life of polycyclic aromatic hydrocarbon-derived adducts in the lung is about 1–2 years. In animals exposed to oxidative stress the levels of, e.g., liver 8oxodG returns to normal values within 24 h (Deng et al. 1998), indicating an elimination half-life of some hours. In cellular systems, elimination half-lives of various types of oxidative damage range from minutes to hours. Some studies suggest that oxidized purines are eliminated faster than oxidized pyrimidines (Spencer et al. 1996); other investigators report the reverse (Jaruga and Dizdaroglu 1996). Injected 8oxodG is eliminated with at half-life of few hours into urine (Loft et al. 1995), and after smoking cessation, urinary excretion of 8oxodG decreases within weeks (Prieme et al. 1998a).

Exposure to environmental tobacco smoke in the workplace resulted in a 63% increase in white blood cell DNA 8oxodG levels. The same study included a nonrandomized and noncontrolled intervention showing that the high 8oxodG levels were mitigated by antioxidant supplementation (Howard et al. 1998b). In another study on occupational exposure of metal fume and residual oil fly ash, urinary excretion of 8oxodG was higher in nonsmokers than in smokers (Mukherjee et al. 2004) at the beginning of the work week, but after 2 days of work, the excretion rates were identical.

Taken together, it appears that oxidative lesions are much more frequent than polycyclic aromatic hydrocarbon (PAH)-derived adducts, i.e., with several orders of magnitude, and that the repair of the PAH adducts occurs with a much slower half-life. In this situation, it is very difficult to infer which of the adducts are most important for carcinogenesis, because all the adducts/modifications show mutagenic properties; rather, such information should rely on the predictive values of the lesions in prospective studies, e.g., case-control or cohort studies. Such studies are not presently published, (reviewed by Halliwell 2002).

With the use of the Comet assay, a long list of studies have showed increased DNA modification in lymphocytes from smokers compared with nonsmokers (Einhaus et al. 1994; Holz et al. 1993; Lam et al. 2002; Park and Kang 2004; Piperakis et al. 1998; Poli et al. 1999; Welch et al. 1999; Zhu et al. 1999), in lung, stomach, and liver of mice exposed to cigarette smoke (Tsuda et al. 2000) and in oocyte-related cumulus cells (Sinko et al. 2005). On the other hand, there are also reports of no differences between smokers and nonsmokers (Hoffmann and Speit 2005; Speit et al. 2003; Wojewodzka et al. 1999), even after taking genetic polymorphisms in the glutathione *S*-transferase mu (*GSTM1*), cytochrome P450 1A1 (*CYP1A1*), xeroderma pigmentosum group D (*XPD*), X-ray repair cross complementing group 1 (*XRCC1*), and X-ray repair cross complementing group 3 (*XRCC3*) genes into account (Hoffmann et al. 2005). Regarding environmental tobacco smoke exposure, there are reports showing increased DNA strand breaks as measured by the Comet assay (Collier et al. 2005; Wolz et al. 2002).

Most animal studies have focused on target tissue of interest in relation to cigarette

smoke-related cancers, whereas human studies mainly use peripheral lymphocytes as a surrogate tissue. However, one study focused on the DNA modification in placenta and found that 8oxodG increased in smokers as well as after exposure to environmental tobacco smoke (Daube et al. 1997). In mice exposed to acute side stream tobacco smoke, heart and lung levels of 8oxodG increased (Howard et al. 1998a). These findings indicate that cigarette smoke also leads to a more general oxidative stress that just to the DNA of the most directly exposed organ, i.e., the lungs.

16.3 Urinary Excretion of Oxidatively Modified DNA Components

Simple back-of-the-envelope calculation from an estimated average of 2,500 hits to bases in each cell's genome per 24 h gives the result that it would take 1 year to oxidize 1% of the genome if the lesions were not repaired (Poulsen et al. 1998), or about 50% at the age of 50. The excretion of such lesions into urine has been proposed to reflect the oxidative stress to DNA and its precursors (Poulsen et al. 2000, 2003).

