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OXYGEN FREE RADICAL AND ANTIOXIDANT DEFENSE MECHANISM IN CANCER

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1. ABSTRACT

The reactive oxygen species (ROS) can damage the nucleic acids. The oxidative modification of the DNA constitutes the fundamental molecular event in carcinogenesis and that is why the interest in the study of the involvement of ROS in that process. On the other hand, oxidative DNA damage-induced mutagenesis is widely hypothesized to be a frequent event in the normal human cell. The enormous evidence suggests an important role of ROS in the expansion and progression of tumor clones, being considered a relevant class of carcinogens. In addition, the use of immunohistochemical techniques has showed that the various types of cancer examined to date manifest an imbalance in their antioxidant mechanisms to respect the primary cell. In the near future new insights in cancer therapies, based on modulation of cellular redox status, may lead the way to additional tools against carcinogenesis from ROS.

2. INTRODUCTION

In the last twelve years there has been a growing interest in understanding the role of free radicals in biomedicine. Particularly interesting are the Reactive Oxygen Species (ROS), of which, the hydroxyl radical ($^{\cdot}OH$) is most damaging of this chemical species; which also include superoxide anions ($O^{\cdot}_2$), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). ROS can be formed in the aerobic life not only during oxidative phosphorylation, through the action of mixed function oxidases, and as byproducts of normal metabolism by enzymes such as superoxide dismutase (SOD), NADPH oxidase, and xanthine oxidase (XO) in neutrophils, but can also be generated from redox cycling of certain drugs and by radiation. Fortunately, the organism is endowed by an antioxidant defense system that allows a balance between the generation of oxidants and antioxidants. When this balance is disrupted a condition referred as oxidative stress develops, and despite the antioxidants defense mechanism to counteract the ROS-related deleterious effects, damage to macromolecules does occur as a result of these reactions. Oxidative damage accumulates during the life cycle and lead to different pathological processes such as: atherosclerosis, myocardial infarction, rheumatoid arthritis, neurodegenerative disorders, and cancer, among others.

Since oxidative DNA damage is considered the most important molecular factor in carcinogenesis (1), recent studies are focussing on the role of ROS in the induction, promotion and progression of this multistage process.

3. ROS IN THE INITIATION, PROMOTION AND PROGRESSION OF CANCER. GENERAL ASPECTS

Cancer development, as a multistage process, requires the cumulative action of multiple events that occur in one cell clone. These events include a three stage model: a permanent change in one somatic cell genetic material (initiation); 2-the expansion of the mutated cell clone (promotion) and 3-the malignant conversion into cancer (progression) (Figure 1). ROS can stimulate carcinogenesis by acting at all three stages (2-6).

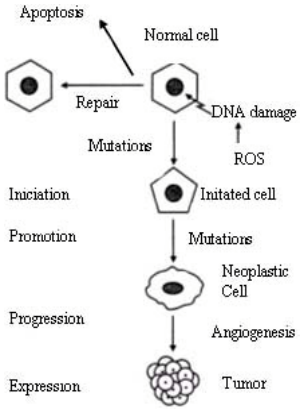

Figure 1. Pathways to cancer. Multistage process, which simplified, comprises initiation (attack by ROS, carcinogen), accumulation of carcinogenic mutations, progresses trough preneoplastic stages by the acquisition of more mutations, promotion by a tumor promoter, progression and development of angiogenic potential leading to expression of tumor

3.1. ROS-induced DNA damage mechanisms (initiation)

The initiation step requires a permanent change in one-cell genetic material. DNA replication and subsequent cell division transform chemical damage to an inheritable change in genetic material (mutation). Oxidative DNA damage can occur by the following processes: 1- Through hydroxyl radical ($^{\cdot}$OH), which is produced from H_2O_2 in the presence of metal ions (Fe^{2+}) or (Cu^{2+}), present or in close proximity to DNA, or released from their normal sequestration sites. 2- Increases in intracellular free calcium (Ca^{2+}), as a result of their release from the intracellular Ca^{2+} stores and through the influx of extracellular Ca^{2+}. A high level of oxidative stress, which may in turn deplete the endogenous antioxidant reserves, is an important signal leading to Ca^{2+} mobilization**.** An effect of ROS-related Ca^{2+} changes is the activation of endonucleases, which can cause DNA fragmentation (a normal process during apoptosis) (7) (Figure 2). These mechanisms may occur simultaneously.

In living cells, there is a steady formation of DNA lesions. $^{\cdot}$OH attack upon DNA generates a whole series of DNA damage by a variety of mechanisms. These include sugar and base modifications, strand breaks and DNA-protein cross-links. Modified DNA bases (pyrimidine and purine) constitute one of the most common lesions. Some of them have mutagenic properties being potentially able to damage the integrity of the genome (2, 8). 8-hydroxyguanine (8-OH-Gua) represents one of the most studied lesions, leading to GC → TA transversions and mutagenesis, unless repaired prior to DNA replication (9). Several other modified bases, which have also been shown to possess miscoding potentials and thus perhaps premutagenic properties, include 2-hydroxyadenine, 8-hydroxyadenine, 5-hydroxycytosine, and 5 hydroxyuracil. DNA base damage is thought to be repaired mainly by base-excision repair (10). At the same time, singlet oxygen can induce DNA damage selectively at guanine residues (11).

A great number of evidences indicate a direct correlation between 8-OH-Gua generation and carcinogenesis *in vivo* (12). Furthermore, the GC → TA transversions have been frequently detected in the tumor suppressor *p*53 gene and *ras* protooncogene. Through the inactivation of tumor suppressor gene or the activation of oncogenes, ROS-related mutations may lead to the initiation.

Recent investigations of benign tumors supported the idea that oxidative DNA damage might be a causative factor in cancer development, and that a positive correlation between the size of the tumor and the amount of 8-OH-Gua, and possibly other base lesions, may be a risk factor that may determine the transformation of benign tumors to malignant tumors (13).

