ORAL CARCINOGENSIS AND ITS PREVENTION

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Abstract

As squamous cell carcinoma is the predominant type of oral malignancy , therefore methods to prevent, detect or treat it in the best way is constantly being searched for.Biomarkers are such agents which reveal genetic & molecular changes related to various endpoints of oral carcinogesis & thereby refine our ability of predicting it's biological course.They will refine our ability in allowing to predict the biologic course of oral cancer and thereby distinguishing individuals at high and or low risk of oral cancer development.Chemopreventives are agents whose curative capacity is defined with help of biomarkers so as to determine their effectiveness & safety.

Keywords – Biomarkers,Chemopreventives, alpha tocopherol, carotene, Retinoid

Introduction

The predominant type of cancer found in the oral cavity is squamous cell carcinoma. Carcinogenesis is a process by which normal cells are transformed into cancer cells and is characterized by a progression of changes on cellular and genetic level that ultimately reprogram a cell to undergo uncontrolled cell division, thus forming a malignant mass. Mutations in DNA that lead to cancer (only certain mutations can lead to cancer and the majority of potential mutations will have no bearing) disrupt these orderly processes by disrupting the programming regulating the processes.Carcinogenesis is,thus, caused by this mutation of the genetic material of normal cells, which upsets the normal balance between proliferation and cell death. This results in uncontrolled cell division and the evolution of those cells by natural selection in the body.

Genes which regulate cell growth and differentiation must be altered and genetic changes can occur at many levels, from gain or loss of entire chromosomes to a mutation affecting a single DNA nucleotide. Two broad categories of genes which are affected by these changes are-

1) Oncogenes, which may be normal genes expressed at inappropriately high levels, or altered genes which have novel properties and therefore, promotes the malignant phenotype of cancer cells.

2) Tumor suppressor genes are genes which inhibit cell division, survival, or other properties of cancer cells. Tumor suppressor genes are often disabled by cancer-promoting genetic changes.

