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Review

Sister Chromatid Exchanges (SCEs), a Biomarker for DNA Damage in Molecular Epidemiology : Methodology and Applications

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INTRODUCTION

Biomarkers are increasingly used in cancer epidemiology to estimate exposure to carcinogens or putative anticarcinogens, to measure preclinical biological effects, and to identify genetic factors that may determine individual susceptibility.[1)] Markers for DNA damage are of special interest since DNA damage is considered to be a crucial element in cancer development.[2)] The Sister Chromatid Exchange (SCE) technique is thought to be a sensitive indicator of DNA damage in mammalian cells.[3)] Thus, increased *in vivo* cytogenetic damage, as determined by SCE in human peripheral lymphocytes, may indicate an increased cancer risk. There is considerable evidence that reactive oxygen species (ROS) contribute to human tumorigenesis,[4,5)] and that antioxidants may often prevent or delay the onset of cancer[6)] ; consequently, a few intervention studies have been implemented with antioxidant supplementation to see if alteration of intakes of antioxidants can decrease SCE and perhaps the incidence of cancer. Because the application of SCE analysis to epidemiologic studies began only recently, many methodological issues require further resolution, and standardization of laboratory techniques across studies has yet to be accomplished. The present paper reviews the principles of the SCE assay and considers some of the issues governing its use in epidemiologic research, particularly in cases of interventions with antioxidant supplementation.

WHAT IS A SISTER CHROMATID EXCHANGE(SCE)?

1. Mechanism of Occurrence

SCE is a reciprocal exchange of DNA sequences at homologous loci between chromatids at the four-strand-stage during replication of chromosomal DNA (Fig 1 and 2). This molecular exchange can easily be visualized under a light microscope at the metaphase stage, following either autoradiography by incorporating ^{3}H-thymidine[7,8)] or differential staining by incorporating thymidine base analogs, 5-bromodeoxyuridine (BrdU), or 5-chlorodeoxyuridine in cells.[9)] SCE seems to be a natural pheno menon occurring only during replication of DNA, since a normal somatic cell produces a certain base level of

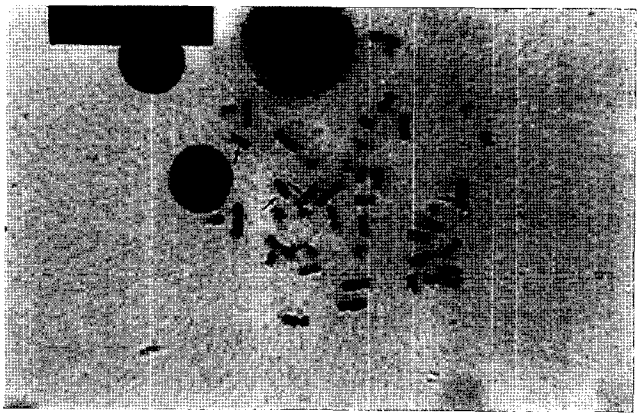

Fig 1. SCEs in human lymphocytes. Cell from a non-smoking healthy male shows 4 SCEs (arrows)

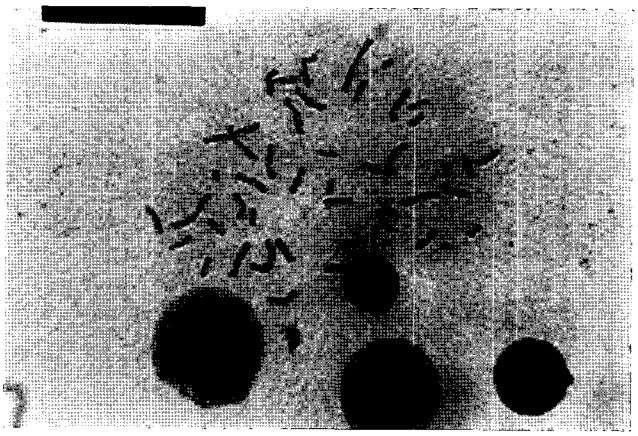

Fig 2. SCEs in human lymphocytes. Cell from a culture exposed to 120 g/ml of Ethyl Methanesulphonate (EMS, a known mutagen) shows approximately 170 SCEs

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SCEs and no SCE can be observed in cells that have not undergone DNA replication in the presence of the precursor. Although the precise molecular mechanisms involved in the exchange process are yet to be elucidated, this must involve DNA strand breakage and rejoining. It has been suggested that the cells, by forming SCEs during replication, may overcome any otherwise unrepaired DNA damage.[10] This explanation seems logical since there is a reversible and concerted DNA double-strand breakage and rejoining which occurs spontaneously during replication of DNA.[11,12] Thus it is expected that increased DNA strand breakages during the S-phase would produce increased SCEs. Although SCEs themselves do not necessarily lead to adverse health outcomes, elevated levels of SCEs apparently indicate that cells have been exposed to a mutagen.[13]

2. Sister chromatid exchange producing agents *in vitro*

A variety of physical and biological agents, including viruses, ionizing radiation, clastogens, and mutagens, can produce SCEs.[14] A wide range of chemicals can induce SCEs in mammalian cell lines.[15] Those most likely to produce SCEs *in vitro* include:

- alkylating agents
- DNA-binding agents
- DNA-base analogs
- chemicals that interfere with DNA repair or cause single-strand breaks in DNA.

In contrast, exposures that cause double-strand DNA breaks such as bleomycin, are efficient producers of chromosome breakage but not of SCEs.[16]

3. Persistence of sister chromatid exchange

The persistence of detectable SCEs depends both on the rate of DNA repair and on the normal half-life of the affected cells.[16] Experiments in rabbits demonstrated that it was possible to measure an increase in SCEs in peripheral blood lymphocytes for many months following repeated low-level exposure to chemicals *in vivo*.[17] Increased SCEs as well as persistent lesions that give rise to SCEs have been subsequently observed in cigarette smokers[18-20] and in individuals undergoing chemotherapy.[21,22] Chronic ethylene oxide exposure, on the other hand, apparently increases SCE levels for at least 1 to 2 years after exposure is reduced or ends.[23,24] These studies suggest that populations of lymphocytes with high SCE frequencies may continue to be present long after any exposure ceases. These cells may represent persistent damage in long-lived lymphocytes or a sensitive subpopulation.[25]

SISTER CHROMATID EXCHANGES (SCES) ASSAY

1. Principle

The SCE assay is a process in which one member of each chromatid pair in replicating cells is stained differently from its sister chromatid. This differential staining enables investigators to detect genetic material exchanged between sister chromatids. The assay is based on the incorporation of 5-bromodeoxyuridine (BrdU), a thymidine analog, into replicating chromosomes, so that BrdU-sensitive staining procedures allow visualization of newly formed SCEs. Equipments and reagents for SCE assay are listed in Table 1.