Finally, while high doses of ROS increase the possibility of cancer initiation through mutagenesis (14, 15), one single ROS may cause cell death if it attack an essential gene for the cell viability, and in dependence on the composition of the ROS involved, the presence of other carcinogens, and the cell cycle position at the moment of exposure (5).

3.2. ROS in tumor promotion

The oxidative stress is strongly involved in this stage of carcinogenesis. In summary, a number of tumor promoter classes are thought to act either by stimulating endogenous oxygen radical production or by altering cellular metabolic processes. Moreover, many tumor promoters have a strong and immediate inhibitory effect on cellular antioxidant defense systems such as SOD, CAT and GSH-Px activities (16). ROS can stimulate the expansion of mutated cell clones by temporarily modulating gene related to proliferation or cell death. While an overload from high levels of oxidative stress halts proliferation by citotoxic effects, low levels can stimulate cell division and promote tumor growth (17). Thus, the stimulation of the intracellular production of ROS is considered the main way to promote the ROS-related tumors (5).

ROS can also induce large increases in cytosolic Ca^{2+} through the mobilization of intracellular Ca^{2+} stores and through the influx of extracellular [Ca^{2+}] (18). The ROS-related changes in intracellular [Ca^{2+}] may account through a direct or indirect action. The induction of the proto-oncogenes *c-fos* was found to be directly while an example of an indirect effect represents the phosphorylation of transcription factors by Ca^{2+}-dependent protein kinases (PKC). The activation of PKC and other protein kinases leads to phosphorylation and the activation of other kinases, which regulate the activity of transcription factors (7, 19).

Other studies have found ROS can directly modulate PKC activity through the oxidation of cysteine residues in the regulatory domain of the enzyme. In mammals, direct effects of ROS have been shown to regulate the activity of the transcription factor NF-kappa B. This factor controls cell growth and oncogenesis, in part, from the induction of gene products that controls proliferative

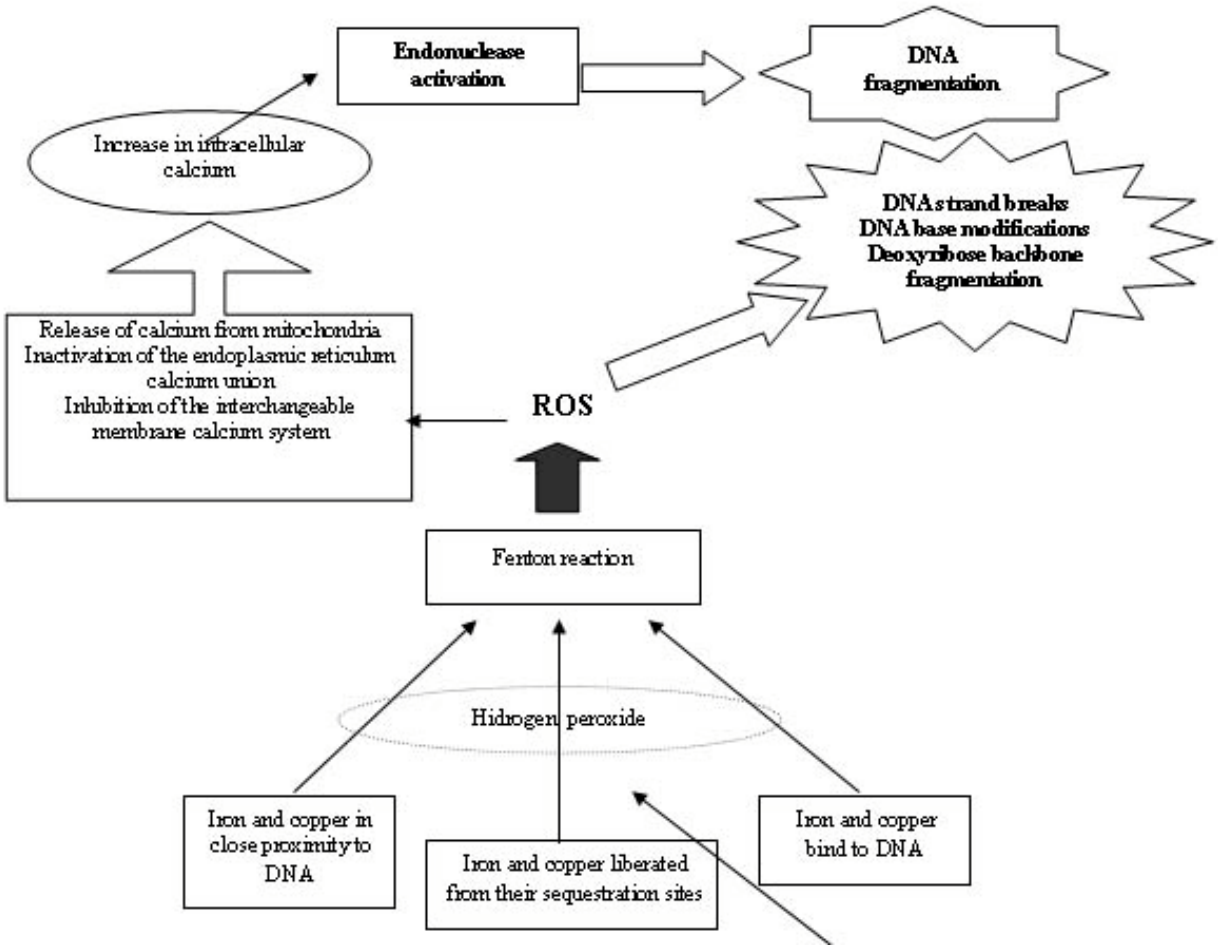

Figure 2. ROS–induced DNA damage. Hypothetical mechanisms are shown in the figure and discussed in the text

responses and suppress apoptotic cascades, such as those induced by tumor necrosis factor (TNF-alpha), expression of oncoproteins and genotoxic stress (20-22). NF-kappa B activation also potentiates proliferation by blocking differentiation in certain settings, and this phenomenon may also promote oncogenesis (22).

