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Forum Review

Oxidative DNA Damage and Human Cancer: Need for Cohort Studies

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ABSTRACT

Research of the role of oxidative DNA damage is well established in experimental carcinogenesis. A large number of human studies on biomarkers of oxidative DNA damage, in particular related to guanine oxidation, have been published. The level of oxidative DNA damage and repair activity can be quite different between tumor and normal tissues; case-control studies have shown increased levels of oxidative DNA damage and decreased repair capacity in leukocytes from cases. Similarly, the urinary biomarkers of oxidative DNA damage may be elevated in patients with cancer. However, such studies are likely to be associated with reverse causality. Case-control studies of genetic polymorphisms in DNA repair enzymes suggest that the common variant Ser326Cys in *OGG1* may be a risk factor for lung cancer, whereas a rare variant in *OGG1* and germ line mutations in the corresponding mismatch repair gene *MYH* are risk factors for hereditary colon cancer. Cohort studies are required to provide evidence that a high level of oxidative DNA damage implies a high risk of cancer. However, this represents a real challenge considering the large number of subjects and long follow-up time required with likely spurious oxidation of DNA during collection, assay and/or storage of samples. *Antioxid. Redox Signal.* 8, 1021–1031.

INTRODUCTION

THE CELLS OF THE HUMAN BODY are continuously exposed to reactive oxygen species from their own mitochondrial respiration, inflammation, radiation, reactions catalyzed by transition metals, metabolism of foreign compounds and other factors, despite a complex network of antioxidant enzymes and scavenger molecules (79). Oxidative damage to DNA is continuously ongoing but is also repaired with high efficiency in the cells in the body (7). Oxidative modifications of DNA are abundant and mutagenic lesions thought to be important in carcinogenesis, as supported by experimental studies in animals and *in vitro* (22, 40). However, the role of oxidative DNA damage in human carcinogenesis has yet to be firmly established in prospective cohort studies.

The mechanistic pathway from exposure and oxidative stress to disease can be investigated by relevant measurements in biological samples, commonly referred to as biomarkers. These can be used to assess exposure, early biological effects, and susceptibility related to the process of carcinogenesis, including oxidative DNA damage (Fig. 1). The biomarkers of exposure and biological effects include measurement of oxidative DNA damage in cells and nucleotide oxidation products in bodily fluids, whereas susceptibility markers describe, for example, antioxidant and DNA repair capacity. Early biological effects include irreparable damage, such as chromosomal aberrations and micronuclei, which lack specificity and can be caused by other factors than oxidative DNA damage.

Validation of biomarkers in this respect includes four steps (89). The first step involves description of dose–response relationships and association with mutations and/or carcinogenesis in experimental systems, which is well documented for several biomarkers of oxidative DNA damage

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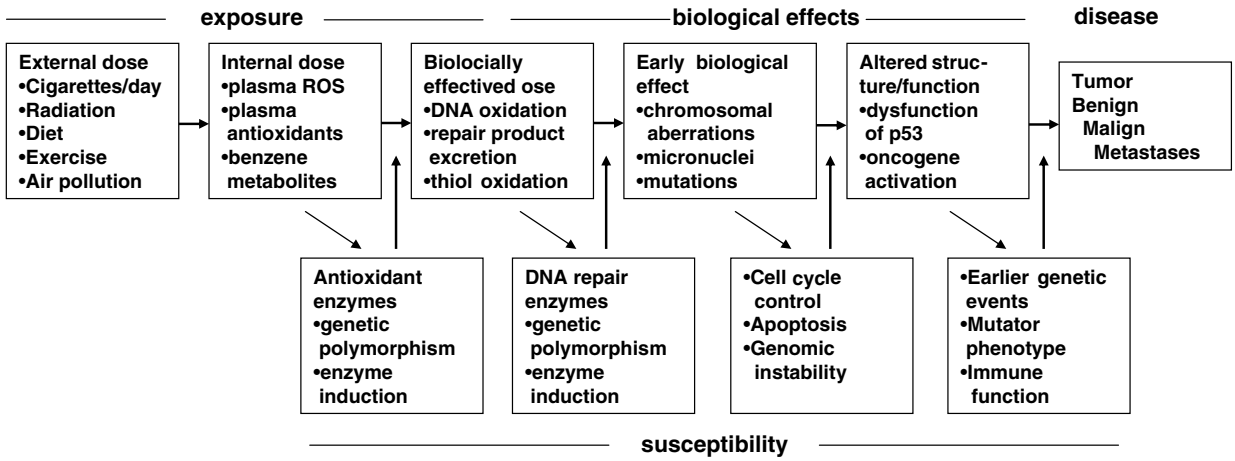

FIG. 1. The causal and mechanistic pathway from exposure to disease described by biomarkers of exposure, biological effects, and susceptibility. *Bullets* indicate examples of biomarkers at the different levels.