Table 1. Equipments and reagents for SCE assays

Equipments	Reagents
Lamina Air Flow Cabinet	Eagle's minimum essential medium (Sigma)
Centrifuge	Penicillin-Streptomycin solution (Sigma)
Suction line with collection flask and trap	$NaHCO_3$ (Sodiumbicarbonate, Sigma)
Vortex mixer	L-Glutamin (Sigma)
Microscope	Fetal bovine serum (Sigma)
37°C CO_2 Incubator	5-Bromo-2-deoxyuridine (BrdU, Sigma)
37°C Waterbath	Phytohemagglutinine (PHA, Sigma)
pH meter	Heparin (Sigma)
Automatic pipettes and tips	Colchicine (Sigma)
Sterile pipettes, disposable pipettes	Giemsa stain stock solution (Sigma)
Pasteur pipettes	Mounting medium (Canada balsam, Sigma)
Glass universal bottles	Dubeccos phosphate buffer saline (Sigma)
Culture flask	KCl (Potassium Chloride, Sigma)
Centrifuge tube	Methanol:acetic acid (3:1,v/v)
Microscope slide and coverslip	Na_2HPO_4 (Disodium phophate, Sigma)
Slide staining racks	NaOH, HCl for pH adjust
Syringe filters, Bottle top filters	Ethyl alcohol
Aluminum foil	

2. Procedure

(1) Blood sampling

Blood for lymphocyte culture is obtained by venipuncture and collected in lithium-heparinized polystyrene tubes. The cultures should be established as soon as possible after the blood is withdrawn, preferably within 24 h.

(2) Setting up cultures

Duplicate 0.8 ml volumes of whole blood are inoculated in 10 ml of Eagle's minimum essential medium (EMEM) supplemented with 100 units/ml penicillin-streptomycin solution and 2 mM of L-glutamine. Cells are washed by centrifuging at 300 g for 5 min. The supernatant is removed and the cells resuspended in 9.5 ml EMEM supplemented with 100 units/ml penicillin-streptomycin solution, 2 mM of L-glutamine and 15% v/v heat-inactivated fetal bovine serum. 0.1ml of phytohemagglutinin and 0.1 ml of lithium heparin, and 0.05 ml of 5 mM 5-bromo-2-deoxyuridine, to give 25 M, are added to each culture. The culture vessels are double-wrapped in foil and incubated at 37℃ in humidified air containing 5.0% CO_2.

(3) Harvesting cultures

The cultures are incubated for 70 hours, and then 0.05 ml of 10 g/ml colchicine is added to each culture. After 2 hours of reincubation, the cells are harvested by centrifugation at 300 g for 5 min and swollen with prewarmed KCl (0.075 M) at 37℃ for 7 min, and fixed with fresh methanol : acetic acid (3:1, v/v). The fixative is changed several times (5-6 times) until it becomes clear and then the cell suspensions are stored for at least twelve hours at -20℃ before the slides are prepared.

(4) Making slides and staining slides

The preparation is applied to the slide (up to 4 slides from each culture) by dropping from a pipette held approximately 30 cm above the slide, and then drying at room temperature. The quality of differential staining is much improved if the slide preparations are allowed to 'age' for 2-3 days before staining for SCEs. The slides are stained with 5% Giemsa solution in a freshly made Sörenson's buffer (42.6 g Na_2HPO_4 in 1L water, pH 10.4) for 12 min. Slides with glass coverslips are mounted using Canada Balsam medium.

(5) Assessment of slides

Slides are coded at random so that scorers are unaware of the origin of the slides. Twenty-five metaphases are scored per culture to determine the mean SCE frequency (50 metaphases per individual) and the number of SCEs noted on the scoresheet. Only diploid second-metaphase (M2) cells with 45-46 centromeres are scored. Every point of exchange is counted as a SCE. Exchanges at the centromere are included only when twisting at this point can be ruled out.

(6) The SCE rate

The SCE rate is expressed in three different ways : ① the mean number of baseline SCEs per cell, ② the highest five SCE means (Top5 HFC), and ③ the percentage of high frequency cells (%HFC), as follows:

① The mean number of baseline SCEs.

② The highest five SCE means (Top5 HFC) are estimated by computing the mean SCE frequency in the highest 5 cells in the SCE distribution for each individual.[26-28]

③ The percentage of high frequency cells (%HFC) for each individual is estimated using the pooled distribution of all SCE cell measurements, ranking them by their SCE frequency, and choosing a number of tolerance level based on percentiles (ex. 99, 90, 85, 70, 60%). Cells with an SCE frequency greater than the corresponding tolerance level are defined as HFCs.[29]

(7) The roles of cell culture components

In the human lymphocyte SCE procedure, a venipuncture whole blood sample is mixed with heparin to prevent clotting and is then placed in a culture medium containing nutrients, BrdU, and Phytohemagglutinin (PHA). PHA is a mitogen (mitosis-promoting agent), that stimulates division of lymphocytes (especially T-cells). Antibiotics are added to the culture, and those most commonly used are penicillin and streptomycin, which restrict bacterial infections. Because cells are scored during their second division, the culture duration should be selected to yield a high percentage of cells in their second division. A culture length of about 72 hours is recommended. Cells are obtained for analyses from cultured cells through a harvesting process. The first step in harvesting is the addition of a very dilute solution of colchicine, which binds specifically to the tubulin of spindle microtubules. Because colchicine arrests mitosis at the metaphase stage, cells in metaphase accumulate in the culture. A hypotonic solution is added to make the cells swell from the influx of water. This allows the chromosomes to disperse freely within the cell membrane, separating the paired chromatids but leaving them still attached at the centromere. The cells are then fixed, to harden the chromatin and enhance the morphology. The fixed cells are spread on slides and stained with Giemsa solution, which shows the differential incorporation of BrdU between the sister chromatids.[13,30,31]

(8) The Cell Cycle (Fig 3)

The majority of lymphocytes are in the G_0 stage of the cell cycle, i.e. with unreplicated chromosomes. Dur-

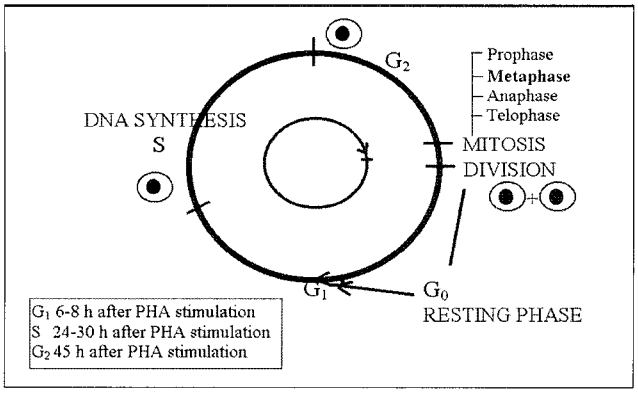

Fig 3. The cell cycle[32]

ing culturing in the presence of mitogen (PHA) cells re-enter the cell cycle (G_1, S, G_2, and mitosis). After mitosis, the new daughter cells enter a postmitotic phase during which no DNA synthesis occurs; this is the G_1 (Gap 1), resting phase. Those cells not preparing for cell division are considered to be in a subphase of G_1 called G_0. The next stage is S, the period of DNA synthesis. During the S phase, the DNA content of the cell doubles, as each DNA molecule acts as a template to make a complementary copy of itself. The S phase is followed by a second nonsynthetic period, G_2 (Gap 2). G_2 ends with the onset of mitosis (M). At the M phase, the chromosomes divide and separate, resulting in two identical daughter cells after cytoplasmic division. The complete cell cycle in cultured human cells may last 12 to 24 hours, only 1 hour of which involves mitosis.[32,33]

(9) BrdU uptake in DNA synthesis

Cells preparing to replicate enter a period of DNA synthesis called the S phase. During this phase, the DNA of chromosomes is duplicated. In the S phase under assay conditions, BrdU partially replaces thymidine, a DNA base that is one of the chromosome's normal components. During the first metaphase in culture, each sister chromatid has one newly formed BrdU-substituted DNA strand and one parent strand of normal DNA without BrdU substitution, as Fig 4 shows.