In *in vitro* experiment, DNA bindings of *p53*, AP-1 and NF- kappa B are all activated in a reductive condition and repressed in an oxidative condition. However, it is noted that certain transcription factors are activated by oxidation while others are repressed by oxidation (23).

Additionally, redox regulation is associated with Ca^{2+} signaling and protein phosphorylation. The key reaction is the reversible reduction/oxidation of the sulfydryl function of the highly conserved cysteine residues in the DNA-binding domain of these proteins.

Recent results from *in vitro* studies have shown H_2O_2 acts as a tumor promoter in non-neoplastic ephitelial cells (T51 B cell line) in rat livers. The induced expression of early response genes c-fos, c-jun, c-myc and egr-1, and the inhibition of gap junctional communication could to be the H_2O_2 –mediated tumor promotion mechanisms (24).

3.3. ROS-induced tumor progression

The third step of carcinogenesis comprises the acquisition of malignant properties by the tumor. Progression is distinguished by accelerate cell growth, escape from immune surveillance, tissue invasion and metastasis (25). Since the generation of large amounts of ROS may contribute to the ability of some tumors to mutate, inhibit antiproteases and injure local tissues, it has together with the increases in the level of oxidatively modified DNA bases (27). Conversely, the increased levels of modified DNA bases may contribute to the genetic instability and metastatic potential of tumor cells in fully developed cancer (28). However, another studies report while on one hand an intense oxidative stress may kill cells being less effective in introducing DNA modifications in a cell population (5), by the other hand there may be cases in which oxidative DNA damage levels are increased, but cancer development does not ensue (29-31). It has been suggested that perhaps oxidative DNA base damage alone is insufficient to cause cancer development, or damage over only a certain range is effective,

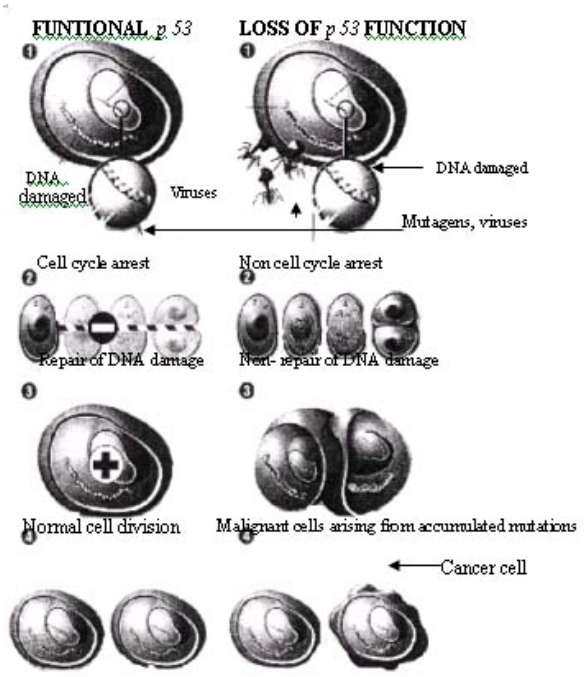

Figure 3. Role of *p53* in the cycle cell. Consequences of *p53* loss functions

excessive damage having an anti-cancer effect by promoting apoptosis (32).

3.3.1. ROS and apoptosis

Over 70% of human cancers have defects in genes upstream or downstream of *p*53 function as one of the most frequent mutations found in human cancer (33, 34). *p*53 controls cell cycle and induces cell death by a multitude of molecular pathways that include apoptosis through transcriptional regulation of pro- and anti-apoptotic proteins. ROS are powerful inductors of *p*53 activity, triggering apoptosis by mechanisms requiring transcription and also by transcription-independent mechanisms (35). Alterations of the *p*53 pathway influence the sensitivity of tumor cells to apoptosis (36). In the presence of ionizing radiation or other sources of ROS-related DNA damage, the expression of *p*53 increases, and a delay of cycle cell does occur, which allows DNA repair before replication (Figure 3). In contrast, cells lacking functional *p*53 proceed with cell divisions and thus permit DNA damage to be carried out in the following generations, leading to continued chromosome rearrangement from the initial DNA damage (Figure 3) (5). Recent reports have revealed a possible ROS-induced apoptosis mechanism. ROS seem to have the ability to signal *p53* translocation to the nucleus and this ROS-induced translocation of *p53* could be an indication of DNA damage by these species. Once in the nucleus *p53*, by DNA repair, maintains the integrity of the genome. This observation is supported by several reports that point to a marked translocation of *p53* to the nuclear compartment after exposure to H_2O_2 (37-39). At the same time, the genotoxic-induced *p53* relocalization appeared to be cell cycle-specific, since cells in the G0/G1 stage had more abundant nuclear-associated *p53* and were also more susceptible to H_2O_2-induced apoptosis than the cells in G1/S phase (35). These findings may contribute to the reports from several studies suggesting one of the role of *p*53 consist of protecting cells from spontaneously generated ROS-induced carcinogenesis (40).

3.3.2. Further mechanisms of ROS-induced tumor progression

Several models of environmentally induced lung endothelial injury have shown the regulatory roles of ROS in the endothelial cells during the vascular phase of cancer metatasis. The authors have suggested that other pathways for ROS involvement in metastasis include the generation of reactive oxygen intermediates by cancer cells, damage to vascular basement membranes mediated by endothelial injury or perturbation, and direct activation of latent matrix metalloproteinases. Human cancer cells exhibit constitutive production of H_2O_2 in levels that are comparable to those formed after perturbation of leukocyte populations' (11).