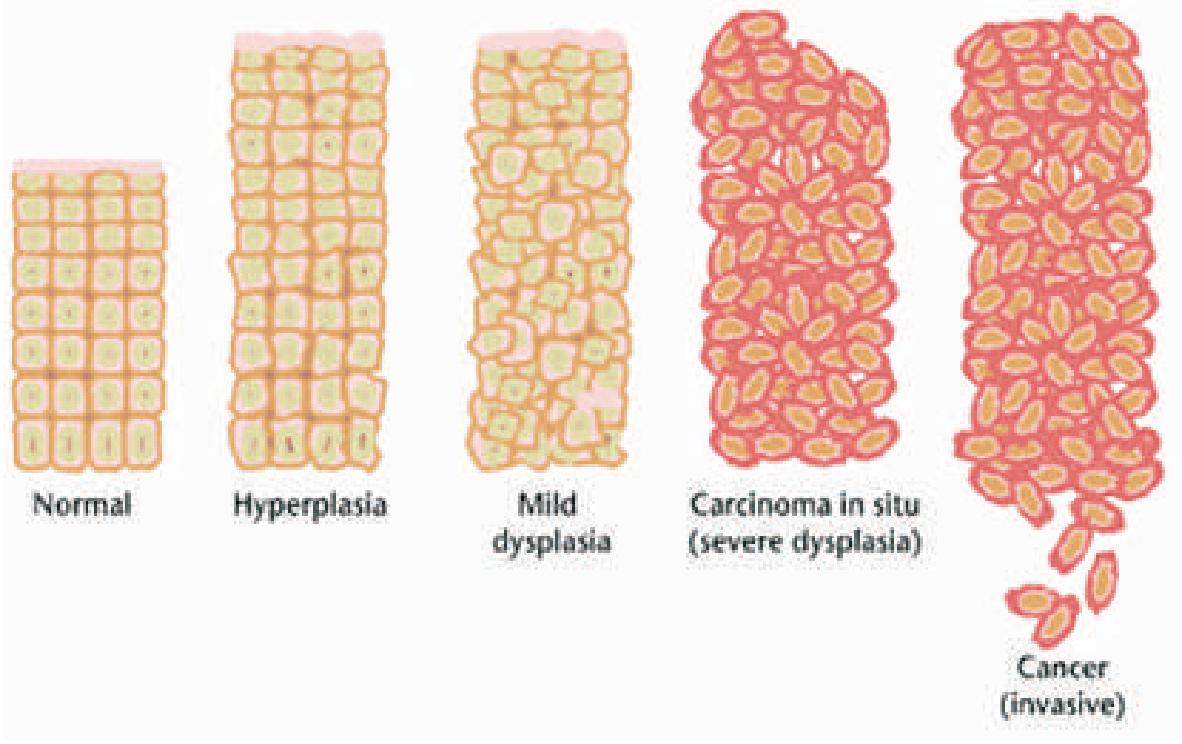

Fig-1 :Cancers are caused by a series of mutations. Each mutation alters the behavior of the cell somewhat.

Biomarkers help in the evaluation of prevention or use of therapies and the detection of the earliest stages of oral mucosal malignant transformation. Biomarkers reveal the genetic and molecular changes related to early, intermediate, and late end-points in the process of oral carcinogenesis. These markers will refine our ability in allowing to predict the biologic course of oral cancer and thereby distinguishing individuals at high and or low risk of oral cancer development. Genetic and molecular biomarkers will also determine the effectiveness and safety of chemo preventives. Biomarkers will also reduce the number of patients and the time for long-term follow-up required to define a significant clinical response to a chemo preventive agent, thereby clarifying the types, doses, frequencies, and regimens to achieve the maximum level of benefit from chemo preventives. This review will focus on the development of genetic and molecular biomarkers, and the use of chemopreventives to intervene in the oral carcinogenesis process[2]

Roles of Cellular Biomarkers

(A) Indicators of DNA repair mechanisms.
(B) Indicators of PCD
(C) Indicators of Tumor development & growth
(D) Indicators of genetic markers of oral cancer

(A) Indicators of DNA Repair Mechanisms

Programmed cell death functions not by itself but in concert with systems that produce DNA repair. Present evidence indicates that cancer cells require a high level of DNA repair. In general, these include repair of the telomeric ends of chromosomes produced through the action of telomerase, and repair of nucleotide sequences, exemplified by mismatch repair, and nucleotide excision repair. Telomeres are heterochromatic structures at the ends of eukaryotic chromosomes and consist of simple, highly conserved, repeated DNA sequences (e.g., TTAGGG, as observed in humans and mice). Every species has a characteristic average of telomeric subunits. In human oral carcinomas, telomerase is

elevated in the proliferative areas of the carcinoma. Defective repair processes and checkpoints are also linked to cancer genomic instability. The types of genomic instability seen in most cases of sporadic cancer suggest a commonality of break types and DNA repair processes. Examples of the more common DNA repair sites are the approximately 20 genes known to be involved in the process of nucleotide excision repair (NER) or the repair/transcription factors, such as TFIIH, that are required to orchestrate the function of incisional proteins, i.e., DNA polymerases, and ligases. These changes may become additional markers for the aggressive and metastatic characteristics of oral carcinoma. DNA repair influences the progression of oral carcinogenesis through the regulation of various growth factors- for example, transforming growth factor, TGF-3. The development of a DNA repair defect and the presentation of a mutated TGF-3 receptor gene (e.g., glutamine and proline replaces a glutamic acid and arginine) have been considered to promote oral carcinogenesis[1]

(B) Indicators of PCD (Programmed Cell Death)