(22, 40). The second step of validation involves description of host factors and exposures affecting the biomarkers. This is also well established for markers of oxidative DNA damage in many biomonitoring and dietary antioxidant intervention studies. The third step documents the association between the biomarker and the disease. A number of case-control studies in this area are severely hampered by the problem that the biomarkers of exposure and early biological effects may be affected by the disease (63). Thus, proof of the role of oxidative DNA damage in human cancer requires prospective studies demonstrating that a high level of oxidative DNA damage is associated with an increased risk of developing cancer. Such studies are difficult because very large numbers of subjects are necessary and the DNA in cells is prone to spurious oxidation during storage. In contrast, urinary biomarkers of nucleotide damage are stable during storage, allowing prospective studies with nested in cohort case-control designs. Support may also come from similar designs involving biomarkers of antioxidant and/or DNA repair capacity, which are stable during storage, and the use of genetic variation in relation to the involved enzymes. The fourth step of validation involves the use of the biomarker in risk assessment and prevention for public health benefits. The present review focuses on the present state of epidemiological validation of biomarkers of oxidative DNA damage and evidence for association with the risk of cancer.

BIOMARKERS OF OXIDATIVE DNA DAMAGE AND REPAIR

Biomarkers of DNA damage

Oxidative damage to DNA can involve any base or sugar moiety and strand breaks, and a very large number of possible lesions have been described (19). Among the many specific oxidative DNA damage products, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is the most studied because of the relative ease of measurement and the mutagenic properties resulting in G–T transversion mutations on replication of DNA containing 8-oxodG (22, 40). The major problem related to measurement of oxidized bases in DNA relates to spurious oxidation occurring during sample preparation, where DNA extraction is a critical issue and the derivatization required for GC/MS analysis is particularly problematic, as described by the European Standards Committee on Oxidative DNA Damage (ESCODD) (21, 30). The comet assay detects DNA strand breaks and abasic sites, whereas it detects base oxidations with the use of repair enzymes, such as formamidopyrimidine glycosylase or endonuclease III, which nick DNA at oxidized purines and pyrimidines, respectively. It is promoted for detection of these lesions with minimum risk of spurious oxidation (15, 21, 30).

A series of repair systems are working in concert for the defence against 8-oxodG in DNA (Fig. 2). Oxidized guanine in DNA is mainly repaired by oxoguanine glycosylase (OGG1) removing 8-oxoguanine (8-oxoGua) opposite cytosine in the DNA strand (1, 8, 61). In addition, repair of 8-oxodG may to some extent occur by nucleotide excision repair, nucleotide incision, and transcription coupled mechanisms (22). A specialized enzyme (MTH1 or NUDT1) sanitizes the nucleotide pool by cleaving phosphates of 8-oxodGTP that, if incorporated during DNA synthesis, is highly mutagenic; mice deficient in that enzyme develop tumors (91). MYH is a base excision repair protein removing adenine misinserted opposite oxidized guanine and working in concert with MTH1 (14, 97).

The urinary excretion of products of damaged nucleotides in cellular pools or in DNA may be important biomarkers of exposure to relevant carcinogens and may predict cancer risk. Among the many oxidative damage products, 8-oxodG is also