Most of the experimental tumors present increased levels of inducible nitric oxide synthase (iNOS); thus, the nitric oxide ($NO^{\cdot}$) released enhance vascular permeability, which enhance tumor progression and angiogenesis (41, 22).

Angiogenesis constitutes one of the most significant events in the development and subsequent expansion of cancer cells (Figure 4). Hypoxia is present in regions of malignant tumors and is though to result from an inadequate rate of angiogenesis. The presence and extent of these hypoxic microenvironments have shown to influence in cancer progression by regulating both cell survival and the expression of key angiogenic molecules (42). Recent studies showed that short hypoxia-reoxygenation episodes over the human endothelial lead to the generation of ROS and stimulated NF-kappa B transcriptional activity. An increase in tubular morphogenesis or neovascularitation, which describes angiogenesis, was found after NF-kappa B been postulated these species may promote tumor heterogeneity, invasion and metastasis (26).

It has been estimated that most human cancers contain a large number of mutations (14). At least 11,000 individual DNA mutations exist in a single carcinoma cell of colorectal tumors (15). ROS-induced DNA damage may represent one potential source of this large number of mutations, which may arise during the development of the disease and may contribute to the metastatic potential of tumor cells. This observation is supported by evidences from breast cancer tissues, in which the potential of metastasis increased stimulation (43). The activation of NF-kappa B may also contribute to a pro-malignant phenotype by upregulating gene products that control cell proliferation and angiogenesis. This transcription factor is known to regulate certain genes associated with metastasis. Thus, it has been postulated a relevant role of NF-kappa B in later stage of oncogenesis may be to promote metastasis (22). In addition, ROS-induced mutations in *p53* may play an important role in regulating the adaptive response of cells to hypoxia by enhancing their survival and release of proangiogenic factors (44).

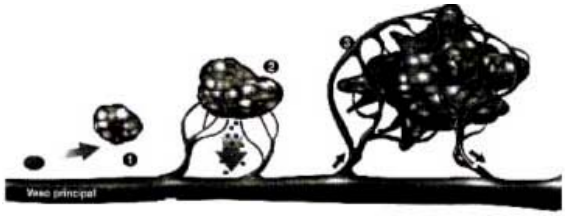

Figure 4. Role of angiogenesis in growth and proliferation of cancer cell. 1-Mutagenic cell forms a clone. 2- Evolution of tumor (releasing mediators increases the angiogenic potential. 3- New blood vessels and capillaries supply tumor irrigation, invasion and metastasis of transformed cell to other organs

Table 1. Tumors strongly related with an oxidant-antioxidant imbalance

Cancer	Key References
Breast	11, 45, 46
Colorectum	11, 45, 47, 48
Oesophagus	49
Blood (acute lymphoblastic leukaemia)	50-52
Pancreas	45
Bowel	11, 53
Lung	11, 54
Prostate	55
Skin	56
Ovary	45
Testis	45
Liver	11
Kidney and Bladder	45
Hepatobiliar	45
Bladder	11
Stomach	45

Finally, a great number of tumors can stimulate a variable intensity of immune response. It depends on the intensity of response and tumor susceptibility, the activated leukocytes-generated ROS can generate a chronic inflammation, which instead of eliminating cancer development may increase tumor progression or induce cell death through citotoxicity or apoptosis (5).

4. ANTIOXIDANTS DEFENSE SYSTEMS IN CARCINOGENESIS

Supporting the idea that ROS may be increased in tumoral cells, the phenomena described above are in consonant with disturbance activities of antioxidant enzyme (40). Table 1 show several kinds of tumors strongly related with an oxidant-antioxidant imbalance.

Antioxidant enzymes and detoxifiers have the ability to inhibit tumor promotion and initiation *in vivo* and *in vitro* assays (57, 58). The initial studies on the antioxidant enzymes, through biochemical methods in tumors homogenates, were contradictory due to, in part, the methods were used could not differentiate between the enzymatic activity of tumoral cells and other types of cells. Recent development of inmunohistochemistry offered a better comprehension in relation to the enzymatic behavior in tumor. So, analysis of these inmunohistochemical studies have revealed that: 1-there is no translocation in the subcellular location of the enzymes in the human cells under study; and 2-in cells with low Mn-SOD levels, a decreased mRNA level for this enzyme was also indicated (59).

Mn-SOD constitutes an enzyme with variable activity in tumors. A significant overexpression of Mn-SOD has been found in gastric and colorectal adenocarcinoma. Similarly, other studies have revealed a significant increase of Mn-SOD mRNA in both oesophageal and gastric cancers, compared to normal tissue (60). Overexpression of Mn-SOD reduces the levels of intracellular ROS and prevents cells death (61). In addition, it has been speculated that tumors with high SOD levels resist ROS-generating therapies through ionizing radiation (62).

However, the increased activity of SOD in some tumor cells is not a characteristic of all tumors (63, 64). Thus, Mn-SOD is reduced in a variety of tumor cells and the lowest activity of total SOD (Cu-SOD and Mn-SOD) has been associated with fastest growing tumor (65).

There are some evidences that tend to ascribe the deficiency of the Mn-SOD activity to a defect in the expression of the gene rather than to its deletion. It is speculated that in the early stage of carcinogenesis an impairment of the signal transduction machinery might cause the defect in the Mn-SOD gene expression, taking into account the second messenger function of ROS which activates transcription factors. Therefore, transition metals have found to be highly reduced in several tumors. This observation, combined with the deficiency of these two transition metals, may result in the limiting the binding of transcription factors like AP-1 and NF-kappa B to the DNA, leading to a defect in the genetic expression of Mn-SOD (66).