In 1992, we showed that smokers excreted on average (big overlapping ranges) about 50% more 8oxodG than nonsmokers (Loft et al. 1992), and later we showed that smokers randomized to smoking cessation decreased their 8oxodG excretion as compared with smokers randomized to continued smoking (Loft et al. 1992; Prieme et al. 1998a). A subsequent study showed that smokers also excreted more of the corresponding base 8oxoG (Suzuki et al. 1995), a finding that could not be repeated in a later study (Harman et al. 2003), and the Poulsen laboratory did not find a difference in 8oxoG excretion between smokers and nonsmokers (unpublished observations). These finding are in agreement with the experimental findings that rats exposed to cigarette smoke for 30 days increased the content of 8oxodG and decreased glutathione levels in all tissues analyszd (Park et al. 1998) and that L-buthionine sulfoximine (BSO) treatment, which depletes glutathione levels, led to a further increase in liver and lung 8oxodG levels. Together with our findings, there is a clear indication that tobacco smoke induces oxidative stress in the lungs but also in other tissues, if not all tissues. In lung cancer patients, the 8oxodG antibody-based assay indicated higher excretion of 8oxodG (Erhola et al. 1997).

Also, urinary 5-(hydroxymethyl)uracil has been reported increased in smokers (Bianchini et al. 1998); however, only when given per excreted creatinine, and only an increase of about 10%. The corresponding nucleoside was also measured in this study, but levels were close to the detection limit. Interestingly, the urinary excretion of 5-(hydroxymethyl)uracil appears higher than other oxidative modifications such as 8oxodG and thymine glycol. A later study from the same group did not find a difference in 5-(hydroxymethyl)uracil excretion, but this time they found a 16% higher excretion of 8oxodG in smokers versus nonsmokers (Pourcelot et al. 1999). A more recent study measured three nucleic acid oxidation products (Harman et al. 2003). In a reasonably sized study, no difference between smokers, ex-smokers, and never smokers could be found with regard to excretion of 8oxodG and the corresponding base 8oxoG, whereas the 5-(hydroxymethyl)uracil was found increased just as in the study mentioned above, however, with a 55% increase. Interestingly, the excretion of the modified base 8oxoG did not differ between never smokers and smokers. In the Malmö Diet and Cancer Cohort (Wallstrom et al. 2003), plasma autoantibodies against 5-hydroxymethyl-2'-deoxyuridine were higher in the smokers lacking glutathione *S*-transferase M1 activity and in

the persons with high alcohol consumption. In urban bus drivers, the urinary 8oxodG excretion rate was higher than in rural bus drivers, regardless of exposure to environmental tobacco smoke and smoking (Loft et al. 1999).

16.4 Analysis of Oxidative DNA Lesions

The analysis of oxidative DNA lesions falls in two distinct categories, specific chemical analysis and unspecific analysis.

16.4.1 Specific Chemical Analysis

This type of analysis is based on a combination of chromatographic separation systems coupled with more or less specific detections systems. A very comprehensive and detailed review of the subject has been published recently (Guetens et al. 2002), and also several more selected reviews are available (Cadet et al. 2004; Cooke et al. 2003; Evans et al. 2004; Halliwell and Whiteman 2004; Jaruga et al. 2001; Loft and Poulsen 1999; Poulsen et al. 2000; Ravanat et al. 1999) including in the forthcoming fourth edition of *Free Radicals in Biology and Medicine, 2006*, by Halliwell and Gutteridge (Oxford University Press).

Choosing a system of analysis depends on a variety of factors. Is the system is to be used for determination of a single or several base oxidation products, and is it to be used to measure on tissue extracts or for urine determinations? Naturally, resources such a price and infrastructure have to be considered.

If a single lesion such as 8oxodG is to be measured in tissue extracts, the system of choice is HPLC with electrochemical detection. This is the most widely used system, and it provides a high sensitivity and specificity. From a cost point of view it is also the most favorable system, and it does not require the special skill that, e.g., mass spectrometry does. HPLC with electrochemical detection can also be used to measure 8-hydroxyadenine, 5-hydroxycytosine, 5-hydroxyuracil and the corresponding 2'-deoxyribonucleosides (Guetens et al. 2002). There are, however, very few reports on simultaneously measured modifications except from the lab of Miral Dizdaroglu by gas chromatography/mass spectrometry (GC/MS), and in human samples such reports are scarce.

If a single lesion such as 8oxodG is to be measured in urine, HPLC with electrochemical detection is also a choice. It should be noted, however, that urinary measurement is a tricky business and only few laboratories have been able to analyze large series of samples. Whereas analysis on tissue extracts is done by a relatively uncomplicated system, i.e., single column with straight or gradient elution, analysis of urine requires separation on a multicolumn system with reverse phase and cation exchange columns combined with selection of relevant fractions (Kasai 2003, 2005; Loft et al. 1993) or reverse phase combined with a proprietary carbon column (Bogdanov et al. 1999). Lin et al. (2004) have reported a GC/MS method that can be used for analysis of 8oxodG in urine.