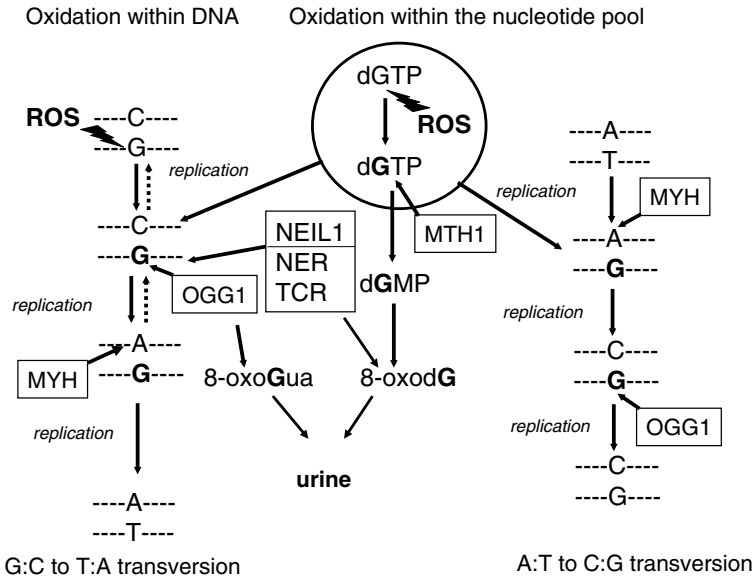

FIG. 2. Oxidation of guanine in DNA and the nucleotide pool with consequences in terms of potential mutations as well as the involved repair pathways. NER, nucleotide excision repair; TCR, transcription coupled repair. **Bold G** indicates oxidized guanine.

the most studied in urine with assays based on HPLC–EC, CE–EC, GC–MS, HPLC–MS/MS and ELISA (18). Oxidative base damage products, including thymine glycol, thymidine glycol, 5-hydroxymethyluracil, 8-oxoGua, 8-oxoadenosine, 8-oxodeoxyadenosine, as well as various repair products of DNA damage secondary to lipid peroxidation, including etheno and malondialdehyde adducts to guanine, cytosine, and adenosine, have also been measured in urine (3, 11, 12, 27, 36, 38, 69, 73, 95). However, the number of studies on each of these products is limited and relationships with oxidative stress and cancer risk factors have not been thoroughly investigated.

8-OxodG from the action of MTH1 as well as from putative nucleotide excision or incision repair of the lesion in DNA and possibly from mitochondrial turnover is excreted unchanged into the urine and may serve as a biomarker of oxidative stress and oxidative damage to nucleotides and probably DNA (47). Recently, an *in vitro* study showed that 8-oxodG is excreted from cells after exposure to oxidative stress in terms of ionizing radiation (31). 8-Oxoguanine excreted in urine should come from OGG1 mediated repair, although the difference in excretion was only 26% percent between *ogg1*$^{-/-}$ and *ogg1*$^{+/+}$ mice (74). However, there are many backup repair pathways, including the NEIL1 base excision enzyme (7). Well-controlled studies have shown that DNA or nucleotides in the diet do not contribute to the excretion of 8-oxodG and 8-oxoGua (17, 27). In steady state, the urinary excretion of 8-oxodG and similar products should in principle reflect the rate of oxidative damage, whereas the level of lesions in DNA from target cells or surrogate cells such as white blood cells should reflect the balance between damage and repair (47). Nevertheless, a study with more than 100 subjects showed no correlation on an individual level between the levels of 8-oxodG in leukocytes and the excretion of 8-oxodG and 8-oxoGua measured by chromatographic methods (24). The correlation between urinary excretion of 8-oxodG measured by ELISA and 8-oxodG or oxidized purines assessed by the comet assay in lymphocytes found among 8 healthy subjects (29) is questionable due lack of specificity of, in particular early versions, the ELISA assays (47, 78).

Biomarkers of DNA repair capacity

A number of biomarkers and assays have been developed for the assessment of the capacity for repair of oxidative DNA damage. The capacity for nicking of labeled oligonucleotides with, for example, oxidized bases or ethenoadducts by extracts of cells or tissues is measured relatively easily (34, 84). Similarly, cell extracts can be applied on nucleoids with a defined level of, for example, 8-oxodG induced by a photosensitizer and nicking assessed by the comet assay (16). In principle, the repair capacity of intact cells would be preferred and may be assessed by monitoring disappearance of base damage after a defined oxidative stress, by radiation, hydrogen peroxide, a photosensitizer and light, or sodium dichromate (45). However, a problem in interpretation of such data for freshly isolated lymphocytes is the ongoing oxidative stress and hence DNA damage occurring during incubation at suddenly increased oxygen tension (90).