On the other hand, some studies have detected a downregulation of Mn-SOD by *p53*. In fact, while a significant increase in the enzyme activity was observed after gene suppression, a significant reduction in Mn-SOD m RNA expression was also confirmed after transient *p53* transfection in Hela cells, which leads to a decrease in the enzyme activity. Since an abnormally increased SOD expression does occur after protein *p53* lack function in most of human cancers, the enzyme represents an attractive target for protein *p53* (35, 36).

Studies in tissue cultures have shown cDNA for Mn-SOD transfection from tumoral cell lines (melanoma, breast carcinoma, squamous cell carcinoma) (59, 62, 67) suppresses tumor development through an increase in the Mn-SOD *in vitro* and *in vivo* activity. Despite the fact the mechanisms by which this suppression occurs are not well established, some evidences appear to indicate a decrease in the $O_2^{-\cdot}$ levels and an increase in the H_2O_2 levels respectively, instead of cell death induction (68). It has been suggested these changes may exert some disturbance on the cell redox environment, which may induce changes in relevant

physiological pathways resulting in some reduction in tumor development (65).

Another important therapeutic repercussion is the low capacity of a variety of tumors for detoxifying H_2O_2 due to a decreased CAT and GSH-Px level (69). However, other findings have shown GSH-Px and CAT activities significantly increased as compared to cancer-free tissues. An overexpression in the case of GSH-Px could explain the obtained results (70-72).

Other studies in relation to antioxidant status in human cervical carcinoma showed a remarkable reduction in the content of GSH, vitamin E and C, GSH-Px and SOD when compared to normal controls ($P< 0.001$). The reduction was more marked in late stages (II, IV) than in early stages (I, II) ($P< 0.001$) (6). However, our results on the activities of GSH and on the antioxidant defense enzymes SOD and CAT in Jamaicans women with cervical cancer, at early stages, showed no substantial changes in the GSH and SOD levels in patients compared to that of controls (normal healthy women). On the contrary, CAT activity was significantly higher in patients than that of the controls (unpublished data).

The GSH/GSSG ratio in blood also decreases in patients bearing breast or colon cancers. This change associates with higher GSSG levels, especially in advanced stages of cancer progression. The results above may be due to the increased peroxide generation, which leads to an affectation in the GSH-related enzymes, and an increased GSSG release from different tissues within the red blood cells (73, 74). In fact, these high GSH and peroxide levels in the cells have been reported when a substantial proliferative activity exits. On the other hand, this antioxidant content decreases when cell proliferation and the rate of protein synthesis in the tumor decreases (74, 75).

Another endogenous antioxidant is coenzyme Q10 (Q). Q levels in tumor tissues of twenty-one breast cancer patients, who underwent radical mastectomy and were diagnosed with infiltrative ductal carcinoma, were lower than corresponding noncancerous tissues (76). This could reflect consumption of Q against peroxidative damage *in vitro* tissues, taking into account the notable antioxidant *in vitro* e *in vivo* activity of the reduced form of Q (QH_2) (77-79).

4.1. Antioxidant Therapy

The ROS-induced disease treatment with antioxidant represents a therapeutic approach. However the mechanisms exerted by most of the chemotherapeutic agents and the ionizing radiation inducing tumor cell death were associated with an enhancement of the oxidative stress effects leading to irreversible tissue damage instead of increased antioxidant actions (64). These observations indicate that relatively low oxidative stress levels promote cellular proliferation often causing degenerative processes and death. Approximately half of all cancer patients receive radiation therapy as their disease management. It has been demonstrated that exposure of cancer patients to therapeutic doses of ionizing radiation cause DNA base modification in lymphocytes.

Another alternative approach in cancer treatment constitutes the use of the anthracycline derivatives. Cytotoxicity of these drugs, such as doxorubicin, has been attributed to the inhibition of topoisomerase II as well as intracellular production of free radicals (80).

Other cancer therapies include the antioxidant enzymes cDNA transfection as a method to modify the redox environment, the use of antioxidants enzymes and low weight compounds and liposoms among others. These therapies should be designed taking into account the tumor specifies and the cell pro-oxidant / anti-oxidant balance (81).

As the role of ROS involvement has become clearer, there has been a general agreement to use antioxidant interventions taking into account the hypothesis of ROS involvement in several pathological conditions. Before proceeding with clinical trials it is essential to consider the following points:

- Oxidative damage involvement in the physiopathology of diseases (measurements of the biological relevance of oxidized molecule concentrations).
- The role of disrupted oxidative balance in pathological conditions (central or secondary).
- Possible impairs in the antioxidant defense systems.
- The oxidative damage location.
- The probability of reaching the desirable concentration in the target by the agent.
- Impact of selected antioxidant in the oxidative conditions.
- Tolerance and safety of dosage.

Growing interest in such therapies represents the association between high antioxidant intake and the incidence of cancer. In fact, the epidemiological literature points to greater consumption of fruits and vegetables to decrease cancer risk (82-84). However, is difficult to clarify fully the effect of dietary components on oxidative stress; thus, a total correlation can not be established between these two factors (35). The use of flavonoids and other related compounds as alternative approaches in relation to mutagenesis prevention are also widely discussed in the lay literature. At the same time, special attention must be put on the molecular biology studies, which makes it possible to increase the antioxidant enzyme's intracellular expression.

The foregoing sections take into consideration several relevant trials about the relationship between dietary micronutrients consumption and cancer risk.

5. MICRONUTRIENTS AS CHEMOPREVENTIVE

Optimum intake of micronutrients reduces the risk of cardiovascular disease and cancer, and influences the long-term health. A deficiency in micronutrients is a plausible explanation for the strong epidemiological evidence that shows an association between low consumption of fruits and vegetables and cancer. The major naturally occurring dietary free-radical scavengers (vitamin

C and E and beta-carotene) have been shown in a number of different studies to inhibit initiation, promotion and progression. However, evidence is becoming stronger supporting these compounds may exert an anticarcinogenic effect through other mechanisms apart from their antioxidant function. The foregoing sections present several micronutrients research studies in attempting to clarify the role of these compounds in cancer risk.