When multiple lesions are to be measured, the prevailing method used is chromatography combined with MS. Initially, GC combined with MS was extensively used. For analysis of tissue extracts, derivatization at high temperature has been used to make the modified bases volatile and thereby suitable for GC. This can introduce artificial oxida-

tion of nonmodified bases. Since nonmodified bases are in concentrations that are about a million times higher than the oxidized, even a minor artificial oxidation can produce erroneous results. For this reason GC with MS, if used, requires modifications of the derivatization process to reduce or eliminate such artifacts, or one can utilize the progress in the coupling between liquid chromatography (LC) and MS and thereby avoid such cumbersome procedures. It is also possible to use GC/MS if the samples are preseparated by liquid chromatography (Gackowski et al. 2003; Rozalski et al. 2004, 2005).

LC coupled with MS (LC-MS) (Dizdaroglu et al. 2001, 2002; Jaruga et al. 2001) or with tandem MS (LC-MS/MS) can be used to measure multiple oxidative modifications (Harman et al. 2003; Ravanat et al. 1998, 1999; Weimann et al. 2001, 2002), in urine as well as in tissue samples.

Whereas much emphasis has been put on the chromatography and detection systems, early investigations did not put much emphasis on the tissue sample preparation. In late 1997, a workshop was held in Scotland, where the problems in the analysis were discussed (Collins et al. 1997) and later compilation of reported values of 8oxodG showed a 5,000-fold range in nuclear estimates and a 60,000-fold range in mitochondrial DNA. It became clear subsequently that this was not correct and that a large part of the variation was because methodological problems and artificial oxidation. This prompted a group to set up a European Union Framework 5-sponsored project, where the problems were identified and a standard protocol for tissue sample preparation established (Collins et al. 2002a, b, 2004; Gedik and Collins 2004; Lunec 1998; Riis and European Standards Committee on Oxidative DNA damage [ESCODD] 2002; Spencer et al. 1996). Also, US researchers took initiative to optimize the quality of assays for the 8oxodG modification (Huang et al. 2001).

It is beyond the scope of this chapter to detail the quality control and optimization of the analysis of 8oxodG, alone or together with other modifications. Caution should be taken when reading the scientific literature on this subject, because results might be because artifacts and poor methodology rather than to real biological events. Particularly, results where the levels are high should be regarded with skepticism, i.e., levels for 8oxodG that are substantially higher than the levels of 1–10 per million dGs found by ESCODD. This applies to analysis of tissue extract levels, i.e., nuclear or mitochondrial DNA extracts. In urine measurements, the level of dG (Weimann et al. 2002) is very low, and artificial oxidation is not a problem even if using GC (Lin et al. 2004).

16.4.2 Nonspecific Analysis

16.4.2.1 The Comet Assay

The most commonly used method is single-cell gel electrophoresis, or the Comet assay. This method uses single cells on a glass slide and is based on the charge on DNA subjected to an electrophoretic field followed by DNA staining. If there is a conformational change or strand breaks in the DNA, this part will move faster in the electrophoretic field than unmodified DNA. When conditions are adjusted properly, intact cells will appear as round, stained nuclei and with increasing DNA modification, the nucleus will shrink as the DNA migrates in the field and a comet-like picture will appear. There are several ways of expressing the results, but a method where, e.g., 100 cells are scored on a damage

scale up to 5 and total cumulative scores for 100 cells as a semiquantitative measure of damage/modification gives reproducible results (Collins 2004; Collins et al. 2002b).

It is evident that strand breaks or conformational DNA changes do not equal oxidative damage, and consequently, the Comet assay cannot be taken as a specific marker for oxidative damage. In addition, the Comet assay may underestimate damage/modification if clustered, say, e.g., that all oxidation occurs on all guanosines in a segment of DNA. The method can be made more specific by the use of DNA repair enzymes that will nick DNA at sites with a certain modification, and measuring with and without incubation with such enzymes, e.g., 8-oxoguanine DNA *N*-glycosylase (hOGG1) (Collins 2005). But this assumes that these enzymes have access to all base lesions (unlikely in chromatin) and thus may tend to underestimate.