There is a considerable body of work that has identified several hundred cellular changes or biomarkers associated with the growth of oral carcinoma and carcinomas of the aero-digestive tract. Many of these indicators are also markers for programmed cell death (PCD). PCD, or gene directed apoptosis, is a common means used in nature to remove unwanted cells. It is characterized by the lack of an inflammation-driven necrosis of the tissue and the histologic appearance of apoptotic cells. PCD is also an important feature of oral keratinocytes undergoing differentiation or transformation during oral cancer development. PCD may also play an important screening role in cancer formation. PCD results in the modification of the surviving cell population in transforming clones by altering the numbers and types of cells in a tumor. The surviving transforming cells appear to have suppressed PCD, and they have a high rate of proliferation, enhanced levels of resistance to different anti-tumor therapies, and elevated levels of DNA repair. There are numerous genetic and molecular changes that are used to identify PCD.[3]

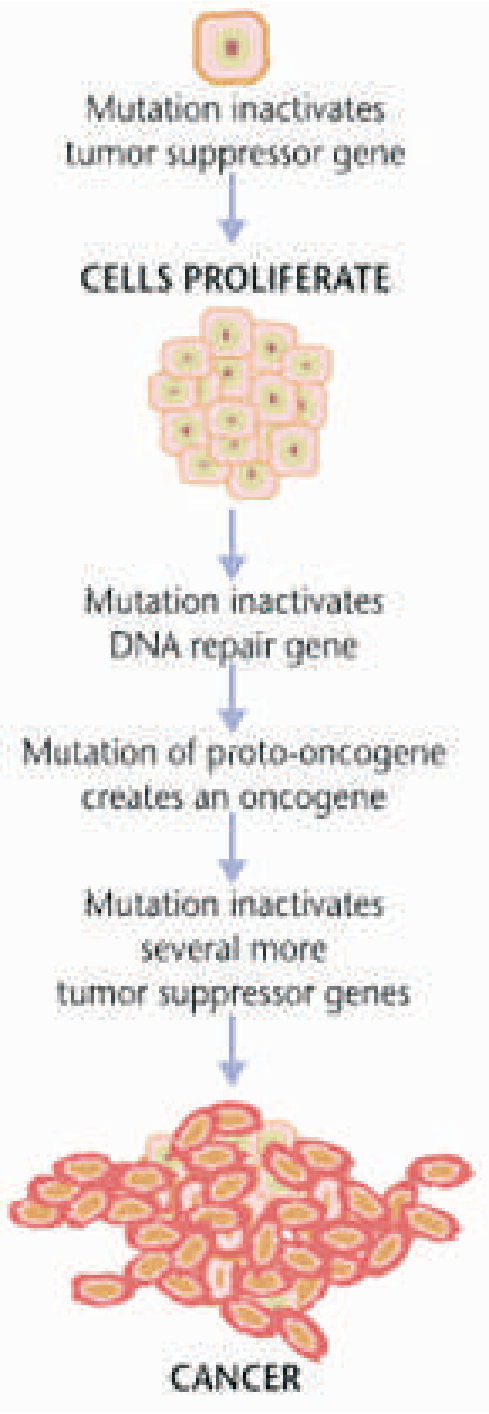

Fig-2 : Steps of Carcinogenesis

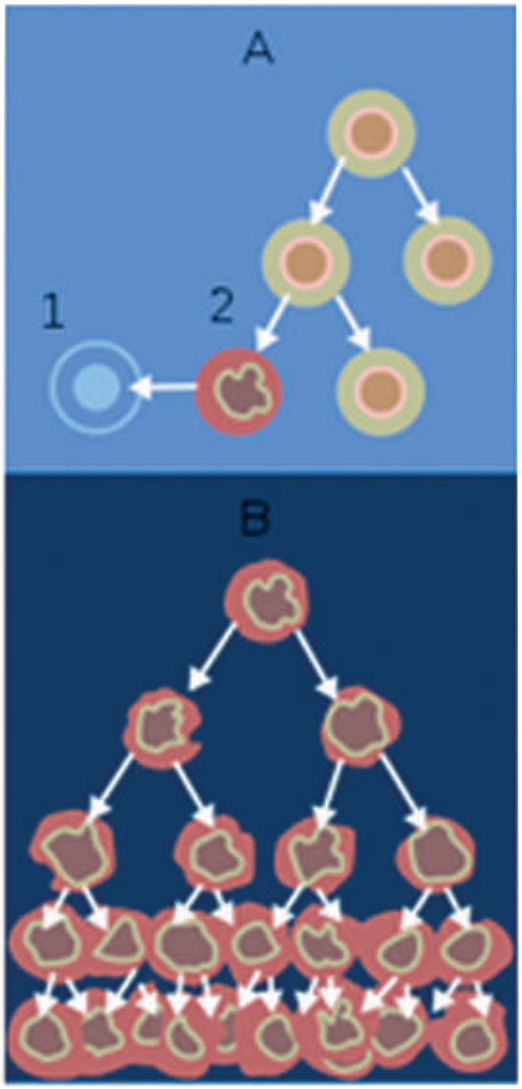

Fig-3 : When normal cells are damaged beyond repair, they are eliminated by apoptosis (A). Cancer cells avoid apoptosis and continue to multiply in an unregulated manner (B).

Oxidation and The Effect on PCD : -

Oral malignant transformation could be a product of oxidative state change. The oxidative state change would then result in changes in DNA repair and PCD. The observed cellular manifestations of these processes are losses of cell growth control and modifications in cell-cell interactions, which could enhance the potential for tumor metastases. Nutrients that act as chemopreventives alter the oxidative state of the oral transforming cell by acting as reducing agents (e.g., anti-oxidant) and/or oxidizing agents (e.g., pro-oxidant).

