Review

Induction of apoptosis in oral cancer cells: agents and mechanisms for potential therapy and prevention

Stephen Hsu*, Baldev Singh, George Schuster

Department of Oral Biology and Maxillofacial Pathology, School of Dentistry, Medical College of Georgia, AD 1443, Augusta, GA 30912-1125, USA

Received 17 September 2003; accepted 24 September 2003

Summary

Oral cancer is one of the most disfiguring types of cancer, since the surgical removal of the tumor may result in facial distortion. Oral cancer is also known to exhibit "field cancerization", resulting in the development of a second primary tumor. Furthermore, the five-year survival rate of this disease has remained approximately 50% during the past 30 years. Prevention and early detection/treatment of oral cancer could significantly improve the quality of life for individuals at risk. Recently, the targeted elimination of oral squamous cell carcinoma cells by inducing apoptosis has emerged as a valued strategy to combat oral cancer. Studies utilizing a variety of chemical or biological interventions demonstrated promising results for induction of apoptosis in oral malignant cells. This review summarizes the results of a number of investigations focused specifically on induction of apoptosis in oral cancer cells by synthetic compounds and naturally occurring chemopreventive agents with apoptotic potential.

© 2003 Published by Elsevier Ltd.

KEYWORDS
Oral cancer; Apoptosis; Phytochemicals; Targeted therapy; Chemoprevention

Contents

Introduction .......................................................... 00
Relationship between cell proliferation and apoptosis in OSCC ......................... 00
Exploiting the apoptotic potential of OSCC ........................................... 00
Chemotherapeutic drugs and apoptosis ............................................... 00
Antibiotics ............................................................... 00
Micronutrients, trace elements and metals ............................................ 00
Biological agents and targeted tumor therapy ....................................... 00

* Corresponding author. Tel.: +1-706-721-2317; fax: +1-706-721-3392.
E-mail address: shsu@mail.mcg.edu (S. Hsu).

1368-8375/$ - see front matter © 2003 Published by Elsevier Ltd.
Introduction

Oral cancer is one of the 10 most frequently occurring cancers world-wide, and its incidence in Europe and the United States ranges from 2% to 6% among all cancer patients.\textsuperscript{1,2} Treatment of oral cancer has primarily relied on classical modalities encompassing surgery, radiation, and chemotherapy or a combination of these methods. Many of the currently used anti-mitotic drugs were developed on the premise that cancer is fundamentally a disease of enhanced or sustained cell proliferation. However, efforts to eradicate disseminated neoplastic cells often have resulted in adverse systemic and cytotoxic effects and development of resistance to therapy. In addition, drug induced cell damage does not inevitably lead to tumor cell death, in part due to evasion of apoptosis by cancer cells.\textsuperscript{3,4} Recently, the discovery of a number of subcellular targets in cancer cells led to the rational development of ‘targeted therapy’. These newly designed drugs are aimed specifically at various components of intracellular signal transduction pathways controlling cell cycle, apoptosis, or angiogenesis. Apoptosis was originally defined in 1972, which stimulated contemporary concepts in the development of cancer and other diseases.\textsuperscript{5} This understanding led to the development of apoptotic-modulating therapies such as antisense of Bcl-2, cFLIP (caspase-8-inhibitory protein, an inhibitor of Fas activation) and surviving, inhibitors of AKT (an anti-apoptotic serine-threonine kinase), nuclear factor k B (NFkB), and recombinant TRAIL (TNF-related apoptosis-inducing ligand) to initiate apoptosis in cancer cells.\textsuperscript{6-9}

It is currently considered that dysregulated cell proliferation and apoptosis lead to the development of cancer.\textsuperscript{4} Therefore it appears that exploiting the apoptotic potential of cancer cells might lead to new therapies that might be less toxic to normal cells due to their physiologically controlled survival pathway.