The *OGG1* gene has a common genetic polymorphism with a variant Ser326Cys, which in complementation assays *in vitro* appears to increase susceptibility to mutagenic properties of reactive oxygen species considerably, whereas 8-oxodG levels and incision activity in leukocytes and some target tissues generally show no difference between the genotypes as recently reviewed (96). However, a more recent study found higher levels of oxidized guanine in human lymphocyte DNA from Cys326 homozygous subjects as compared with heterozygous or homozygous wild-type subjects after *ex vivo* treatment with sodium dichromate (45). Differences in effects of the polymorphism in extracts and intact cells may be due to disruption of dynamic relocalization of *OGG1* during the cell

cycle in cells harboring the Cys326 variant (50). Although *OGG1* has been considered a housekeeping gene, a number of *in vitro* and *in vivo* studies have shown that it may be upregulated at the mRNA level by oxidative stress (70, 71). The *OGG1* mRNA level in blood cells has also been shown to be upregulated in humans exposed to sunlight and cooking oil (13, 93). So far, no biomarker is available for other base excision enzymes that can excise 8-oxoguanine (7).

MTH1 is also important for the level of 8-oxodG (91), although even less is known of its regulation. The activity can be measured by formation of the monophosphate from 8-oxodGTP (6).

It is likely that people with low repair capacity have higher lifetime cancer risk, although that needs confirmation from epidemiological studies. The actual DNA repair capacity may be a consequence of genetic polymorphisms, possible in promoter regions, and in other genes, as well as modifying effects of environmental exposure and style factors.

FACTORS AFFECTING BIOMARKERS OF OXIDATIVE DNA DAMAGE IN HUMANS

Environmental and lifestyle factors have been extensively studied for effects on biomarkers of oxidative DNA damage, and exhaustive review will not be attempted here. A number of studies have found the expected higher levels of 8-oxodG and other oxidative bases or strand breaks in leukocyte DNA from smokers as compared with nonsmokers, although this is far from consistent (10, 28, 47, 52). Inconsistencies may be due to possible induction of OGG1 activity by smoking (28, 34). Exposure to ambient air particles and benzene has consistently been associated with high levels of 8-oxodG in lymphocytes (2, 81, 82, 92). Many occupational exposures have been shown to increase the level of strand breaks in leukocytes (52). Attempts to reduce the level of oxidative DNA damage in leukocytes by means of antioxidant supplements in well-nourished people have to a large extent been negative, whereas some studies with antioxidant-rich food show positive effects (53, 54). Exercise may also modulate oxidative DNA damage, such that strenuous activity, for example, at hypoxic conditions, can increase the levels (55, 58), whereas moderate daily exercise has been shown to increase MTH1 mRNA expression and reduce levels of 8-oxodG in leukocytes (76).

The urinary excretion of 8-oxodG and 8-oxoGua has relatively consistently been found to be increased among smokers (28, 47, 49). Heavy exposure to air pollution in occupational settings in terms of diesel exhaust, polyaromatic hydrocarbons, and benzene has been associated with increased 8-oxodG excretion (49, 59, 60), whereas nonoccupational exposure to ambient air pollution was not associated with 8-oxodG excretion (43, 81). Exercise of very high intensity (marathon running or in high altitude) has been associated with increased 8-oxodG excretion (55, 66, 67). Cancer treatment with radiation and/or chemotherapy may also increase 8-oxodG excretion (4, 32). A number of studies have investigated potential effects of diet and antioxidants, including interventions on urinary 8-oxodG excretion, showing generally limited effect (48, 56).

ASSOCIATIONS BETWEEN BIOMARKERS OF OXIDATIVE DNA DAMAGE AND CANCER IN HUMAN

Case-control studies of DNA damage and repair in cancer tissue

A number of studies have compared the level of oxidative DNA damage in tumor tissue with the levels in the surrounding tissues or in similar tissue from patients without cancer. As summarized in a recent review, such studies show that the level of oxidative DNA base damage may be elevated in tumor tissue involving the skin, breast, colon/rectum, cervix, stomach, liver, and kidney, whereas three studies on lung cancer tissue are conflicting, and there was no increase in one study on larynx cancer (22, 83). These studies are small and some suffer from rather high levels of oxidized DNA bases, probably due to the use of outdated methodology with improper control of spurious DNA damage during sample preparation. A recent study showed that DNA strand breaks measured by the comet assay were increased in Barretts' esophagus and the risk of adenocarcinoma was increased among the patients with highest level of damage (64).