Chemopreventives and PCD:-

The treatment with chemo preventives, during malignant transformation or in fully transformed malignant oral mucosal cells, results in the induction of PCD and the observed inhibition of oral carcinogenesis and malignant tumor growth. Chemo preventives induce PCD because of their oxygen-responsive characteristics, which trigger inducers such as the tumor suppressor gene p53, modifiers of PCD such as the bcl-2 family, and immune-derived cytokines-for example, TNF Chemopreventives, as exemplified by retinoids, carotenoids, tocopherols, bioflavonoids, isothiocyanates, indoles, and polyphenols, induce PCD. [8]

C)Indicators of Tumor Development & Growth

Biomarkers establish the level of risk for individuals in a cohort or target group of patients, and they may provide information concerning the etiology and the process of carcinogenesis. The primary goal for the use of early, intermediate, and late biomarkers is to identify individuals and their level of risk. [1,6]

(D) Indicators of Genetic Markers of Oral Cancer

A developing solid clone of transforming cells found in an oral carcinoma arrives at the state of malignancy by proceeding through stages of transformation. The transformation rate is dependent on the location of the clone in the spherical tumor mass and the oxygen states of the cells. [7]

Chemopreventive

Chemopreventives are chemicals of natural or synthetic origin. Unlike other drugs, which do not prevent disease, chemo preventives reduce the incidence of diseases such as cancer before clinical symptoms occur. This development is critical for the understanding of early oral mucosal transformation.