After mitosis, the double-stranded DNA of chromosomes in the two daughter cells also contains one BrdU-substituted strand and one parent strand. During DNA synthesis in the second cell division in culture, BrdU again partially replaces thymidine in the newly synthesized strands of DNA in each chromatid. Then, when

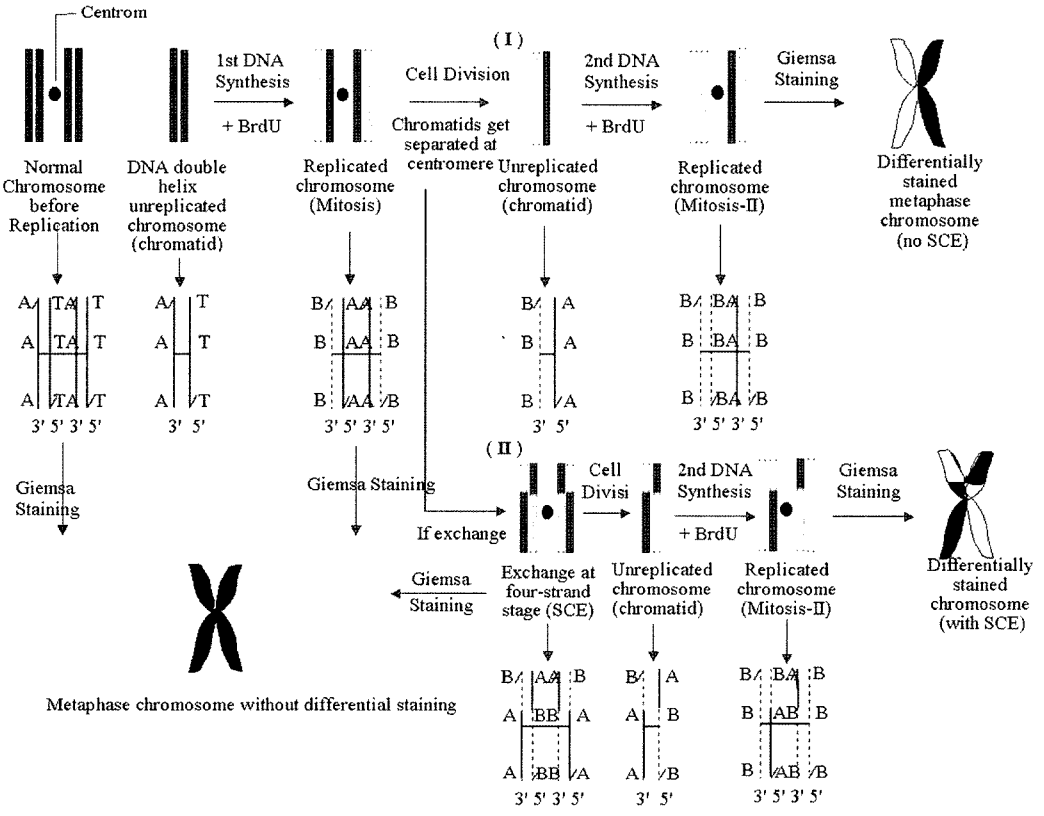

Fig 4. Sister chromatid differentiation and SCE formation as revealed by BrdU-Giemsa techniques during first and second cycle[43]

the daughter cells enter metaphase, one of the sister chromatids includes a parent strand of DNA and a BrdU-substituted strand of DNA, and the other contains two strands of BrdU-substituted DNA. The asymmetrical distribution of BrdU-substituted DNA in the second metaphase allows visualization of SCEs : the chromatid, which contains the parent DNA strand stains with a different intensity compared to the chromatid with BrdU in both strands. If an SCE has occurred, it appears as a discontinuity in the stain intensity along the chromatid.[13]

CONFOUNDING FACTORS OF SISTER CHROMATID EXCHANGES

The baseline SCE frequency in human peripheral lymphocytes averages about 7-10 per cell but has been reported to range from about 4 to 14 per cell in non-exposed individuals.[34] Even within the same laboratory, the baseline frequency among individuals may vary by a factor of two or more.[35] Several potential sources of variation have been identified and they generally fall into two categories : (1) culture factors associated with the *in vitro* growth of the lymphocytes : and (2) biological factors associated with the genotype, lifestyle, or general health of the individuals.

1. Effect of cell culture factors

(1) Sample storage before culture

The cultures should be established as soon as possible after the blood sample is collected, preferably within 24 h.[36] Although heparinized blood can be stored for several days at 4 - 37C, storage can lead to lymphocyte death, reduction of mitogenic response, and selective loss of cells with lesions that can result in SCE formation.[37]

(2) BrdU concentration

Because BrdU itself promotes SCEs in a dose-dependent manner, the BrdU concentration in the culture medium is a factor of great importance in determining the measured background level of SCEs. BrdU induction of SCEs apparently depends on the ratio of BrdU concentration to the number of dividing cells per culture; thus, this ratio needs to be standardized across cultures to minimize the effect of BrdU.[37,38]

(3) Time of harvest

The influence of harvest time reflects the presence of different populations of lymphocytes with different cell progression times and different SCE levels. Santesson et al.[39] found that lymphocytes in T-cell enriched cultures exhibit more SCEs than lymphocytes in B-cell enriched cultures. It has also been suggested that the slow and fast replicating lymphocytes exhibit different SCE levels.[40]

(4) Additional factors in the Assay

Other variations in SCE assays can also affect the measured frequency. For example, the sera used in the culture medium may affect the SCE frequency[41] and a reduction in the baseline may be achieved if the serum is heat inactivated or dialyzed prior to use.[42] Since BrdU is most sensitive to light (other than red or yellow), which can cause photolysis of BrdU-containing DNA, the frequency of SCEs increases as a function of time and intensity of light exposure to cells. The cultures must be protected from light exposure during incubation of growth.[43] Different culture media and culture temperatures also influence frequencies.[38,40]

2. Effect of biological factors

(1) Effect of Age

Since the accumulation of mutation load is one of the causes of aging, and since the mutation frequency in human lymphocytes increases with age, increases in variation of SCE frequency with age is understandable given that individuals are constantly being exposed to a variety of environmental agents in nature or otherwise.[43] Recently, Bolognesi et al.[44] have reviewed several biomonitoring studies which explored the relationship between age and baseline SCE frequency in control populations. They have, however, found that age appears to have only a minor influence on baseline SCE frequency, although significant differences are observed between newborns and the elderly.[45]