Many agents, either naturally occurring or synthetic/genetically engineered, have demonstrated apoptosis-inducing properties. These agents often induce tumor cells to undergo certain types of programmed cell death (PCD), with limited or tolerable damage to surrounding normal cells. PCD may occur in various forms such as apoptosis, apoptosis-like PCD, and necrosis-like PCD involving different mechanisms, depending upon the PCD inducers and/or responding cells.\textsuperscript{10,11} When cells undergo typical process of apoptosis, morphological alterations can be observed such as chromatin condensation, apoptotic body formation, phosphatidylserine translocation, or cellular shrinkage and blebbing prior to cell lysis.\textsuperscript{5,12} At the molecular level, there are two major pathways leading to apoptosis involving caspases. The intrinsic apoptotic pathway involves mitochondrial membrane permeabilization, release of cytochrome c into the cytosol, apoptosome formation, and activation of caspase-9 and downstream caspases, leading to DNA fragmentation. The final stages of apoptosis generally employ caspase-3-dependent mechanisms, wherein caspase-3 acts as the executor for cell death by cleaving multiple structural and repair proteins. Caspase-3 has been shown to be the primary executioner caspase, necessary for cleavage of PARP (Poly(ADP-Ribose) Polymerase), fodrin, gelsolin, ICAD (inhibitor caspase-activated deoxyribonuclease), X-IAP (X-linked inhibitor of apoptosis protein), STAT1 (signal transducer and activator of transcription-1), topoisoisomerase-1, vimentin, lamin-B, Rb (Ratinoblastoma tumor suppressor protein) and others.\textsuperscript{12,13} Furthermore PARP and lamina-A also may be cleaved by caspases 7 and 6. The other pathway (designated extrinsic...
pathway) for activation of procaspases is initiated by TNF-α (tumor necrosis factor-α) or Fas ligand. In this pathway, the initiator procaspase-8 becomes activated by proximity-induced autoactivation due to recruitment by the adaptor protein FADD (Fas-associated death domain) to the death domain-containing receptors, e.g. TNF-R1 (tumor necrosis factor-receptor) and Fas. The activated caspase-8 leads to activation of the executioner caspase-3. Interestingly, activated caspase-8 also cleaves and activates BID, which controls release of cytochrome-c from mitochondria by complexing with BAX and lipids.14-16 The non-apoptotic cell death pathways, such as necrosis, autophagic cell death, and caspase-independent cell death, lack one or several of these characteristics; especially activation of caspases and DNA fragmentation.10,17 A mechanistic link between apoptosis and necrosis has been reported involving cleavage of calcium pump by caspases.11

### Relationship between cell proliferation and apoptosis in OSCC

At certain stages in tumor development, the balance between proliferation and apoptosis is interrupted, resulting in deregulated cell proliferation.18,19 In OSCC, approximately 50% of tumors exhibited dysfunctional p53, resulting in the loss of a check point control. Thus, cells with damaged genomes would not be able to undergo apoptosis, allowing the defective genome to persist and replicate in the offspring cells.20 Chromosomal aberrations and accumulation of mutations in many genes encoding crucial proteins or oncoproteins that control cell growth and apoptosis may also induce neoplastic formation.21-23 These genes include those coding for cell cycle regulators such as cyclins, cyclin-dependent kinases (CDKs), cyclin-dependent kinase inhibitors (CKIs), and Rb;21,22,24,25 pro-survival genes encoding telomerase, growth factors or their receptors, inhibitors of apoptotic proteins (IAPs), NF-κB, Bcl-2 and Bcl-xL;20 the pro-apoptosis genes encoding caspases, BAX, BAK, Bid, Fas, and receptors for TNF; and genes encoding key transcription factors or elements for signal transduction leading to apoptosis/survival.14,20 The neoplastic cells bearing mutations or defects that are pro-survival would outgrow their normal counterparts leading to the development of OSCC. However, it has been considered that when tumors first arise, they are actually more prone to induction of apoptosis than their normal counterparts.

### Exploiting the apoptotic potential of OSCC

OSCC is the preferred tumor model as it comprises 90% of oral malignant neoplasms.26 Molecular alterations causing oral carcinogenesis have been linked to genetic factors involving chromosomal aberrations, tumor suppressor genes, oncogenes, DNA mismatch repair genes, environmental and viral factors.23,27-29 These mutations or other mechanistic dysregulations allow OSCC cells to proliferate at a rate that exceeds cell death, to migrate and penetrate the basement membrane, and to initiate angiogenesis.21,22 It is likely that sequential and/or concomitant mutations or defects enable the transformed epithelial cells to proceed through precancerous progression and malignant conversion with a metastatic potential.30,31 Thus, elucidation of putative biomarkers would lead to detection of tumors at a stage appropriate for intervention.21,22 The biomarkers in OSCC may exist as genetic or molecular indicators identified as mutated, silenced, overexpressed, amplified, or altered genes or gene products. These biomarkers may represent the loss of functional tumor suppressors, cell cycle regulators, or apoptosis regulators resulting in dysregulation of cell growth and/or cell death.32 For example, the absence of functional CKIs such as p16, p21, p27 and p57 were often found in OSCC.33-35 Over-expression of these CKIs results in growth arrest.39-42 In addition, over-expression of p16, p21, or p27 was shown to induce apoptosis.43 These identified biomarkers could be used as targets for chemoprevention or therapeutic treatment. Furthermore, defects in OSCC, which enabled them to evade the regulatory system, could be exploited by therapeutics targeting these proliferation/apoptosis regulators.18,44,45