5.1. Carotenoids and Vitamin A

Carotenoids are members of a family of widely distributed pigments in fruits and vegetables. The actual knowledge of the human toxicology of carotenoids is derived almost exclusively from works on beta-carotene. The absorption, uptake and tissue distribution may differ among the different carotenoids, some of which are bioavailable and others of which are not (85).

Of the 600 or so carotenoids that have been identified, beta-carotene has received special attention. Several clinical trials have shown reduced lung cancer incidence after increments of beta-carotene ingestion. Despite the promising results revealed in the studies, there appears another ambiguous clinical trials. Thus, the Functional Food Science and Defense against Reactive Oxidative Species (85) summarizes several experimental trials in relation to beta-carotene and the risk of cancer. The first one, a Linxian (China) trial of 29, 584 adults over a five year period revealed significant reductions in mortality from total and stomach cancer (13 % and 21 % respectively) in the group randomized to 15 mg beta-carotene in combination with 30 mg vitamin E and 50 mg Selenium. However, a Finnish study of 29, 133 chronic heavy smokers tested the effects of 20 mg beta-carotene, either alone or in combination with vitamin E, for an average of six years. There was a significant increase of lung cancer incidence (16 %) in the group that received beta-carotene. People smoking for over an average of thirty years were more sensitive to an increased risk of lung cancer. Another study of 18, 314 subjects at a high risk for lung cancer (heavy smokers, and asbestos-exposed workers), assayed the combination of 30 mg beta-carotene and vitamin A over a four years period. The experimental group had a significantly increased risk of lung cancer. A reduced risk of lung cancer (relative risk 0.80) was seen in subjects who were former smokers at the beginning of the study. On the other hand, participants with high serum beta-carotene levels had a 31 % reduction in risk of lung cancer, when compared with the group they were randomized to. These observations were supported by a Physicians' Health Study, which was conducted over 12 years in 22, 071 male physicians who consumed 50 mg beta-carotene every other day. There was no beneficial influence of this micronutrient on cancer incidence.

Beta-carotene has also received special attention with respect to photoprotection. Experimental studies had shown that beta-carotene provided significant protection to UV-carcinogenesis (86-88). At the same time, almost all of the large number of prospective and retrospective epidemiological studies of either the intake of foods rich in beta-carotene, or high levels of blood beta-carotene, have reported a strong association with reduced risks of skin cancer (89, 90). However, in this case the role of beta-carotene as an anticancer dietary supplement has also been questioned as a result of a clinical trial in which incidence of nonmelanoma skin cancer was unchanged in patients receiving 50mg/day of beta-carotene supplement for a five years period (91). Further, recent experimental studies have failed to demonstrate a photoprotective effect of beta-carotene to UV-carcinogenesis (92) and, on the contrary, a significant exacerbation of UV-carcinogenic expression, with respect to tumor latent period and multiplicity, has been reported (93).

Carotenoids beta-criptoxanthine, lycopene, lutein and zeoxanthine may also have a protective role in breast cancer as suggested by a recent finding (94).

The mechanisms by which carotenoids carry out their protective effects in cancer are not completely understood. Scientific results indicate that beta-carotene is a powerful singlet oxygen quencher and exhibits strong antioxidant properties (95, 96). In addition, it has also been reported their inhibitory properties on the arachidonic acid metabolism, on chromosome instability, and on ornitin decarboxilase, adenilate and guanilate ciclase activities (97). But, by the other hand, beta-carotene can act as a prooxidant at high oxygen concentration and it has been seen that, under oxidative stress conditions, carotenoids exhibits either limited antioxidant properties or a prooxidant effect (98-100). Therefore, the response to beta-carotene supplementation might depend on the presence and interaction with other dietary compounds, as well as the concentration (ineffectual or exacerbated) of these dietary factors and the absent of some of them. In fact, a short-term phase I toxicity trial of supplemental beta-carotene in a small number of human volunteers demonstrated a continued statistically significant decrease in serum vitamin E concentration during supplementation for 9 months with 15, 30, 45 and 60 mg beta-carotene/d. Although, other studies have demonstrated no such interaction (85).

Additionally, a mechanism indicating a possible interaction between among beta-carotene, alpha-tocopherol and vitamin C was proposed. In this context alpha-tocopherol, in terminating the radical-propagating reaction, forms alpha-tocopherol radical cation, which in turn, would be repaired by beta-carotene to form the carotenoid radical cation. This radical would be repaired by ascorbic acid (101). Because of it is hydrophilic characteristics it is anticipated that the ascorbate radical would be formed in the hydration shell surrounding the membrane (102).

Another factor to consider could be the life style, taking into account the high risk for lung cancer in heavy smokers and asbestos-exposed workers as discussed above.

Finally, since the expression and function of gap junctions is very frequently downregulated in cancer cells, and growth inhibitory signals is believed to pass through these connexons facilitating aberrant proliferation, it could be interesting to evaluate the carotenoids gap junctions-related effects. This observation is based on carotenoids-induced increase communication and thus, limiting aberrant proliferation (65). In addition, it has been postulated from *in vivo* studies conducted in rats liver, protective effects of

alpha-carotene, beta-carotene and lycopene in cancer promotion could be due to their enhancement of gap junctional communications. At the same time these effects appear to be critically dose-dependent, with suboptimal doses having no effect and excessive doses causing inhibition instead of enhancement (103). However, in view of the complexity of the measure of nutrients in human tissues, the studies on diet-related effects on gap junctional communications appear to be difficult.

5.2. Vitamin E

Vitamin E is the major fat-soluble antioxidant in the cellular membranes. The hepatic alpha-tocopherol transfer protein recognizes only the 2R-alpha-tocopherol forms and not 2S or other naturally ocurring vitamin E forms, such as ganma-tocopherol or tocotrienols. The RDA is 15 mg alpha-tocopherol, or 22 IU of natural RRR-or 33 IU of synthetic all rac-alpha-tocopherol (104).