(2) Effect of Sex

Based on published data, a general recommendation to analyze separate groups of female and male donors may be proposed. This recommendation is supported by the following findings : (a) females have more SCEs than males,[46] (b) the frequency of SCEs in female lymphocytes may be influenced by pregnancy,[47-49] specific stages of the menstrual cycle[50] and oral contraceptive use, which are known to induce SCEs.[48,49,51,52] In an ingenious approach, Wulf and Neibuhr[53] showed in blood lymphocyte cultures from a pair of chimeric twins that the XX cells are more sensitive to SCE-inducing agents than XY cells. Another possible explanation may be a difference in the level of hormones between the sexes.[43]

Menstrual cycle : The prominent variation in SCEs during different stages of the menstrual cycle in women may be attributed mainly to their specific cyclic changes in hormones, particularly steroid hormones; for example, progesterone and estrogen are known to play an important role in increasing the sensitivity of the individuals or cells to genetic damage.[47,54]

Pregnancy : A profound alteration in physiological status, particularly in respect to the levels of certain sex hormones such as progesterone and estrogen, occurs in

females during pregnancy. Hill and Wolff[55] did not find a significant difference in the frequency of SCEs between the blood lymphocyte cultures of pregnant and nonpregnant women. Sharma and Das[47] have shown that the frequency of SCEs is higher at the late stages or at the last trimester of pregnancy in women; they have also observed a similar enhancement in SCEs in blood cultures of pregnant female muntjacs as compared to the nonpregnant females or males. Most interestingly, when natural estrogen, progesterone, and human chorionic gonadotropin (HCG) - which are present at higher levels during pregnancy, were added exogenously to lymphocyte cultures of nonpregnant females, these did induce SCEs. The SCE-inducing effects of steroid hormones have also been seen in Chinese hamster lung cells and human lymphocytes *in vitro*.[54] It therefore suggests that female mammals that have menstrual or estrus cycles are more prone to such rhythmic variation because of precise hormonal cycles and are perhaps more sensitive to SCE induction and/or genetic damage when the hormones are at a higher level. The fluctuations in baseline frequency of SCEs as a function of time observed in men[35,56] are not due to hormones but occur because of some other factors such as circadian (day) rhythms or as yet unknown sources of variation.[43]

(3) Effect of Smoking

The smoking habit was found to be by far the most effective lifestyle factor affecting SCEs in general populations, with a strong dose-effect relationship.[57] Higher SCE frequencies in smokers have been found in a majority of the relevant studies, with an approximately 30% increase being most common.[57,58] Tobacco smoke consists of a mixture of genotoxic agents, such as polycyclic aromatic hydrocarbons, *N*-nitrosamines, various organic compounds including benzene and acrylonitrile, and inorganic compounds including various carcinogenic heavy metals and polonium- 210.[59] The genotoxic activity of tobacco smoke has been reported to be due to covalent interactions with DNA, as well as to the generation of reactive oxygen species, either directly or phagocyte-mediated.[60] Exposure to cigarette smoke and its condensate can cause SCEs and tumors in laboratory animals, as well as inhibiting DNA repair.[61]

(4) Effect of Alcohol and Coffee consumption

Reports on the possible effects of alcohol and coffee consumption are few, and the conclusions largely discordant.[57] Individual contributions of these habits cannot easily be assessed for linkage with smoking habits. However, there is plausible biological evidence suggesting that such drinking habits could have genetic effects *in vivo* : e.g., organic extracts from the urine of heavy coffee drinkers were demonstrated to be mutagenic in *Salmonella typhimurium*,[62] while acetaldehyde, the first metabolic product of ethanol, induced SCEs both *in vitro* and *in vivo*.[63]

SISTER CHROMATID EXCHANGES AS BIOMARKERS FOR OXIDATIVE DAMAGE

1. Reactive oxygen species and antioxidants

In living cells, reactive oxygen species (ROS)-including free radicals such as hydroxyl radicals and superoxide radicals, and molecules such as hydrogen peroxide - are formed continuously as a consequence of both biochemical reactions and external factors. Oxidative stress, which occurs in a cell when the production of ROS overwhelms the cell's natural antioxidant defenses, is thought to play a role in carcinogenesis by oxidative damage to DNA (Fig 5).[64] ROS can damage DNA, and division

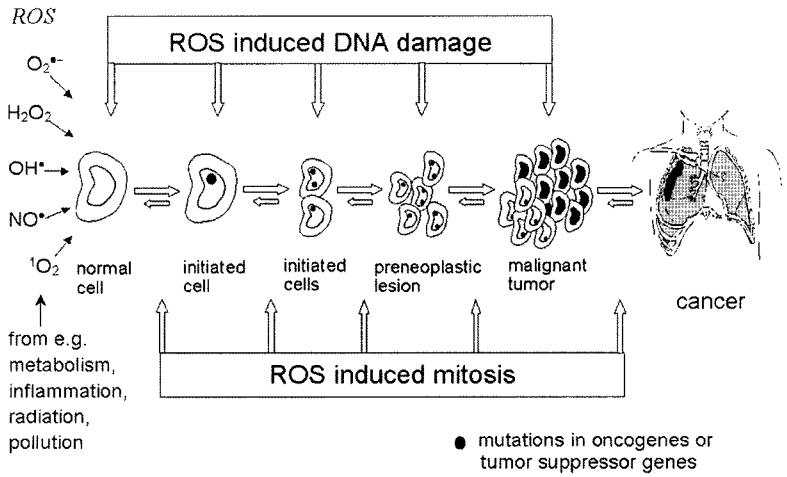

Fig 5. Possible roles of ROSs in multistage carcinogenesis[64]

of cells with unrepaired or misrepaired damage leads to mutations. If these relate to critical genes such as oncogenes or tumor suppressor genes, initiation and/or progression may result. ROS can interfere directly with cell signaling and growth,[65] and cellular damage inflicted by ROS can induce mitosis, increasing the risk that damaged DNA could lead to mutations and increasing the exposure of DNA to mutagens, including ROS.[66] Mutations may also be induced by recombination, gene conversion, and nondisjunction during mitosis.[67] Moreover, mitogenesis increases the possibility of clonal expansion of cells transformed by oncogene activation and/or tumor suppressor inactivation. In support of this, high spontaneous SCE frequencies have been found in peripheral lymphocytes of patients with various cancers, such as prostate cancer,[68] lung cancer,[18] ovarian cancer,[69] cancer of cervix uteri,[70] breast cancer,[71] and acute leukemia.[72]

Antioxidants can be defined as substances that delay or inhibit, oxidative damage to a target molecule.[73] Human tissues are protected from oxidative damage by a variety of mechanisms, including small molecular weight antioxidants (e.g. vitamins C and E), and enzymes that destroy the reactive species (e.g. SOD, catalase, GSH-Px) (Fig 6). These antioxidants play an important part in preventing cancers by acting as scavengers of ROS.[74]