(A) Overview

A concept common to chemo

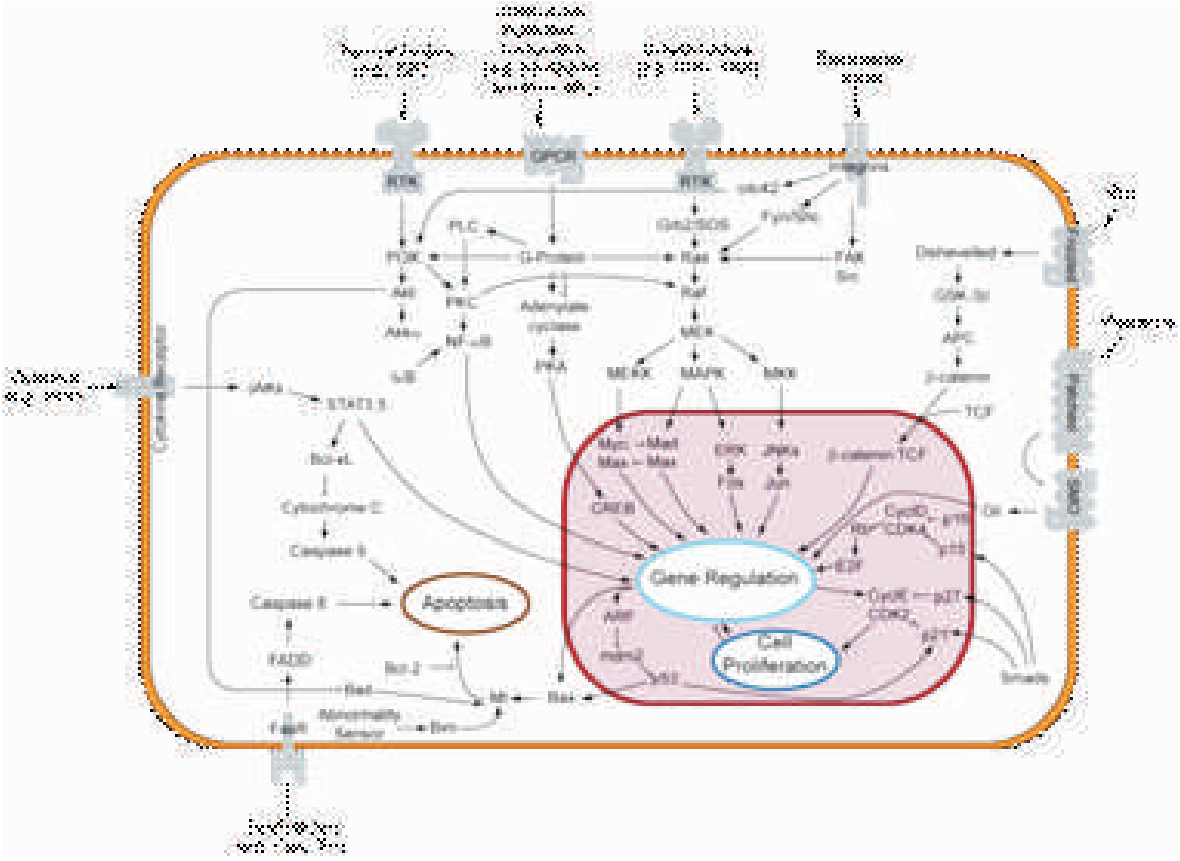

Fig-4 : Many tumor suppressor genes effect signal transduction pathways which regulate apoptosis, also known as "programmed cell death

prevention is the ability of chemical agents to function as reducing agents, anti-oxidants, or oxidizing agents (pro-oxidants). The chemical character of the chemopreventive will depend on the partial pressure of oxygen and the level of oxidative metabolites produced or derived in the cell.

β carotene, a carotenoid, acts as an anti-oxidant but can also act as a pro-oxidant, depending on the oxygen state of the cell. β -carotene as well as ellagic acid (from garlic) are carcinogen-blocking agents that either suppress promotion or act as anti-oxidants, which are reducing agents. Their specific mechanism of action is unknown, but blocking agents prevent carcinogenic compounds from reacting with critical target sites of DNA by inhibiting the metabolic activation of carcinogens catalyzed by cytochrome P450 (Phase I enzymes).

Other chemopreventive agents, such as δλ-alpha tocopherol (vitamin E), are strong antioxidants that enhance the cellular detoxification system by increasing the levels of glutathione-S-transferases (Phase II enzymes). The activation of these enzymes may also lead to the trapping of reactive carcinogen metabolites or the triggering of apoptosis.

Some chemopreventives may suppress the promotion of cancer and prevent the transformation of pre-malignant cells by altering differentiation. For example, retinoids enhance differentiation while affecting housekeeping genes and oncogene expression.

Another class of chemopreventives, the terpenes, may also inhibit oncogenic expression and cell proliferation, reducing dedifferentiation.