### Chemotherapeutic drugs and apoptosis

Chemotherapeutic drugs currently being used for head and neck cancers and found to induce specific apoptotic pathways include cisplatin (CDDP, cis-[PtCl2(NH3)2]), 5-fluorouracil (5-FU), and Teniposide (Vm-26). 5-FU was initially used in chemotherapy for gastrointestinal, breast, pancreas, and skin neoplasms. Yoneda et al. found that 5-FU-induced apoptosis in OSCC through a p53/p21-independent pathway while the cells accumulated in S phase.46 Tong et al. suggested that the apoptosis induced by 5-FU in OSCC could be caspase-dependent, and indicated that the effectiveness of 5-FU-induced apoptosis might vary in different tu-
or cell lines. Furthermore, other investigators showed that 5-FU-induced apoptosis in OSCC involves caspases 1, 8 and 3. Additional mechanisms in 5-FU-induced apoptosis could be through the inhibition of NFκB by stabilizing IκB, accompanied by activation of caspases 8 and 3.

OSCC cells resistant to one drug may be susceptible to a combination of drugs due to availability of alternate apoptotic pathway activation. In this regard, the platinum-containing CDDP-induced apoptosis in metastatic oral carcinoma cells (B88) differs from the 5-FU activated pathway associated with down-regulation of NFκB and anti-apoptosis proteins TRAF (TNF receptor-associated factor), and cFLIP. The CDDP-induced apoptosis occurs via mitochondrial-mediated activation of caspases 9 and 3, and NFκB suppression; whereas the caspase-8 activation and down regulation of anti-apoptotic proteins were not induced. CDDP efficiently caused permeabilization of the mitochondrial membrane and cytochrome c release within 12 h, accompanied by up-regulation of apoptotic protease-activating factor-1 (Apaf-1), indicating apoptosome formation and subsequent caspase activation cascade. In addition, carboplatin, a CDDP analog, induced Fas-dependent apoptosis of human tongue carcinoma cells. These data suggest that each chemotherapeutic drug may activate specific intracellular pathway(s) and induce apoptosis in target cells. Clinical applications combining different drugs may exhibit synergistic effects and reduce resistance and/or cytotoxicity. A new platinum compound, caused a lower degree of cytotoxic effect when used with 5-FU and radiation therapy in oral cancer patients.

Taxol (paclitaxel), the well known plant-derived anticancer drug used for breast and other cancers, was isolated from the Pacific yew tree Taxus brevifolia. Currently, there are numerous taxane family members of anticancer drugs developed from the prototype paclitaxel, which target the microtubules and centrosome, causing growth arrest and apoptosis.

Another chemotherapeutic drug, teniposide (Vm-26), is a podophyllotoxin derivative, a natural product that specifically targets topoisomerase (TOP) II and leads to DNA damage. Similarly, TOP I inhibitors such as camptothecin (CPT) are used as chemotherapeutic drugs to induce tumor cell apoptosis. Therefore, topoisomerases appear to be suitable targets for anticancer drugs. A recent report described that a diterpenoid quinone compound, Salvicine, also a TOP I inhibitor, possesses potent caspase-3-dependent apoptotic properties against multidrug-resistant tumor lines from different origins. However, these chemotherapeutic drugs produce considerable cytotoxicity in normal cells, which pose limitations for the therapeutic/preventive purposes.

Antibiotics

An antifungal antibiotic, trichostatin, isolated from the bacterium Streptomyces hygroscopicus, was found to inhibit histone deacetylation and plays a role in cell growth regulation. Synthetic histone deacetylase (HDAC) inhibitors, e.g. MS-27-275, may be of potential value for specifically inducing apoptosis in OSCC. Trichostatin A (TSA) up-regulated p21, BAK, and BAX; reduced levels of E2F-1, Rb, and p53; and induced caspase-3-dependent apoptosis in oral carcinoma cell lines. The results suggested that TSA induced apoptotic cell death through alteration of the expression of cell cycle regulators and apoptosis-regulating proteins. However, TSA is considered toxic to normal tissues.