Epidemiological studies have been shown that people taking vitamin E supplements appear to lower their risk for different types of cancers, such as colon cancer (105, 106), esophageal cancer (107) and prostate cancer (82, 108, 109). Several reports have demonstrated that the intake of vitamin E (200 UI/day) reduces the incidence of colon cancer (110, 111). It has been postulated that alpha-tocopherol can lead to apoptosis in colorectal cancer cells by inducing p21wafi/cip1, a powerful cell cycle inhibitor (107). On the other hand, promising results have been obtained in the esophageal cancer reduction by consuming vitamin E in combination with Selenium (Se) and beta-carotene (112).

The protective effect of vitamin E in cases of oxidative DNA damage may result from the inhibition of free radical formation and activation of endonucleases that can be triggered by intracellular oxidative stress, as well as by increasing the rate of the removal of damaged DNA (113-115).

Alpha-to-copherol has been shown to reduce the generation of $^{\cdot}OH$ induced by H_2O_2 with the subsequent DNA base modification in human cells from oral epithelium (116) and the DNA strand breaks in VH 10 cell lines from the skin (117). Furthermore, it has been demonstrated the inhibition exerted by this micronutrient against lipid peroxidation in HL-60 cells and the prostaglandin F_2 and LPS-induced apoptosis in ovine corporal luteal cells and in human endothelial cells respectively (118-120). The mechanisms underlying the apoptosis inhibition by vitamin E could be explained through their antioxidant actions, taking into account that apoptosis constitutes one of the possible mechanisms for the preventive effect of antioxidants on cancer development (115).

Despite the data available that collectively suggest vitamin E protects against cancer, several reports point to perplexing results obtained from interactions of vitamin E and vitamin C. Thus, when alpha-tocopherol (30 μM) or ascorbate (600 μM) are added separately, each vitamin has a protective effect against the oxidative DNA damage in human sperm (121). Nevertheless the same study reported the addition of both vitamins together caused damaging effects. When vitamins were mixed by using a concentration of 30 μM and 60 μM for vitamin E and vitamin C respectively, while neither a harmful nor beneficial activity on H_2O_2-induced DNA damage in human lymphoblastoid cells was revealed, an increased radiation-induced genome damage was evident (122). Other study showed negative results for vitamin E in combination with vitamin C and beta-carotene to prevent colorectal cancer adenoma over a period of 4 years (123). And another trial revealed no effects of supplementation with vitamin E, ascorbic acid, and coenzyme Q on oxidative DNA damage, estimated by 8-oxo-7, 8-dihydro-2'-deoxyguanosine excretion in smokers (124). Finally several others data point to disappointing results in the vitamin E afforded protection against oxidative damage in humans (125-128). Since vitamin C regenerates vitamin E from it's reduced form (tocopheroxy radical), it has been suggested that the addition of vitamin E hinders the protective effect of vitamin C against the oxidative DNA damage (5). However, an exhaustive analysis in the vitamins high dosage effects and the time of treatment in cancer patients should be carried out.

5.3. Vitamin C

Vitamin C (ascorbate), a water-soluble antioxidant, has a prominent *in vitro* antioxidant action. At the same time several studies have pointed out ascorbate inhibits the formation of carcinogenic nitrosamines, stimulates the immune system, protects against chromosomal breakage, and regenerates vitamin E as part of the antioxidant defense system (85). The epidemiological evidence for a risk-reducing role of vitamin C in cancer is not very strong. However, a consistent protective effect of vitamin C has been found in studies of some types of cancer such as: oesophageal, lung, stomach, colon and rectal (129-132). Moreover, additional studies showed that subjects with low serum levels of vitamin C have a 50 % increased risk of gastric metaplasia or chronic gastritis, which are both precancerous lesions (133).

Another studies have revealed the protective effects of this micronutrient against the oxidative DNA damage *in vivo.* Thus, perhaps, the antioxidative properties could be, in part, responsible for the effects of ascorbate in cancer, though other properties of ascorbate appear to contribute (134, 135). However, data from biomarkers of oxidative DNA damage are not sufficiently convincing, except perhaps in subjects with very low vitamin C intakes such as the heavy smokers (137-139). A plausible explanation of these observations appears to be due to the variability of tissue saturation (84, 125, 139).

Other reports suggest vitamin C also offers protection from stomach cancer (125), but limited evidence was provided by several populations (140). Such reduction may be exerted through vitamin C-induced inhibitory action in the generation of n-nitroso compounds by interrupting the reaction between nitrites and amine groups (141).

An study in which 2 g/day 5, 6-benciliden-L-ascorbate (BA) was intravenously administered in patients with advanced malignant tumor, showed a fast and important reduction in the tumor size with no adverse reactions. A plausible explanation could be the BA-induced *in vivo* tumor apoptosis, which was not blocked either by CAT or cysteine analogues (142).

A controlled intervention trial with daily doses of vitamin C (500 mg), giving in combination with alpha-tocopherol (200 mg) and coenzyme Q10 or placebo did not reveal any change in the rate of DNA oxidation, as measured by urinary excretion of 8-oxodG (124). By the other hand, recent studies suggest that ascorbate sometimes increases DNA damage in humans. Although there is no evidence that these effects are deleterious to humans, it is an important point to consider in future studies, taking into account other reports has postulated vitamin C exhibits prooxidant properties (143, 144).

Finally, the conclusion from an exhaustive survey of the literature is that oral intake of high (up to 600 mg/day, i.e. six times the current RDA) levels of vitamin C are safe and entirely free from side effects. At the same time, levels up to 2000 mg/day have not been consistently reported to result in side effects (85). But, taking into consideration that minor reports have shown vitamin C may exert prooxidant actions, an attentive precaution should be considered with the use of high intake of this antioxidant vitamin.