DNA repair capacity has also been studied in tumor tissues. In seven lung cancer patients, OGG1 activity was closely correlated between lung tissue and lymphocytes, in which low levels of activity was shown to be a risk factor for lung cancer in a larger set of 68 cases and 68 controls (65). The OGG1 activity was also found to be lower in lung tumor tissue compared with the surrounding tissue from 56 patients (83). In contrast, a small study with 8 patients showed higher OGG1 activity in lung tumor tissue, whereas the activity of NTH1, which excises oxidized pyrimidines from DNA, was lower in tumor as compared with surrounding tissue (67a). The 8-oxodGTPase activity by MTH1 was higher in tumor than in normal lung tissue from 33 patients (83). Interestingly, for both OGG1 and MTH1 the activities correlated between tumor and normal tissues within the same individual, whereas none of the activities correlated with the level of 8-oxodG in the DNA on an individual basis (83). The capacity for repair of DNA damage secondary to lipid oxidation in terms of adenine and cytosine etheno adducts assessed by nicking assays was not different between tumor and surrounding tissue, although adenocarcinoma tissue had lower activity than squamous cell carcinoma (84).

Apparently, the level of oxidative DNA damage and repair activity can be quite different between tumor and normal tissues. However, it is highly likely that the tumor process with possible inflammation, poor perfusion, changes in signalling pathways, repair and other defense mechanisms *per se* can induce oxidative stress and subsequently increase the level DNA damage. Thus, these findings are not proof of involvement of oxidative DNA damage in the initiation of cancer (63). Nevertheless, the combination of high MTH1 activity, low OGG1 activity, and low 8-oxodG levels in tumor tissue may suggest that oxidation in the nucleotide pool is more important than oxidation within the DNA as source of 8-oxodG in DNA also in humans (83).

Case-control studies of DNA damage repair in white blood cells

A number of studies have compared the level of oxidative DNA damage and repair capacity biomarkers between white blood cells from patients with cancers and control subjects. These studies have in general been small and just compared levels of biomarker between cases and controls, who have frequently been selected for convenience (5). Moreover, in epidemiological studies, the risk associated with a factor should be expressed as a relative risk, usually estimated as an odds ratio or incidence rate ratio. In this respect, the dose–response relationship describing the risk associated with increasing level of biomarker in categories or as a continuous variable should also be assessed.

Several studies have found increased levels of 8-oxodG in white blood cells from patients with cancer of the lung (26, 28, 94), colon (25), and esophagus/cardia (10), compared with healthy subjects. A number of case-control studies report DNA strand breaks measured by the comet assay on lymphocytes of which only two with report their findings as odds ratios. These two studies showed an increased odds ratio for bladder cancer (77) and breast cancer (80) associated with a high level of SB.

A large number of studies have compared the ability to repair oxidative DNA damage induced in lymphocytes by radiation or hydrogen peroxide after isolation from blood between cancer patients and controls. The use of such tests has relatively consistently shown lower repair capacity among patients with many types of cancers (5, 57, 68, 98). Similarly, the repair activity for 8-oxodG in DNA by nicking assays with leukocyte extract was reduced in two studies with lung cancer patients compared with controls (28, 65). Moreover, the capacity for repair of DNA damage in terms of adenine and cytosine etheno adducts assessed by nicking assays was reduced in leukocytes from lung cancer patients compared to controls (84). For prostate cancer the mRNA expression of *MTH1* and *OGG1* after phytohemagglutinin stimulation was higher in lymphocytes from cases than from controls, although a significantly increased odds ratio was only found for *MTH1* and the there was no difference for the *MYH* expression (46).

The case-control studies of biomarkers of oxidative DNA and repair in leukocytes point rather consistently at increased levels of damage and decreased repair capacity in cases, although few of the studies have proper epidemiological design and reporting. However, it should be stressed that biomarker-based case-control studies are likely to be associated with reverse causality, in particular with respect to oxidative stress and other alteration, which can easily be induced by cancer and thus influence both the level of DNA damage and repair (63).

Case-control studies of urinary biomarkers of oxidative DNA damage

A number of small studies have assessed the excretion of biomarkers of oxidative DNA damage among cancer patients compared with healthy controls (22), although none reported results as odds or incidence rate ratio of disease associated with the level of excretion. Urinary excretion of 8-oxodG has been found elevated among patients with gynecological cancers (99), lung cancer in one study but not in another (20, 28), prostate cancer (51), or other various cancers (85). Urinary excretion of 8-oxoGua was 50% higher in 42 cancer patients with bone metastasis than in 38 healthy controls (72), whereas there was no difference in excretion between 51 patients with lung cancer and healthy controls when smoking was taken into account (28). The urinary excretion of thymine glycol and thymidine glycol was higher in 20 cancer patients compared with 10 healthy controls (11).