Staurosporin, a protein kinase inhibitor isolated from the bacterium Streptomyces staurosporaeus, was initially suggested to be a potential chemotherapeutic agent. Staurosporine has been tested on the OSCC line SAS, in which it induced rapid apoptosis via activator protein-1 (AP-1) dependent pathway by cell detachment, up-regulation of both c-fos and c-jun, and DNA fragmentation. However, the effects of staurosporin are not specific for tumor cells, which could be more resistant to staurosporin than normal cells. Thus, Staurosporine alone may not be suitable as a chemotherapeutic agent for OSCC. Other antibiotics such as pinyangmycin (PYM), bleomycin (BLM), peplomycin (PEP, derivative of BLM), and epi-adriamycin (E-ADM) are still under investigation. PYM has been shown to induce apoptosis by regulating the mitotic phase via cytochrome c release.

Micronutrients, trace elements and metals

The title for "the most precious metal in the fight against cancer" must belong to selenium (SE). It is a micronutrient and an inorganic metal found in soil, Brazil nuts, tuna fish, pink salmon,
Induction of apoptosis in oral cancer cells

beef, and liver. Selenium and its derivatives/metabolites have been linked to a reduced cancer incidence. Clinical trials pioneered by Clark and colleagues indicated that dietary selenium supplements may reduce the prevalence and mortality of certain human cancers, particularly prostate cancer among populations with a low baseline plasma selenium. Recent findings indicated that selenium as a dietary supplement may prevent DNA damage and promote apoptosis in aged canine prostate epithelial cells, and reduce DNA damage and oxidative stress in rat lymphocytes. Clinical trials and animal studies suggested that the chemoprevention effects of selenium are associated with growth arrest and apoptosis in tumor cells. A well-controlled-in vitro study using primary oral mucosal cells and OSCC cells suggested that selenium compounds, selenodiglutathione (SDG) and 1,4-phenylenebis (methylene) selenocyanate (p-XSC), activate the MAPK pathways and Fas ligand expression in OSCC, while SDG also induces apoptosis preferentially in OSCC as compared to oral mucosal cells. In addition, selenium compounds also induced stress response in OSCC cells by up-regulating stress pathway kinases JNK and p38 that may further accelerate the apoptotic process, and gene expression profiles identified by cDNA microarray confirmed these mechanisms. It is of interest that selenomethionine, an organic selenium compound and the major component of dietary selenium, is able to activate p53-dependent DNA repair. In conclusion, the chemopreventive effects of selenium compounds works on three fronts as: (1) a scavenger for reactive oxygen species, (2) an activator of DNA repair in normal cells, and (3) an activator of apoptosis via Fas specific pathways in tumor cells. These properties would enable selenium compounds to be potential cancer preventing dietary supplements to combat oral cancer. Recently, Hatfield and co-workers have developed two model systems encompassing (a) transgenic mice carrying mutant selenocysteine SEC0 tRNA, and (b) Iox-Cre techniques selectively removing Sec-tRNA gene in order to further assess the role of selenoproteins in human health.

Other metal compounds also have emerged as potential anticancer agents. Among them, platinum (Pt) and ruthenium (Ru) containing compounds top the list. Since Pt is the backbone of CDDP, a group of Pt compounds that target tumor cell DNA have been developed as anti-tumor agents. Calcium is another potential candidate for application to combat cancer, and its anticancer effects are under investigation. As an intracellular messenger, calcium ion is involved in a number of signaling pathways, which link it to apoptosis. Scorrano et al. reported that BAK/BAX-mediated apoptosis of certain cells depend on Ca\(^{2+}\) transfer from the endoplasmatic reticulum (ER) to the mitochondria. Therefore, traffic of Ca\(^{2+}\) from ER to the mitochondria could be used as a trigger for ER-mediated apoptosis. Elucidation of the role of calcium in oral cancer prevention would be of immense value.