2. Effect of Oxidants and Antioxidants on SCEs

(1) in vitro Study

A number of *in vitro* studies have been conducted to examine the effects of oxidants and antioxidants on sister chromatid exchanges. Emerit et al.[75] have reported that superoxide anion radicals ($O2^{\cdot-}$) generated either photochemically (by photoreduction of flavinmononucleotide by near UV radiation) or enzymatically (with xanthine oxidase) gave rise to both SCE and chromosome aberrations in human lymphocytes, and that superoxide dismutase (SOD) afforded almost complete protection against chromosome breakage as well as SCE induction. Weitberg and Weitzman[76] also reported that enzymatically generated superoxide anions induced SCEs in Chinese hamster ovary (CHO) cells, and that the antioxidant ascorbic acid (0.1 mM) significantly suppressed SCE induction. In higher concentrations of ascorbic acid, however, significant augmentation of oxygen radical-induced SCEs occurred. Stimulated human phagocytes produce SCEs in CHO cells by a mechanism involving oxygen metabolites.[77] SOD, catalase, hydroxyl radical scavengers (benzoate, mannitol), *N*-acetylcysteine, and β-carotene protected target CHO cells from phagocyte-induced SCE, implicating the involvement of hydroxyl radicals in this chromosomal damage. α-Tocopherol (>5 μM) protected target cells exposed to phagocytes but not to enzymatically generated oxidants when the vitamin was added just before the source of oxygen radical, suggesting that the principal action of tocopherol is to inhibit the release of oxidants from phagocytes. On the other hand, cultivation of target cells with supplemental tocopherol protected them from the toxic effects of the enzymatic oxidant-producing system, indicating a role for membrane-associated free radicals in the mechanism of SCE induction.

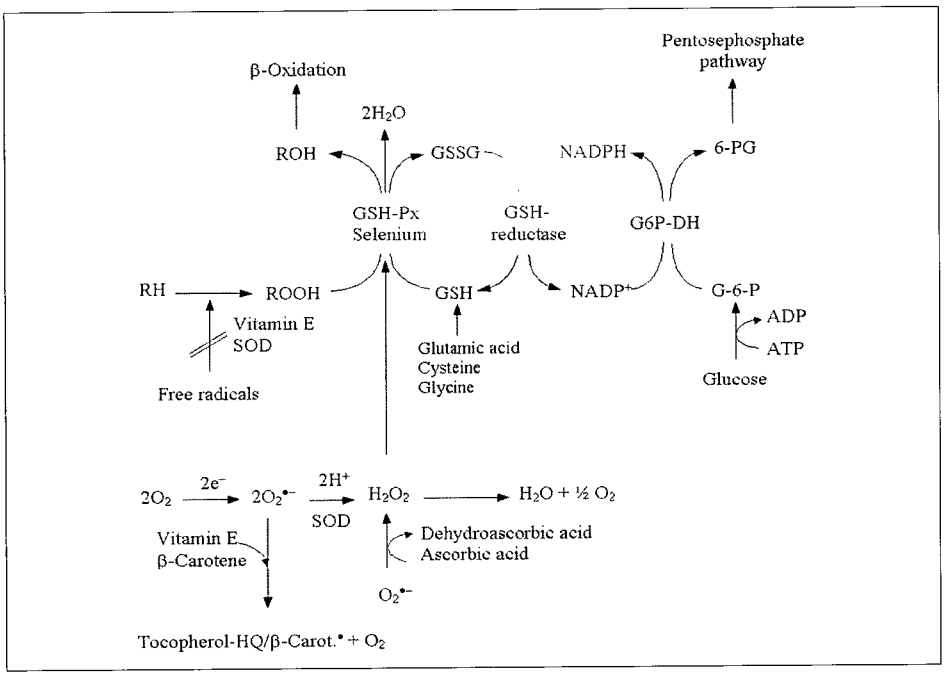

Fig 6. The cellular antioxidant defense system

Low concentrations of sodium selenite (0.1- 1.0 μM) protected the target cells. However, higher concentrations (>10 μM) of selenite had no effect on oxidant-induced SCE formation or increased the number of SCEs.[77] Larrmendy et al.[78] have found that superoxide anion plus hydrogen peroxide generated enzymatically induced SCEs in Chinese hamster fibroblasts and that the iron-complexing agent-phenanthroline completely inhibited the production of these SCEs, indicating that the Fenton reaction is responsible for the production of SCEs. Cigarette smoke condensate (CSC) has been reported to produce SCEs in mammalian cells.[79] Lee et al.,[80] however, have found that SOD and/or catalase or ascorbate failed to reduce the SCE frequency in the presence of either CSC or smoke-bubbled through phosphate buffered saline solution (smoke-PBS), suggesting that SCE induction by CSC or smoke-PBS is caused by pathways not involving free radicals or oxidation. β-Carotene and retinoic acid yielded lower frequencies of chromosomal aberration and SCEs in CHO cells induced by pan masala - a dry complex mixture of tobacco and areca nut which is closely associated with oral cancer.[81] In mouse mammary cell culture, -carotene has been shown to reduce SCEs induced by MNU (N-methylnitrosourea), a direct acting carcinogen.[82] Ascorbic acid reduced SCEs induced by carcinogens such as cyclophosphamide (CP) and mitomycin C (MMC) in bone marrow and spleen cells in mice, indicating that ascorbic acid acts as an anti-SCE agent in both *in vivo* and *in vivo/in vitro* conditions in mice.[83] Edenharder et al.[84] have recently performed studies on carcinogen induced SCEs *in vitro* using several chemoprevent agents, including-carotene, retinal, riboflavin, α-tocopherol and vitamin C and K_1. β-Carotene, retinal and α-tocopherol caused highly significant reductions of SCE frequencies, which were to a large extent independent of the inducer and the treatment schedule.

(2) Human biomonitoring studies

Only a few investigators have examined the association of dietary or plasma antioxidant concentrations and the frequency of SCEs in humans. In one study,[85] sixty asymptomatic cigarette smokers were randomly allocated into three treatment groups. Smokers in Group 1 received 900 mg of dl--tocopheryl acetate daily for 6 weeks, whereas 40 mg of -carotene were administered daily to those in Group 2 for the same period. Subjects in Group 3 were treated with a matched placebo. The following measurements were taken : plasma levels of vitamin E and -carotene; circulating leukocyte counts; SCEs; and reactive oxidant generation by blood phagocytes using luminol- enhanced chemiluminescence (LECL) responses of blood phagocytes activated with phorbol myristate acetate (PMA), N-formyl- L-methionyl-L-leucyl-L-phenylalanine (FMLP), and cytochalasin B (CB). The administration of vitamin E or β-carotene during 6 weeks had no influence on the frequency of SCEs in lymphocytes from smokers; for example, SCE and leukocyte counts remained unchanged throughout the trial in all three treatment groups, although vitamin E appeared to inhibit the generation of oxidants by activated phagocytes and β-carotene scavenged oxidants generated by the myeloperoxidase/H_2O_2/halide system.