Indomethacin, an anti-inflammatory drug, blocks prostaglandin synthesis and

reduces tumor development, resulting in a normal differentiation pattern.

Cells also protect themselves from reactive oxygen substances (ROSs) by activating anti-oxidant pathways and molecular systems that use enzymes. Examples are superoxide dismutase, which controls the level of the superoxide anion (e.g., 0_2-)' whereas catalase modifies the levels of hydroxyl radicals (e.g., OH-), and glutathione-S-transferase (GSTs) alters the level of the intracellular anti-oxidant glutathione.

Less obvious cellular anti-oxidants are proteins such as Bcl-2, which is comprised of a family of proteins that modifies PCD.

There are also several protein families that function as redox, anti-oxidant/pro-oxidant molecules that also regulate PCD (e.g., p53). Perhaps the most important common feature of chemopreventives is their ability to trigger PCD in transformed cells. The retinoids, carotenoids, tocopherols, isothiocyanates, and polyphenols induce PCD in various cell types. Clinically, there is an advantage to the combination of chemopreventives with chemotherapeutics, especiallywith the alkylating agents. [8]

(B) Genetic, molecular, and biochemical activities of chemopreventives

(I) Retinoid Chemopreventives:-

The retinoid molecule family consists of vitamin A and its derivatives. The primary source of retinoids in the diet is retinyl esters from animal tissues and retinol from the conversion of carotenoids (e.g., 3-carotene) derived from a vegetable source. Retinol tends to be the biologically active form, but retinoic acid may substitute for retinol for many functions, and both can be found in the serum linked to proteins. Retinol binds to proteins designated as retinol-binding proteins, while retinoic acid is associated with albumin. The normal plasma level for retinol is approximately 2 mmol/L, while that of retinoic acid is 10 to 20 nmol/L, about 150-fold lower than retinol Retinoic acid is metabolized by hydroxylation of the cyclohexenyl ring, a reaction mediated by cytochrome P450, producing a 4-hydroxy metabolite which can undergo further oxidation. These oxygenreactive molecules could enhance genetic instability and result in abnormal cell growth, differentiation, and cell - cell interactions In cells, retinol and retinoic acid bind to specific cytoplasmic binding proteins, which include CRABP-I/II (cytoplasmic retinol binding proteins I and II). The cellular binding proteins mediate transfer of retinol and retinoic acid from the cytoplasm to the nucleus. The nuclear retinoic acid receptors (RAR) are members of the steroid-thyroid superfamily of nuclear receptors. Retinoid modulation of gene expression is the result of the activation of one of four possible receptors. The RARs are found in many different isoforms, as shown by mRNA, which indicates that the isoforms arise from different promoters or by alternative RNA splicing in a region at the 5' end of the mRNA. Isoforms appear to be tissue-specific, and their expression is stage-specific, suggesting distinctive roles for each isoform. The expression of mRNA for RAR-, -β, -γ, and -x has been found in normal oral mucosa. The development of premalignant change and smoking or alcohol use do not appear to change the general distribution of these receptors in oral tissue. However, recent studies have indicated that with retinoid treatment there is an increase in the level of RAR-β in pre-malignant lesions. Lotan et al. (1995) presented a study where RAR-1 was depressed during pre-malignant change and retinoid treatment, and remission of oral leukoplakia was coincident with an increase in RAR-β. It appears that RAR-β may be an important indicator for a retinoid response in premalignant or malignant oral mucosa. The aberrant response to retinoic acid, as reflected by RAR-β modulation, is also echoed by changes in related growth factor receptors such as epidermal growth factor. Retinoid ligand specificities are found in recombinant RAR-α and RAR-β receptors, with at least two retinoid analogs binding preferentially to either RAR-α or RAR-β. Initial studies suggested that RAR-β was approximately 10-fold more sensitive to all-trans retinoic acid than RAR-α. The results of these studies indicated that the blockage of the RAR changed tissue oxidation of retinol to retinoic acid, but other oxidation