Accordingly, the urinary biomarkers of oxidative DNA damage may be elevated in patients with cancer. However, this could very well be a consequence of the disease, in particular in advanced stages, with ongoing oxidative stress, inflammation, and tissue turnover (63). It should also be noted that the urinary excretion of these products represent contributions from the whole body.

Genetic polymorphisms of DNA repair enzymes

Differences in risk for cancer between subjects with genetically determined differences in repair of oxidative DNA damage would support the importance of this in carcinogenesis. The disease would not be expected to affect the measurement of genotypes, and bias in this respect is not likely to occur in case-control studies.

The Ser326Cys polymorphism of *OGG1* has inconsistently been associated with risk of lung cancer as recently reviewed with odds ratios above unity for subjects with two mutant alleles in five studies, of which two were statistically significant, and two studies showing odds ratios below unity (96). A new study included >2,000 cases and showed an overall odds ratios of 1.34 for the mutant genotype that was close to statistical significance (39), whereas an increased risk of adenocarcinoma in the lung was significant and robust. No formal meta-analysis has been published. While the published studies do not indicate an interaction between the *OGG1* genotype and smoking with respect to lung cancer (39, 96), a single study suggests that the Cys326 variant implies increased susceptibility to polyaromatic hydrocarbon-rich coal combustion emissions (44). As recently reviewed and with a few supplementary studies, the Ser326Cys polymorphisms of *OGG1* do not appear to be associated with the risk of breast, colon, stomach, squamous cell head and neck and cervical cancer (35, 62, 96). The review also list single small studies showing an elevated risk of esophageal, nasopharyngeal, and orolaryngeal cancers, and inverse risk of prostate cancer associated with the Ser326Cys variant, whereas other variants of *OGG1* were associated with increased risk of prostate cancer (96). Accordingly, the risk associated with the Cys326 genotype of *OGG1* is limited, even for lung cancer. This may be due to a limited effect on the activity and the presence of multiple alternative pathways as described above.

For some forms of colorectal cancer, there is other genetically based support for the involvement of guanine oxidation in the pathogenesis. The mismatch repair gene *MYH* show biallelic mutations in a subset of familiar adenomatous polyposis with late onset (14, 97). *MYH* is a base excision repair protein removing adenine misinserted opposite oxidized gua-

nine and working in concert with MTH1 and OGG1 (14). Even heterozygosity for *MYH* appears to convey risk for colon cancer (23). A rare R154H variant of *OGG1* was recently identified among patients with hereditary sporadic colorectal cancer and was found to be a possible risk factor for sporadic cancers (41). *MTH1* has also been investigated for mutations in relation to hereditary colon cancers, finding very few variants (41) There is some suggestion that the Ser326Cys variant of *OGG1* may be potential effect modifier with respect to meat intake and smoking and risk of colon cancer (23, 42).

Cohort studies of oxidative DNA damage and cancer risk

Prospective studies demonstrating that a high level of oxidative DNA damage is associated with an increased risk of developing cancer in the future are required to provide convincing proof of the role of oxidative DNA damage in human cancer. Such studies are difficult because cohorts of very large numbers of subjects are necessary to accrue a sufficient number of cancer cases during follow up (Fig. 3). At present the large number of subjects preclude that oxidative DNA damage is measured with the present time-consuming assays in suitable cells at entry, whereas a nested approach with analysis of only samples from cases and a comparable disease-free control group from the cohort is feasible in principle. However, that approach requires that the cells (e.g., lymphocytes) are carefully isolated at entry and preserved in special media during storage at very low temperature to reduce the risk of spurious oxidation of the DNA (21, 30). The comet assay with detection of oxidative base damage by means of repair enzymes may be suited for such an approach, although it has not yet been tested in a large-scale long-term setting. Another and possibly more feasible approach would be to assess the capacity for repair of oxidative DNA damage in terms of expression, protein levels, and/or activity in stored samples in a nested in cohort design. For these approaches the relevance of lymphocytes in relation to target organs can also be discussed. In a large cohort study it is unlikely that target tissue can be sampled. However, cohort studies of other biomarkers related to DNA damage, including chromosomal aberrations, micronuclei, and polyaromatic hydrocarbon adducts in lymphocytes or total white blood cells have shown that high levels predict the risk of cancer (9, 33, 87).