16.4.2.2 Alkaline Elution

Alkaline elution is a technique where DNA is eluted through filters. If DNA is fragmented, it will elute earlier because of small molecular size, compared with nonfragmented DNA. Such methodology has been applied to oxidative damage (Osterod et al. 2001; Pflaum et al. 1997), and the same argument about specificity as for the Comet assay can be done. We have only been able to find a single report relating to tobacco smoke and oxidative DNA modification using this method. Human lung cells exposed to smoke in buffered saline showed strand breaks that were abolished by catalase (Fielding et al. 1989; Mukherjee et al. 2004).

16.4.2.3 Immunological-Based Methods

Several commercial enzyme-linked immunosorbent assay (ELISA)-based assays have been marketed, and some investigators have produced similar assays. As of today there has not been an assay developed based on immunological methods that has shown sufficient specificity. Testing out an assay, Poulsen et al. found both lack of specificity and sensitivity (Prieme et al. 1996), and recently we tested a newer commercially available assay on different HPLC fractions of urine samples and found that several of the eluted fractions other than 8oxodG reacted in the ELISA kit (unpublished data). Comparison between an ELISA method and HPLC-electrochemical detection (ECD) showed that for quantification HPLC, clean up was necessary (Shimoi et al. 2002). Nevertheless, this paper has been quoted for a demonstration that there is agreement between the HPLC-ECD and ELISA measurements. We have doubt about what the ELISA kit measures besides 8oxdG. It has been suggested that oligonucleotides in urine containing 8oxodG are comeasured by ELISA; however, we demonstrated that such oligonucleotides do not exist in urine at measurable concentrations. We believe that it is not possible to make an antibody that is sufficiently specific for detection of 8oxodG because of other unknown substances in urine. Consequently, data based on such ELISA methods should be interpreted with caution. Antibodies can be very useful for upconcentrating samples.

16.5 Planning In Vivo Experiments to Investigate Oxidative DNA Lesions: Design Considerations

According to the design used scientific evidence can be graded for quality (Concato et al. 2000) into five groups:

- Grade 1: Evidence obtained from at least one properly randomized, controlled trial
- Grade 2-1: Evidence obtained from well-designed controlled trials without randomization
- Grade 2-2: Evidence obtained from well-designed cohort or case–control analytical studies, preferably from more than one centre or research group
- Grade 2-3: Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.
- Grade 3: Opinions of respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees

Taking starting point in the studies cited in Sects. 16.4.2.2 and 16.4.2.3, it is evident that the studies belong to grade 2-2 or lower. The only study with quality grade 1 is that of Prieme et al. (1998b), who used a design where smokers were randomized to continued smoking, later followed by a smoking cessation program, or immediately entering a smoking cessation program. This design is the ethically acceptable alternative to randomize nonsmokers to smoking or nonsmoking. Evidence from comparing cases and controls most often overestimates the effects (Kunz and Oxman 1998), and indeed, when we compare our cohort study (Loft et al. 1992) with the randomized intervention study (Prieme et al. 1998b), we find a 2- to 3-fold difference in the effect of smoking.

Although we have not performed a complete survey of studies on oxidative DNA markers and smoking with regard to quality, it is clear that most researchers use a design that is inferior regarding quality of design.

Regarding the total evidence available, however, it is quite clear, that the single grade 1 quality design and several grade 2-2 and grade 3 quality studies performed provides strong evidence that tobacco smoking induces oxidative modification to DNA

16.6 Some Aspects of DNA Repair of Oxidative Modifications

Whereas focus has been on the modification to DNA, it is becoming increasingly clear that DNA repair processes may have equal importance. Repair of oxidative modification in DNA is extensive, and individual differences in DNA repair are proposed to be important for development of cancer and premature aging (Hoeijmakers 2001a, b).

There are a large number of enzyme systems that can recognize oxidative DNA modifications and start a multistep process of repair that seems important for the modulation of oxidative mutagenesis and carcinogenesis (Nohmi et al. 2005).