5.4. Selenium

Several investigations have revealed the preventive role of selenium (Se) against cancer in a variety of organs and species. Despite the association between low selenium level and advanced tumor disease, it yet to be decided whether this phenomenon is more likely to be a consequence or a causative factor for development and course of the disease (145-147).

Foods such as meat, seafood, grains and poultry, constitute the major source of Se. Some of the studied functions for this micronutrient in mammals include that to be an essential component of the GSH-Pxs and thioredoxin reductase. The structures of the organoselenium compounds in foods have not been completely elucidated. Currently, the US Food and Drug Administration only approve selenium-enriched yeast as a supplement for human usage. Several clinical intervention trials (148, 149) have used Se compounds such as selenomethionine, Se-methylselenocysteine, selenocysteine and selenoethionine which have been identified in selenium enriched yeast. However, the form of Se that is responsible for cancer prevention remains undefined (145).

Two clinical intervention trials, both conducted in China, used dietary supplements containing 50 μg Se/day in combination with other nutrients to prevent esophageal cancer. The former, giving 50 μg Se/day as selenium-enriched yeast and combined with beta-carotene and alpha-tocopherol, showed a significant reduction and a lower mortality from stomach cancer in relation to placebo groups (150). The other trial, giving 50 μg Se/day as inorganic selenium, in combination with another 25 vitamins, did not detect a significant effect on the development of esophageal cancer (151). Another randomized controlled trial was carried out in the US to 1312 patients with a history of basal cell or squamous cell carcinomas of the skin. The aim consisted in to determine whether a nutritional supplements of Se (200 μg/day) decreases the incidence of cancer. The results of this study did not show that Se supplementation reduces the risk of carcinoma of the skin. However, Se supplements revealed to be associated with significant reductions in secondary end points of total cancer incidence (lung, colorectal and prostate) and lung cancer mortality (148). Since supplementation in rodents with inorganic selenium or various forms of organoselenium compounds have shown inhibit formation of 8-OH-dG, it has been postulated the reduction of lung, colon and prostate cancers in the clinical trials that employed selenium-enriched yeast could be due to, in part, to a reduction in various kinds of oxidative stress, including 8-OH-dG (145).

Several *in vitro* studies and others, conducted in rodents, have shown that various levels and forms of Se compounds inhibit carcinogen-induced covalent DNA adduct formation and DNA oxidative damage (152-155). However, since some features of Se metabolism are specific to humans, these results need to be demonstrated in human populations (145, 156).

In a study of Se intake and colorectal cancer individuals in the lowest quartile of plasma Se had four times the risk of colorectal adenomas compared to those in the highest quartile (157). By the other hand, in a nested, case-control prospective study on ovarian cancer, serum Se was associated with decreased risk (158).

Selenium and GSH-Px levels were found to be lowered in patients with carcinoma of uterine cervix (159, 160). Since GSH-Px is a Se-dependent enzyme, it seems to be clear a Se deficiency may be the cause of the reduction in their levels, and thus contribute to cancer development among probably other mechanisms unrelated to the activities of the selenoenzymes.

Experiments employing cell cultures have showed Se induces apoptosis and inhibits cell growth in transformed cells (161, 162). In addition, induction of the *p*53 gene by selenium compounds was demonstrated. However, another reports indicate the induction of apoptosis by Se may not be entirely due to the response of *p*53 (163).

Finally, the recommended daily allowance is 50-70 μg Se per day for healthy adults, but intake of 200 μg Se per day have been used with safety in clinical trials in relation to cancer. However, despite the use of up to about 700 μg Se per day with no apparently adverse clinical symptoms (164-166), the intake of an

excess of Se may result in oxidative damage and genome instability (167).

6. DIRECTIONS FOR FUTURE RESEARCH

While many details regarding the role of ROS-induced DNA damage in the etiology of complex multifactorial diseases like cancer are yet to be discovered, it is evident that oxidants act at several stages in the malignant transformation, since they can induce permanent DNA changes. Thus, it is becoming increasingly clear that oxidative DNA damage plays a role in the development of cancer. In addition, most human cancers contain large numbers of mutations and severe oxidative stress may serve as an efficient source of mutations during tumor progression. However, to date no one has successfully proved that oxidative DNA damage constitutes a valid biomarker for cancer development. It would need measuring DNA of healthy human subjects over many years to see who develops cancer (168).

Diets rich in fruits and vegetables are commonly associated with decreased incidence of the major human cancers. As described above, multiple mechanisms can account for this, but some other studies have revealed no effects in decreasing oxidative DNA damage**,** at least in the population examined, which was supplemented with high intake of micronutrients in respect to current RDA values. The response to antioxidant supplementation might depend on the presence and interaction with other dietary compounds, as well as the concentration of these dietary factors and the absent of some of them. Since tumors generally appear in about a decades after a permanent change in one-cell genetic material occur, it is important to take into account that clinical trials need to be long enough to evaluate the effect on cancer. It also might be difficult to clarify fully the effect of dietary components on oxidative stress, because many other factors (endogenous and exogenous) have an influence. Exogenous factors affecting oxidative stress include ionizing and non-ionizing radiation, smoking, certain diseases such as autoinmune problems, chronic hepatitis, alcohol intake, air pollution, physiological stress and extraneous exercise among others**.**

The mechanism trough ROS plays an important role in the initiation and progression of cancer, is not yet fully understood. By the other hand, ROS mediated–tumor promotion needs for further experimental evidences in humans.

Finally, in the near future the use of valid biomarkers will provide new insights in the experimental studies in relation to the qualitative and quantitative importance of the oxidative DNA modification and cancer development in humans. These may lead the way to elucidate possible preventive tools.

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