In contrast to cellular DNA, urinary biomarkers of nucleotide damage are stable during storage, allowing prospective studies with nested in cohort case-control designs. So far, no published study has assessed the urinary excretion of oxidized nucleotides or corresponding bases as predictors of cancer in a prospective setting. In collaborative studies including the authors' laboratory, several of such studies involving a cohort of more than 57,000 subjects are ongoing.

There is one published cohort study association between cancer risk and possible secondary oxidative damage to DNA in terms of the exocyclic adduct ethenodeoxyadenine (37). In a cohort study of 1,956 men, 47 cases of all cancers were identified after 12–17 years follow up. In a nested case-control design, no difference in urinary excretion of the repair product ethenodeoxyadenosine at the entry into the cohort was found between the cases and 31 cancer-free smoking matched control subjects. Obviously, this small study cannot exclude even major associations related to specific cancers. For the urinary excretion of markers oxidation of nucleotides,

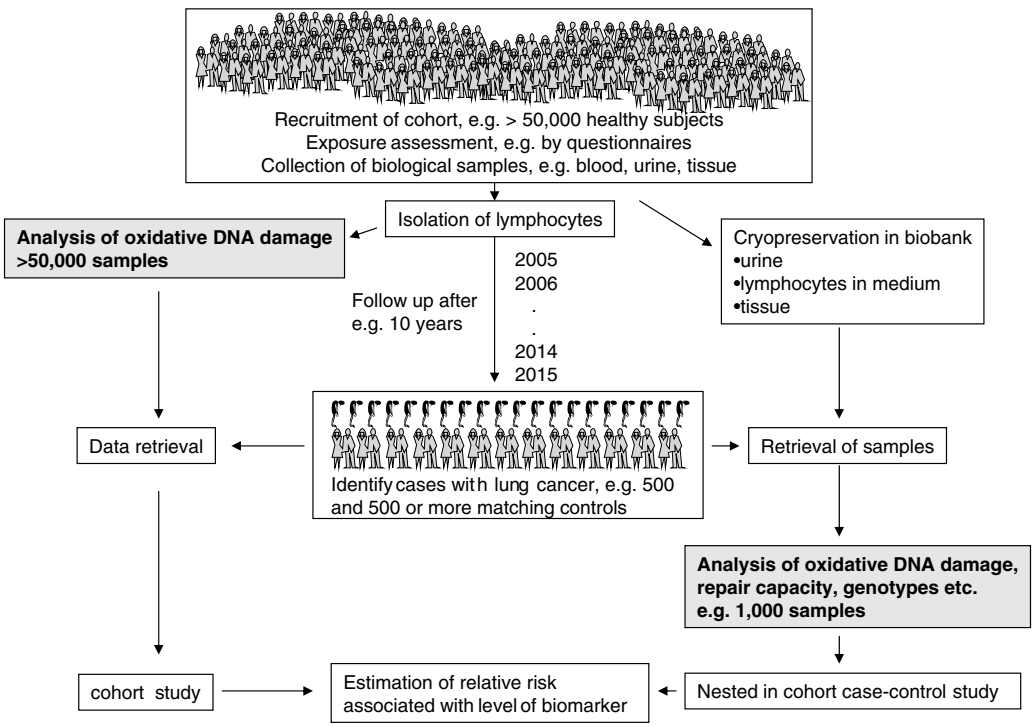

FIG. 3. Proposed design of cohort-based study of association between oxidative DNA damage and risk of cancer.

it should also be recognized that the level reflect the sum of the whole body exposure and the relevance to specific target organs can be discussed.

For cohort studies it is important that the biomarker reflects the level of damage over the long time period of possible carcinogenesis. That can be assessed in small scale studies with repeated sampling in the same subjects. For example, the urinary excretion of 8-oxodG is relatively constant within an individual showing coefficients of variations <20% over prolonged periods of time (54, 55). The long-lasting step-wise process of carcinogenesis with initiation, promotion, and progression should be considered in the interpretation of associations in cohort studies. An increased level of oxidative DNA damage may have different importance during each of these stages. The relevance of the biomarkers to the target tissues should also be considered, It should also be possible to provide some evidence for the relevance of biomarkers of oxidative DNA damage in lymphocytes and urine by comparing with target tissue levels in small scale case-control or case only studies, although the effect of the disease *per se* in that respect cannot be excluded.