Another study also did not show reduced SCEs in smokers after 14 weeks of supplementation of β-carotene (20 mg).[86] Researchers conducted a randomized, double-blind, placebo-controlled intervention trial with 143 heavy smokers. Plasma β-carotene levels increased 13-fold in the treatment group during the intervention, whereas SCEs decreased almost identically in the treatment and placebo groups (4.37 0.38 vs. 4.24 0.37 SCE/cell). On the other hand, micronuclei in the sputum were reduced in the β-carotene- treated group of smokers in the same intervention trial.[87] In an intervention study using multivitamin/mineral supplementation in pregnant women, increased plasma levels of antioxidants in the multivitamin/mineral supplemented pregnant group did not decrease SCE rates. However, the supplementation could have prevented an increase in SCE rates which may have been induced by ROSs generated from the enhanced steroid hormones in the last trimester, suggesting that multivitamin/mineral-supplement during pregnancy may prevent DNA damage due to the altered hormonal profile.[88] Van Rensburg et al.[89] investigated the relationships between plasma levels of ascorbate, vitamin E and β-carotene, and the frequency of SCEs in cigarette smokers. They found no correlations between SCE and plasma levels of these antioxidants, indicating that normal antioxidant levels may be inadequate to protect cells against DNA damage caused by the oxidant overload generated by the hyperreactive phagocytes of smokers. On the other hand, Hageman et al.[90] have found that SCE frequencies of females (n=30) significantly inversely correlated with plasma α-tocopherol concentrations suggesting that α-tocopherol may exert a reducing effect on tobacco smoked-induced SCE frequencies. Cheng et al.[91] have reported that higher intakes of vitamin A (sum of the consumption of carotenoids and retinol) and selenium were also inversely associated with SCE levels.

CONCLUSIONS

Molecular epidemiology offers an alternative, more focused, and economical approach to the study of cancer prevention. Biomarkers for DNA damage are now gaining importance as a useful indicator of exposures to genotoxic agents in epidemiologic studies; the SCE test

in lymphocytes is considered to be one of the most sensitive indicators of DNA damage. Therefore, a regimen of antioxidant supplementation using SCE assays, with well controlled confounding factors such as smoking, age and sex, can be expected to yield definitive answers to questions concerning the efficacy of antioxidant protection in cancer prevention.

Literature Cited

1) Santella RM. DNA damage as an intermediate biomarker in intervention studies. *Proc Soc Exp Biol Med* 216 : 166-171, 1997
2) Weinstein IB. The origins of human cancer; molecular mechanisms and their implications for cancer prevention and treatment. *Cancer Res* 310 : 633-638, 1988
3) Tucker JD, Auletta A, Cimino MC, Dearfield KL, Jacobson-Kram D, Tice RR, Carrano V. Sister chromatid exchange; Second report of the gene tox program. *Mutat Res* 297 : 101-180, 1933
4) Ames BN. Endogenous oxidative DNA damage, aging and cancer. *Free Res Commun* 7 : 121-128, 1989
5) Cerutti P. Oxy-radicals and cancer. *Lancet* 344 : 862-863, 1994
6) Gey KF. Optimum plasma levels of antioxidant micronutrients. Ten years of antioxidant hypothesis on arteriosclerosis. *Bibl Nutr Dieta* 51 : 84-99, 1994
7) Taylor JH, Woods PS, Hughes WL. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labelled thymidine. *Proc Natl Acad Sci* 43 : 122-138, 1957
8) Taylor JH. Sister chromatid exchanges in tritium-labelled chromosomes. *Genetics* 43 : 515-529, 1958
9) Perry PE, Wolff S. New Giemsa method for differential staining of sister chromatids. *Nature* 251 : 156-158, 1974
10) Shafer DA. Replication bypass SCE mechanisms and the induction of SCE by single-strand adducts or lesions of DNA. *Basic life Sci* 29(Part A) : 245-265, 1984
11) Wang J, Liu LF. DNA topoisomerases : enzymes that catalyze the concerted breaking and rejoining of DNA backbone bands. In : Taylor JH, ed. Molecular Genetics, Vol.3. pp.65-88, Academic Press. New York, 1979
12) Liu LF, Liu CC, Alberts BM. Type II DNA topoisomerases : enzymes that can unknot a topologically knotted DNA molecule via a reversible double-strand break. *Cell* 19 : 697-707, 1980
13) Wilcosky TC, Rynard SM. Sister chromatid exchanges. In : Hulka BS, Wilcosky TC, Griffith JD, eds. Biological Markers in Epidemiology, pp.105-124, Oxford University Press, 1990
14) Gebhart E. Sister chromatid exchange (SCE) and structural chromosome aberration in the mutagenicity testing. *Hum Genet* 58 : 235-254, 1981
15) Latt SA. Sister chromatid exchange formation. *Ann Rev Human Genet* 15 : 11-55, 1981
16) Carrano AV. Chromosomal alterations as markers of exposure and effect. *J Occup Med* 28 : 1112-1116, 1986
17) Steika DG, Minkler JL, Carrano A. Induction of long-lived chromosome damage, as manifested by sister chromatid exchanges in lymphocytes of animals exposed to mitomycin-C. *Mutat Res* 51 : 383-396, 1987
18) Hopkin JM, Evans HJ. Cigarette smoke-induced DNA damage and lung cancer risk. *Nature* 283 : 388-390, 1980
19) Wulf HC, Husum B, Niebuhr E. Cessation of smoking enhances sister chromatid exchanges in lymphocytes. *Hereditas* 102 : 195-198, 1985
20) Tucker JD, Ashworth LK, Johnston GR, Allen NA, Carrano AV. Variation in the human lymphocytes sister-chromatid exchange frequency : Results of a long-term longitudinal study. *Mutat Res* 204 : 435-444, 1988
21) Gilian Clare M, Jones WG, Taylor JH. Sister chromatid exchanges in human lymphocytes exposed to single cytotoxic drugs in vivo or in vitro. *Eur J Cancer Clin Oncol* 18 : 979-989, 1982
22) Tucker JD, Wyrobek AJ, Ashworth LK, Christensen ML, Burton GV, Carrano AV, Everson RB. Induction, accumulation, and persistence of sister chromatid exchanges in women with breast cancer receiving cyclophophamide, adriamycin, and 5-fluorouracil chemotherapy. *Cancer Res* 50 : 4951-4956, 1990
23) Stolley PD, Soper KA, Galloway SM, Nichols WW, Norman SA, Wolman S. Sister chromatid exchanges in association with occupational exposure to ethylene oxide. *Mutat Res* 129 : 89-102, 1984
24) Sarto F, Cominata I, Pinton AM, Brovedni PG, Faccioli CM, Bianchi V, Levis AG. Cytogenetic damage in workers exposed to ethylene oxide. *Mutat Res* 138 : 185-195, 1984
25) Carrano AV, Moore DH. The rational and methodology for quantifying sister chromatid exchange in humans. In : Heddle JA, ed. Mutagenicity : New Horizons in Genetic Toxicology. pp. 267-304, Acadimic Press. New York, 1982
26) Wiencke JK, Christiani DC, Kelsey KT. Bimodal distribution of sensitivity to SCE induction by diepoxybutane in human lymphocytes. I. Correlation with chromosomal aberrations. *Mutat Res* 248(1) : 17-26, 1991
27) Wiencke JK, Wrensch MR, Milke R, Petrakis NL. Individual susceptibility to induced chromosome damage and its implications for detecting genotoxic exposures in human populations. *Cancer Res* 51 : 5266-5269, 1991
28) Kelsey KT, Christinani DC, Wiencke JK. Bimodal distribution of sensitivity to SCE induction by diepoxybutane in human lymphocytes. II. Relationship to baseline SCE frequency. *Mutat Res* 248 : 27-33, 1991
29) Carbonell E, Peris F, Xamena N, Creus A, Marcos R. SCE analysis in human lymphocytes of a spanish control population. *Mutat Res* 335(1) : 35-46, 1995
30) Perry PE, Thomson EJ. The methodology of sister chromatid exchanges. In : Kilbey BJ, Legator M, Nichols W, Ramel C,