Lastly, organosulfur compounds in garlic, represented by diallyl sulfide (DAS) and diallyl disulfide (DADS), showed apoptosis-inducing ability in a skin carcinogenesis model and in colon cancer cell lines expressing either wild-type or mutant p53. It also has been reported that DADS-induced apoptosis in human leukemia HL-60 cells was associated with caspase-3 activation. Furthermore, HL-60 cells incubated with DADS produced significant amount of hydrogen peroxide, which further increased the stress to the cells. The role of fresh garlic in combating cancer is discussed later in this review.

**Biological agents and targeted tumor therapy**

Cancer is increasingly viewed a disease of deregulated cell cycle. Therefore, key factors regulating cell cycle and/or apoptosis may be targeted by specifically designed biological agents to destroy cancer cells. Biological agents potentially used in cancer therapy targeting the cell cycle or apoptosis generally refer to either genes constructed in expression systems such as viruses to produce intracellular or cell surface proteins (gene therapy), or monoclonal antibodies, which specifically recognize and bind targets either on cell surface or in situ (immunotherapy). Both types of agents are able to destroy targeted cells through regulation of endogenous biological pathways. Other agents obtained by biological techniques, such as antisense oligonucleotides and ribozymes also belong to this category. Expression of tumor suppressor p53 in OSCC is often aberrant; therefore introduction of wild-type p53 into the tumor cells could restore the apoptotic mechanism. Clayman et al. developed an adenovirus carrying a cytomegalovirus (CMV) promoter-driven wild-type p53 gene, and demonstrated that infection of the adenovirus reduced the size of subcutaneous tumor in animal models. These authors also tested the adenoviral-p53 in recurrent advanced head and neck SCC (including OSCC) patients in a phase I trial by direct injection of the adenovirus CMV-p53 into the tumors. Results of this study...
showed improvement of prognosis in several patients with partial success.99

Recently, various types of non-viral vectors have been developed as alternative gene transfer technologies for gene therapy.100 Merlin et al. reported that p53 gene was transfected into human carcinoma cells by complexing with glucosylated poly-ethylenimine derivatives in order to avoid immune response and viral infection.101

Expression of exogenously introduced cell surface proteins can change cell behavior in OSCC leading to self-destruction. When retinoic acid receptor β was transfected into OSCC, growth arrest and apoptosis were observed, accompanied by up-regulation of pro-apoptotic proteins p21, p27, BAX, BAK, caspase-9, and caspase-3.102 The stress-responding proteins, such as heat shock protein 70 (HSP70 or DnaK) also can serve as targets to induce apoptosis. Kaur et al. suppressed the expression of HSP70 in OSCC by antisense oligonucleotides, which induced typical caspase-dependent apoptosis. They suggested that the possible mechanism might be the interruption of HSP70-Bcl-2 interaction.103

Cell surface receptors, especially the receptors responsible for cell growth and/or angiogenesis, e.g. epidermal growth factor (EGF) receptor and vascular endothelial growth factor (VEGF) receptor, are the primary targets for these agents.104–106 The EGF receptor expression is linked to aggressive tumor behavior.107 Synthetic inhibitors of tyrosine kinase activity of EGF receptor successfully induced apoptosis in a human gingival squamous cell carcinoma line Ca9-22, as measured by cell growth and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assays. Furthermore, transfection of an antisense oligodeoxynucleotide of protein kinase CK-2 inhibited the growth of some OSCC for up to 96 h.108

Phosphatases and protein kinases, especially the tyrosine kinases also are targeted to induce cancer cells death.109 Two protein phosphatase inhibitors, okadaic acid (OA) and calyculin A (CA) induced apoptosis in SCC25 cells.110 Muraki et al. reported that in oral cancer specimens, expression of the apoptosis signaling protein Fas antigen is correlated with prognosis,111 and application of monoclonal antibody (mAb) that specifically binds to the Fas antigen in SCC25 cells triggered nuclear fragmentation and DNA ladder formation, markers of apoptosis.112

Although the potential for molecular targeting therapy has been investigated intensively in recent years, only a small number of “targeted therapies” exhibited therapeutic potential clinically and were approved as anticancer drugs, represented by Gleevec, Herceptin (mAb), Rituxan, and Iressa, which target Abl protein tyrosine kinase, HER-2 receptor, CD20 of B cells and EGF receptor tyrosine kinase respectively.113–115 A reason for the failures of other agents is that many tumors simply do not rely on a single receptor or signal transduction pathway for growth, but previously developed targeted therapies often depended upon the alterations of a single element or target in tumor cells.116 Nevertheless, there are a number of targeted drugs still under clinical trials.117