The human homologue of the MutT protein (hMTH1) enzyme hydrolyses 8oxodGTP and prevent its incorporation into DNA. In lung cancer tissue, the activity of hMTH1 is lower than in normal lung tissue (Speina et al. 2005), and expression, i.e., mRNA levels, in lung cancer cells parallels cellular levels of 8oxodG (Kennedy et al. 1998).

hOGG1 is the initial step in recognition and incision of the 8oxodG lesion and in a case control study, the activity of hOGG1 was lower in peripheral lymphocytes from lung cancer patients than in those from controls (Paz-Elizur et al. 2003). hOGG1 shows a single nucleotide polymorphism (SNP), Ser326Cys, that gives relative risk (RR) of 5.8 in women and 2.0 in men for developing lung cancer in smokers with occupational exposure to smoky coal (Lan et al. 2004), as also observed inanother study where an odds ratio (OR) of 2.1 was found (Le Marchand et al. 2002). Lung tumors with loss of heterozygosity at loci associated with *hOGG1* and the glutathione peroxidase 1 (*GXP1*) genes had about double levels of 8oxodG in nuclear DNA (Hardie et al. 2000).

The xeroderma pigmentosa type a (XPA) protein is involved in nucleotide excision repair and a SNP A-G in the 5' non–coding region of the *XPA* gene and one or two G alleles is associated with a reduced lung cancer risk from smoking (Wu et al. 2003).

In a recent study where several polymorphisms was studied simultaneously, including the repair genes *XRCC1* (one polymorphism) and *ERCC2* (two polymorphisms), their interaction with smoking was studied, and the most striking finding was that the adjusted ORs for lung cancer of individuals carrying five or six variant alleles was 0.3, whereas it was 5.2 for the wild-type alleles/nonsmokers (Zhou et al. 2003).

The *XRCC1* has a SNP, Arg194Trp, and this allele also seems to lower the risk of lung cancer, RR 0.4 in cases with high serum retinol values (Ratnasinghe et al. 2003); however, a SNP, Arg399Gln, gave an OR of 1.4 for smokers developing bladder cancer (Kelsey et al. 2004).

DNA ligases also plays a role in DNA repair, but a SNP (A to C) in exon 6 of DNA ligase I (*LIG1*), seems not related to a changed risk of lung cancer (Shen et al. 2002).

16.7 How Important Are Oxidative DNA Lesions for the Causal Relation between Cigarette Smoke and Development of Cancer and Other Diseases?

The bulk of evidence indicates that tobacco smoking and exposure to environmental smoke lead to increased levels of oxidative modifications in DNA and to increased excretion of repair products into urine. As argued above, urinary excretion does not reflect repair but the total "stress-burden," i.e., the rate of oxidation of DNA and its precursors, and therefore smoking cessation reduces this oxidative stress to DNA. The lesions have been shown to be promutagenic, and it can therefore be concluded that tobacco smoking leads to increased promutagenic oxidative lesions in DNA, as discussed by Halliwell (2002).

The quality of the evidence is strong, and some of the studies demonstrating the oxidative stress to DNA in humans are of the highest grade of scientific evidence, as detailed above.

The subsequent question is: How important is this increased oxidative stress in the development of tobacco related diseases? In this aspect, oxidative DNA modification by mechanistic implications relates mostly to cancer development. Several facts are important to realize. (1) The oxidative DNA modifications are not specific for tobacco exposure; rather, tobacco smoke exposure increases preexisting oxidative modifications. (2) Tobacco smoke contains many chemicals that may modify DNA and form premutagenic lesions, or other types of disease-relevant DNA modifications. (3) The locations within

DNA of the oxidative modifications are not known, and location may be very important in disease processes. (4) The relative contribution of oxidative DNA modifications to the overall number of mutational events in DNA is not known, and the number of mutational spots in DNA from oxidative modification may represent a large or small fraction of other mutations/modifications and therefore also a minor or large contribution to disease. (5) Other endogenous and/or exogenous factors may be important for development of cancer.

Based on the data available as of today, it is clear that oxidative stress to DNA can be an important initial part of the pathogenesis of tobacco-related cancer development as well as in the later stages after malignant clones has been formed. Whereas there is a clear biological plausibility that oxidative stress could be important, it is important to make quantitative estimations of the importance of oxidative stress among all other biological possible mechanisms. One way of doing this is from trials or epidemiological studies where the relative risk of cancer risk from measures of, e.g., high or low oxidative stress to DNA are estimated; such studies are presently not available, but are under way. Even if such data reveal a low cancer risk from oxidative stress, this will not rule out its importance. It could very well be that oxidative stress to DNA in combination with other factors may be very important. Such factors could be DNA repair activity, inflammatory response, baseline DNA damage in nonsmokers, and so forth. Clearly, there is continued need for considering oxidative stress to DNA in the conquest for deciphering the mechanisms of tobacco-related diseases, particularly cancer.

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