eds. Handbook of Mutagenic test procedures, pp.495-529, Elservier Science Publishers. New York, 1984
31) Schwartz GG. Chromosome aberrations. In : Hulka BS, Wilcosky TC, Griffith JD, eds. Biological Markers in Epidemiology, pp.147-172, Oxford University Press, 1990
32) Alberts B. Zellwachstum und Zellteilung. In : Bray D, Lewis J, Raff M, Roberts K, Watson JD, eds. Molecularbiologie der Zelle, pp.679-747, VCH. Weinheim, 1987
33) Lewin B. Cellcycle and growth regulation. In : Lewin B. ed. Genes, pp.1089-1129, Oxford University Press. Oxford, 1997
34) Ashby J, Richardson CR. Tabulation and assessment of 113 human surveillance cytogenetic studies conducted between 1965 and 1984. *Mutat Res* 154 : 111-133, 1985
35) Dewdney RS, Loveli DP, Jenkinson PC, Anderson D. Variation in sister-chromatid exchanges among 106 members of the general U.K. population. *Mutat Res* 171 : 43-51, 1986
36) Carrano AV, Natarajan AT. Considerations for population monitoring using cytogenetic techniques. *Mutat Res* 204 : 379-406, 1988
37) Bloom AD. Guidelines for Studies of Human Populations Exposed to Mutagenic and Reproductive Hazards. March of Dimes Birth Defects Foundation. White Plains. New York, 1981
38) Crossen PE. SCE in lymphocytes. In : Sandberg AA, ed. Sister Chromatid Exchange, pp.175-193, Alan R. Liss Inc. New York, 1982
39) Santesson B, Lindahl-Kiessling K, Mattsson A. SCE in B and T lymphocytes : Possible implications for Bloom's syndrome. *Clin Genet* 16 : 133-135, 1979
40) Abdel-Fadil MR, Palmer CG, Heerema N. Effect of temperature variation on sister-chromatid exchange and cell-cycle duration in cultured human lymphocytes. *Mutat Res* 104 : 267-273, 1982
41) MCfee AF, Sherrill MN. Mitotic response and SCEs in lymphocytes cultured in sera from different sources. *Experientia* 37 : 27-29, 1981
42) Kato H, Sandberg AA. The effect of sera on sister chromatid exchanges in vitro. *Exp Cell Res* 109 : 445-448, 1977
43) Das BC. Factors that influence formation of sister chromatid exchanges in human blood lymphocytes. *CRC Crit Rev Toxicol* 19 : 43-86, 1988
44) Bolognesi C, Abbondandolo A, Barale R, Casalone R, Dalpra L, De Ferrari M, Degrassi F, Forni A, Lamberti L, Lando C, Migloire L, Padovani P, Pasquini R, Puntoni R, Sbrana I, Stella M, Bonassi S. Age-related increase of baseline frequencies of sister chromatid exchange, chromosome aberrations, and micronuclei in human lymphocytes. *Cancer Epidemiol Biomarkers Prev* 6(4) : 249-256, 1997
45) Sardas S, Karahalil B, Akyol D, Kükner S, Karakaya AE. The effect of smoking on sister chromatid exchange rate of newborn infants born to smoking mothers. *Mutat Res* 341 : 249-253, 1995
46) Lazutka JR, Dedonyte V, Krapavickaite D. Sister-chromatid exchanges and thier distribution in human lymphocytes in relation to age, sex and smoking. *Mutat Res* 306 : 173-180, 1994
47) Sharma T, Das BC. Higher incidence of spontaneous sister-chromatid exchanges (SCEs) and X-ray-induced chromosome aberrations in peripheral blood lymphocytes during pregnancy. *Mutat Res* 174 : 27-33, 1986
48) Ghosh PK, Ghosh R. Effect of betel chewing on the frequency of sister chromatid exchanges in pregnant women and women using oral contraceptives. *Cancer Genet Cytogenet* 32 : 211-215, 1988
49) Ghosh R, Ghosh PK. Sister chromatid exchanges in the lymphocytes of control women, pregnant women, and women taking oral contraceptives : Effects of cell culture temperature. *Environ Mol Mutagen* 12 : 179-183, 1988
50) Joseph-Lerner N, Fejgin M, Ben-Nun I, Legum C, Amiel A. The correlation between the frequency of sister-chromatid exchange and human reproductive hormones. *Mutat Res* 300 : 247-252, 1933
51) Murthy PBK. Frequency of sister chromatid exchanges in cigarette smokers. *Hum Genet* 52 : 343-345, 1979
52) Murthy PBK, Prema K. Further studies on sister-chromatid exchange frequency in users of hormonal contraceptives. *Mutat Res* 119 : 351-354, 1983
53) Wulf HC, Neibuhr E. Different sister chromatid exchange rates in XX and XY cells of a pair of human chimeric twins. *Cytogenet Cell Genet* 39 : 104-108, 1985
54) Lukic B, Barajaktarovic N. Sister-chromatid exchanges in newborns after normal and progesterone-treated pregnancies. *Mutat Res* 191 : 121-124, 1987
55) Hill A, Wolff S. Increased induction of sister chromatid exchange by diethystilbestrol in lymphocytes from pregnant and premenopausal women. *Cancer Res* 42 : 893-896, 1982
56) Tucker JD, Christensen ML, Strout CL, MCgee KA, Carrano AV. Variation in the human lymphocyte sister chromatid exchanges frequency as a function of time : results of daily and twice-weekly sampling. *Environ Mol Mutagen* 10 : 69-78, 1987
57) Barale R, Chelotti L, Davini T, Del RY S, Andereassi MG, Ballardin M, Bulleri M, He J, Baldacci S, Di Pede F, Gemignani F, Landi S. Sister chromatid exchange and micronucleus frequency in human lymphocytes of 1,650 subjects in an Italian population : II. Contribution of sex, age, and life style. *Environ Mol Mutagen* 31 : 228-242, 1988
58) Sbrana I, Luccini A, Landi S, Barale R. Enhancement of SCE frequency by Alpha-naphthoflavone in cultured lyphocytes in relation to environmental factors. *Environ Mol Mutagen* 26 : 331-337, 1995
59) International Agency for Research on Cancer (IARC). Tobacco smoking. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to human, Vol.38, IARC. Lyon, 1986
60) Pryor WA, Stone K. Oxidants in cigarette smoke : radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. *Ann NY Acad Sci* 686 : 12-28, 1993
61) De Marini DM. Genotoxicity of tobacco smoke and tobacco