The interpretation of associations between biomarkers of oxidative DNA damage and the risk of cancer in epidemiological studies relies on the understanding of possible causal pathways (88). The level of DNA damage is in principle a product of the extent of exposure to oxidative stress, antioxidant defenses, and DNA repair capacity, which can be genetically determined and modified by many factors, including diet. If DNA damage measured by a biomarker is an intermediate step between an exposure and lung cancer, an unadjusted estimate of the effect of the biomarker level on risk of lung cancer would simply express the cancer predictive value of the biomarker, whatever caused or modified the level. However, such unadjusted estimates may also be subject to confounding if the exposure (e.g., smoking increases the level of biomarker and the risk of cancer), although no association between the biomarker and lung cancer exists. If the estimate of the effect of the level biomarker on cancer risk is adjusted by available information on exposure, it would express the cancer predictive effect of the proportion of the variation in the biomarker level, which is not caused by the exposure as assessed. This would express the cancer predictive value of the biomarker beyond that of the exposure. However, such a simple confounder analysis approach cannot be used to demonstrate, for example, gene–environment interactions and may not be satisfactory (75, 86). Preferably, more complex mathematical models based on Bayesian principle and physiologically based pharmacokinetic concepts of the involved pathways should be applied (88). An advantage of such approaches is the integration of experimental knowledge allowing a better understanding of the involved mechanisms.

CONCLUSION

The role of oxidative DNA damage is well established in experimental carcinogenesis (22, 40) and biomarkers of oxidative DNA damage have been used for human studies for more than 20 years (12). While a large number of studies with such human biomarkers, in particular related to guanine oxidation, have been published, the process of validation is far from complete, and evidence that a high level of oxidative DNA damage implies a high risk of cancer is still lacking. The level of oxidative DNA damage and repair activity can be quite different between tumor and normal tissues, although this may be caused by the tumor process with possible inflammation, poor perfusion, oxidative stress, and changes in signaling pathways, repair, and other defense mechanisms.

A large number of cancer risk factors, including smoking and exposure to air pollution, have been shown to affect the level oxidative DNA damage or the repair capacity in leukocytes or the urinary excretion of repair products. Case-control studies of biomarkers of oxidative DNA and repair in leukocytes point rather consistently at increased level of damage and decreased repair capacity among cases. Similarly, the urinary biomarkers of oxidative DNA damage may be elevated in patients with cancer. However, it should be stressed that biomarker-based case-control studies are likely to be associated with reverse causality, in particular with respect to oxidative stress and other alteration that can easily be induced by cancer and thus influence both the level of DNA damage and repair (63). Case-control studies of genetic polymorphisms in DNA repair enzymes and cancer risk are not likely to be biased in that respect. The common variant Ser326Cys in *OGG1* with yet unresolved influence on the function of the protein may be a risk factor for lung cancer, whereas a rare variant in *OGG1* and germ-line mutations in the corresponding mismatch repair gene *MYH* appear to be important risk factors for hereditary colon cancer. These data certainly indicate the importance of oxidative DNA damage in some cancers and epidemiological studies combining polymorphisms, functional aspects, and level of damage in surrogate and target tissues would be highly interesting.

Cohort studies with biomarkers of oxidative DNA damage are required to provide evidence that a high level of oxidative DNA damage implies a high risk of cancer. However, this represents a real challenge, considering the large number of subjects and long follow-up time required with likely spurious oxidation of DNA during collection and/or storage of samples. To the best of the authors' knowledge, the necessary and extremely costly precautions have not been applied in the ongoing large cohort studies and few of the related biobanks include urine. Accordingly, these matters should be considered carefully when establishing new cohorts. Until they are available we can focus on biomarkers of DNA repair functions (e.g., in leukocytes) and urinary excretion of repair products in cohort studies. Fortunately, many new assays of repair capacity and oxidized DNA bases and nucleosides in urine are available. Hopefully, such studies will provide evidence and understanding of the role of oxidative DNA damage in human cancer. Ultimately, we may focus cancer prevention guided by responses in biomarkers of oxidative DNA damage.

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ABBREVIATIONS

ESCODD, European Standards Committee on Oxidative DNA Damage; MTH1, MutT homolog–8-oxodTPase; MYH,

MutY homolog; OGG1, oxoguanine glycosylase; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGua, 8-oxoguanine; ROS, reactive oxygen species.

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