processes, perhaps linked to cytochrome P450 activity, may also be altered. Recent studies have also indicated that the conversion of retinol to retinoic acid might be mediated by the RAR. The ability of retinoids to inhibit or increase the growth of cancer cells could be dependent on the accumulation and rates of conversion and non-conversion of retinoic acid in the cell. It is well-known that head and neck squamous cell carcinoma (HNSCC) patients, particularly those who smoke tobacco, have low levels of serum retinoids. Their low intake of fruits and vegetables may play a role in the development of oral cancer, but the cellular state of the oral mucosa must also be considered. The concentration of any retinoid metabolite may be initially dependent on the type, number, and affinity of the RARs present on the cell. Changes in the binding and concentration of the CRBP (cytoplasmic retinal binding protein) or CRABPs might also influence the triggering of retinoic acid response elements (RARE) and associated genes. Exogenous influences affect the conversion of retinol to retinoic acid by altering the activity of alcohol dehydrogenase and the cytrochrome P450 system noted above.In addition, retinoic acid receptors appear to be involved in the differentiation of myeloid cells and keratinocytes. Retinoic acid binding to its receptors has also been shown to induce apoptosis in the differentiating cell population.[9,10]

The finding that programmed cell death occurred following treatment with retinoic acid indicates that a cascade of definitive genetic changes was set into motion. RA induction of PCD may be different from other chemopreventive inductions of PCD by triggering BAG-1 and internal phosphorylation signals that trigger the Bcl-2 system of proteins. Other chemopreventives, such as BC or vitamin E, appear to activate the oncogene-inductive process (e.g., p53) and/or the immune-inductive process (e.g., the tumor necrosis factor ITNFI receptor family, FAS/FASL) for PCD. Retinoid-induced genetic changes will not only inhibit the growth of developing cancer cells or established cancer but also possibly promote the growth of pre-malignant or malignant cancers. These results again indicate that the responses of tissues to retinoids are more complex than is apparent from studies that emphasize RAR expression.[10,11]

(ii) Genetic and molecular activities of carotenoid and tocopherol:-

The carotenoids and tocopherols produce genetic and molecular responses similar to those observed with the retinoids.The specificities of these responses have been previously reviewed Animal studies with the hamster buccal pouch tumor model have further confirmed some of the similarities between these chemopreventive groups and the retinoids. These include the over-expression and halflife of p53, enhanced differentiation of the oral mucosa, reduction in neovascularization of developing oral cancers, and the induction of apoptosis. The major cellular differences between the retinoid response and the carotenoids or tocopherols is the lack of a complex receptor system described above for the retinoids. In general, the major biochemical similarity among these groups (e.g., carotenoids, retinoids, tocopherols) is their isoprenoid structure and biochemical sterol effects. Their sterol chemistry is derived from their hydrophobic cyclic structures that contain unsaturated double bonds.

Chemopreventives, as a group, respond to changes in oxygen partial pressure that influences their ability to act as oxygen free radical quenchers or reactive oxygen molecules.[13] In a well-oxygenated environment, thecarotenoid, β-carotene, can inhibit the growth of oral cancer cells, because the carotenoid induces an oxidative stress in the tumor cell. At the identical partial pressure of oxygen, tocopherol tends to have an anti-oxidant activity and reduces the oxidative stress. These oxygen-handling characteristics could affect the triggering of PCD, the cell cycle, by modifying kinase activities and eventually lead to an alteration in activities such as c-fos and other cellular processes linked to differentiation or DNA repair. The responses of the oral mucosal cells to nutritional agents such as retinoids, carotenoids, or tocopherols will also depend on the degree of