smoke condensate. *Mutat Res* 114 : 59-89, 1983
62) Nehlig A, Derby G. Potential genotoxic, mutagenic and antimutagenic effects of coffee : A review. *Mutat Res* 317 : 145-162, 1994
63) Obe G, Gobel D, Engeln H, Herha J, Natarajan AT. Chromosomal aberrations in peripheral lymphocytes of alcoholics. *Mutat Res* 73 : 377-386, 1980
64) Loft S, Poulsen HE. Cancer risk and oxidative DNA damage in man. *J Mol Med* 74 : 297-312, 1996
65) Cerutti P, Ghosh R, Oya Y, Amstad P. The role of the cellular antioxidant defense in oxidant carcinogenesis. *Environ Health Perspect* 102 (Supp 10) : 123-129, 1994
66) Ames BN, Shigenaga MK, Gold LS. DNA lesions, inducible DNA repair, and cell division : three key factors in mutagenesis and carcinogenesis. *Environ Health Perspect* 101 (Supp 5) : 35-44, 1993
67) Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of ageging. *Proc Natl Acad Sci* 90 : 7915-7922, 1993
68) Dhillon VS. Dhillon IK. Chromosome aberrations and sister chromatid exchange studies in patients with prostate cancer : possible evidence of chromosome instability. *Cancer Genet Cytogenet* 100(2) : 143-147, 1998
69) Dhar PK, Devi S, Rao TR, Kumari U, Joseph A, Kumar MR, Nayak S, Shreemati Y, Bhat SM, Bhat KR. Significance of lymphocytic sister chromatid exchange frequencies in ovarian cancer patients. *Cancer Genet Cytogenet* 89(2) : 105-108, 1996
70) Dhillon VS, Kler RS, Dhillon IK. Chromosome instability and sister chromatid exchange (SCE) studies in patients with carcinoma of cervix uteri. *Cancer Genet Cytogenet* 86(1) : 54-57, 1996
71) Dhillon VS, Bhasker R, Kler RS, Husain SA. Sister chromatid exchange (SCE) studies in breast cancer patients : a follow-up study. *Cancer Genet Cytogenet* 80(2) : 115-117, 1995
72) Tuna M, Artan S, Gezer S, Sayli BS, Basaran N. Sister chromatid exchange analysis in acute leukemia patients. *Cancer Genet Cytogenet* 79(1) : 86-88, 1995
73) Gutteridge JM, Halliwell B. Antioxidants in Nutrition, Health, and Disease. Oxford University Press. Oxford, 1994
74) Byers T, Perry G. Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. *Annu Rev Nutr* 12 : 139-159, 1992
75) Emerit I, Keck M, LEVY A, Feingold J, Michelson AM. Activated oxygen species at the origin of chromosome breakage and sister-chromatid exchanges. *Mutat Res* 103 : 165-172, 1982
76) Weitberg AB, Weitzman SA. The effect of vitamin C on oxygen radical-induced sister chromatid exchanges. *Mutat Res* 144 : 23-26, 1985
77) Weitberg AB, Weitzman SA, Clark EP, Stossel TP. Effects of antioxidants on oxidant-induced sister chromatid exchange formation. *J Clin Invest* 75 : 1835-1841, 1985
78) Larramendy M, Mello-Filho AC, Leme Martins EA, Meneghini R. Iron-mediated induction of sister-chromatid exchanges by hydrogen peroxide and superoxide anion. *Mutat Res* 178 : 57-63, 1987
79) Lee CK, Brown BG, Reed EA, Lowe GD. The effects of treatment conditions on sister chromatid exchange induction by cigarette smoke condensates. *Environ Mutagen* 9 : 57-58, 1987
80) Lee CK, Brown BG, Rice Jr WY, Doolittle DJ. Role of oxygen free radicals in the induction of sister chromatid exchanges by cigarette smoke. *Environ Mutagen* 13 : 54-59, 1989
81) Patel RK, Trivedi AH, Jaju RJ, Kukreti MS, Bhatavdekar JM, Shah PM, Patel DD. Protection from pan masala induced genomic damage by beta-carotene and retinoic acid - an in vitro experience. *Neoplasma* 45 : 169-175, 1998
82) Manoharan K, Banerjee MR. β-carotene reduces sister chromatid exchanges induced by chemical carcinogens in mouse mammary cells in organ culture. *Cell Biol Intern Rep* 9 : 783-789, 1985
83) Krishna G, Nath J, Ong T. Inhibition of cyclophosphamide and mitomycin C-induced sister chromatid exchanges in mice by vitamin C. *Cancer Res* 46 : 2670-2674, 1986
84) Edenharder R, Kerkhoff G, Dunkelberg H. Effects of β-carotene, retinal, riboflavin, α-tocopherol and vitamin C and K_1 on sister- chromatid exchanges induced by 3-amino-1-methyl-5*H*-pyrido [4,3-*b*]indole (Trp-P-2) and cyclophosphamide in human lymphcyte cultures. *Food Chem Toxicol* 36 : 897-906, 1998
85) Richards GA, Theron AJ, Van Rensburg CEJ, Van Rensburg AJ, Van der Merwe CA, Kuyl JM, Anderson R. Investigation of the effects of oral administration of vitamin E and beta-carotene on the chemiluminescence responses and the frequency of sister chromatid exchanges in circulating leukocytes from cigarette smokers. *Am Rev Respir Dis* 142 : 648-654, 1990
86) Van Poppel G, Kok FJ, Duijzings P, De Vogel N. No influence of beta-carotene on smoking-induced DNA damage as reflected by sister chromatid exchanges. *Int J Cancer* 51 : 355-358, 1992
87) Van Poppel G, Kok FJ, Hermus RJJ. Beta-carotene supplementation in smokers reduces the frequency of micronuclei in sputum. *Br J Cancer* 66 : 1164-1168, 1992
88) Park E, Wagenbichler P, Elmadfa I. Effects of multivitamin/mineral supplementation, at nutritional doses, on plasma antioixdant status and DNA damage estimated by Sister chromatid exchanges in lymphocytes in pregnant women. *Internat J Vit Nutr Res* 69(4) : 27-31, 1999
89) Van Rensburg CEJ, Theron A, Richards GA, Van der Merwe CA, Anderson R. Investigation of the relationchips between plasma levels of ascorbate, vitamin E and ß-carotene and the frequency of sister-chromatid exchanges and release of reactive oxidants by blood leucocytes from cigarette smokers. *Mutat Res* 215 : 167-172, 1989
90) Hageman G, Van den Oetelaar A, Welle I, Beurskens-Comuth P, Kleinjans J. Influence of smoking behavior in relation to

plasma retinol and α-tocopherol on sister chromatid exchanges in human peripheral blood lymphocytes. *Cancer Epidemiol Biomarkers Prevent* 4 : 289-293, 1995

91) Cheng TJ, Christiani DC, Xu X, Wain JC, Wiencke JK, Kelsey KT. Glutathione S-transferse genotype, diet, and smoking as determinants of sister chromatid exchange frequency in lymphocytes. *Cancer Epidemiol Biomarkers Prevent* 4 : 535-542, 1995