transformation that has occurred in the cell. Normal cells appear to react to these agents by differentiating, while transforming cells or fully transformed cells may also differentiate, but they continue toward programmed cell death. Some of the more common membrane-related responses to β-carotene and/or tocopherols are the reductions in membrane-associated enzymes such as serine and threonine kinases, connexin 43, and a reduction in ras proteins. Other previously discussed proteins, such as proto-oncogenes c-fos, cmyb, N-myc, and the tumor suppressor p53, have been shown to be affected by treatment with these chemopreventives. Stress proteins, acting as cellular chaperonins, can complex to p53, and, following treatment of oral carcinoma cells with β -carotene, their levels of expression increase and affect the movement of proteins. In addition, growth factors and immune regulatory factors such as EGF and TGF-α have a reduced expression, while TGF-β1 and TNF- α expression are elevated during the inhibition of oral carcinogenesis. The EGFR's affinity, number, and protein expression were reduced following β -carotene treatment and suppression of oral cancer growth. Studies reveal the broad effects of β -carotene and other similar chemopreventives on many different cellular functions. Importantly, the carotenoids and tocopherols do not appear to produce the toxicity seen with retinoids, but their effectiveness at preventing or reversing pre-malignant change is unclear.[11, 14]

(D) Oxidative biochemistry of chemopreventives

Chemopreventive agents can be defined by their suppression or blocking of mutagenic activity, initiation, and/or promotion during oral carcinogenesis. Agents that alter the mutagenic process are generally enzymes that enhance the solubilization or degradation of mutagenic or carcinogenic agents. This process usually activates the intracellular anti-oxidant, glutathione, and its associated system. The glutathione pathway regulates free radical activity by producing reducing agents, such as thiol-containing proteins, with cysteine-glycine residues. The primary anti-oxidant in this pathway is glutathione, which can react with electrophilic sites produced on a carcinogen molecule through the action of cytochrome P450 hydrolyses. Glutathione also acts to block the nucleophilic attack of the carcinogen on DNA. Chemopreventive agents, such as glutathione, may also function by enhancing the elimination of the genotoxic agent from the liver and other tissues. In these tissues, the hydroxylation of the cytochrome P450 system and mixed-function oxidases (MFO) eventually terminates with conjugates of reduced protein salts containing glucuronate and sulfates.[9]

Unfortunately, the activation of this system by the chemopreventive agents may also increase carcinogenesis by producing more highly electrophilic epoxides. The result could be the conversion of procarcinogens to their genotoxic form, which could generate oxygen free radicals. Many of the chemopreventives may work not only through this pathway but also by altering the level of glutathione-S-transferases (GSTs) which mediates electrophile scavenging. The carotenoids, tocopherols, and retinoids all appear to be capable of modifying GSTs. The carotenoid, β-carotene, has been shown to depress GST levels, while other chemopreventives, such as alpha tocopherol acid succinate or dithiolthione oltipaz, elevate the level of GST.

Chemopreventives have been shown to inhibit both initiation and promotion during oral carcinogenesis. The inhibition of initiation may occur by preventing the carcinogen from becoming fully active by enhancing DNA repair and/or the activation of tumor suppressor genes. The inhibition of promotion could result by triggering differentiation. Initiation and promotion may also be affected by the elimination of transformed malignant clones of cells. The induction of an inflammatory cytotoxic immune reaction or the generation of apoptosis could accomplish this. Examples of chemopreventives that have exhibited anti-promotional activity include tamoxifen (an anti-estrogen), retinoids, and carotenoids,

which are also inhibitors of proliferation. In addition, alpha tocopherol acid succinate and piroxicam are known to suppress arachidonic acid metabolism, which, in turn, could affect apoptosis or tumor development by reducing prostaglandins. Alpha tocopherol can inhibit the activity of prostaglandins by blocking their synthesis, and this occurs by a reduction in the function of cyclo-oxygenase. Leukotriene activity can also be regulated by reducing the activity of lipoxygenase. The inhibition of prostaglandins has been shown to be associated with the suppression of oral carcinogenesis.[12]

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Source of Support - Nil

Conflict of Interest - None declared

How to cite this article:

Shah Sonali, Kaur Manpreet, Oral carcinogensis and its prevention: *Oral Max Path J*, 4(1), Jan-Jun 2013: 293-301