Phytochemicals: selected chemopreventive agents with potential to induce apoptosis

Cancer might be one of the diseases linked to alterations in diet among humans. In developed countries, the typical diet is no longer mainly fruits and vegetables, unlike that of other primates.118,119 It has been considered that a diet high in vegetables (more than 440 g/day) and fruits could prevent at least 20% of all cancers. The convincing evidence for benefits of consuming fruits and vegetables derives from the reduced risk of gastrointestinal cancer such as mouth, pharynx, esophagus, stomach, colon, and rectum.120 It is conceivable that effective plant-derived chemoprevention agents might target molecules that regulate the cell cycle, cellular senescence, and apoptosis.121

The term ‘phytochemicals’ refers to a wide variety of compounds produced by plants. Phytochemicals are one of the major sources of anticancer drugs.122 However, among the more than 4000 phytochemicals cataloged, only about 150 have been studied for their medical benefits.123 Phytochemicals with potential for chemoprevention have been categorized in the following groups: (1) carotenoids (e.g. α-carotene, β-carotene, γ-carotene, lutein, lycopene), in tomatoes and other yellow/orange vegetables; (2) isothiocyanates (e.g. sulphoraphane), in cruciferous vegetables—cabbage, broccoli; (3) glucosinolates (e.g. glucobrassicin, sinigrin), in cruciferous vegetables—brussel sprouts; (4) sulfides (e.g. allyl sulfide), in garlic, onions, scallions and broccoli; (5) diarylhepanoids (e.g. curcumin), in ginger, turmeric; (6) saponins in soybeans and other legumes; (7) vanilloides in capsaison found in red pepper and chili pepper; (8) phenols/phenolics: (i) catechins in green tea; (ii) cinnamic acid in coffee; (iii) ellagic acid in berries, walnut and pecans; and (iv) resveratral—a phytoestrogen in wine and grapes, and (9) flavonoids, which can further be classified as...
follow: (I) flavones (e.g. luteolin), in apple skin, celery; (II) acanthocyanins (e.g. cyanidin), in berries; (III) flavanons (e.g. hesperitin), in citrus fruits, grapefruits; (IV) isoflavones (e.g. genistein), almost exclusively in soybeans; (V) flavonols (e.g. quercetin), in onions, apples; (VI) flavanols (e.g. catechin, ECGC), in green tea.124–126

Many leafy plants, either fruits or vegetables, are high in phenolic compounds (also referred to as phenols, phenolics and/or polyphenols), which have been studied extensively in recent years. The phenolic compounds form the largest category of phytochemicals and are the most widely distributed in nature.127–129

Green tea catechins/polyphenols

One of the richest sources for polyphenols is from the tea leaves of Camellia sinensis. The tea leaves contain approximately 40% polyphenols by dry weight. The majority of the tea consumed in the world is black tea (78%) while green tea consumption comprises 20%.130

In vitro studies showed that green tea causes reversible G1 arrest of the cell cycle by inhibition of Rb phosphorylation in oral leukoplasia.131 The most potent component of green tea polyphenol, (-)-epigallocatechin-3-gallate (EGCG) was able to induce growth inhibition of transformed cells by activating apoptosis132 while normal cell growth was not affected.133 We have demonstrated that EGCG alone or green tea polyphenols were able to induce apoptosis in oral squamous carcinoma cells, while normal human epidermal keratinocytes survived.134,135

Studies in human head and neck squamous cell carcinoma cell lines showed that EGCG induced growth arrest and apoptosis, associated with increased p21, p27, decreased cyclin D1, and increased pro-apoptosis factor BAX protein levels, and decreased anti-apoptosis factors Bcl-2 and Bcl-xl protein levels.136 EGCG or a mixture of green tea polyphenols (GTPP) induced TNF-α gene expression and TNF-α release from cells;137 inhibited tumor promoter-induced AP-1 activation and tyrosine phosphorylation of platelet-derived growth factor (PDGF) β receptor;138 inhibited EGF receptor phosphorylation;136 induced p16, p18, p21 or p27 accumulation-mediated growth arrest and subsequent apoptosis;136,139–141 and induced caspase-3-activated apoptosis in tumor cells.142,143

We have found that EGCG elicits a second signal pathway in normal epithelial cells, in addition to the apoptotic pathway activated in tumor cells. We observed that EGCG is a potent inducer of p57 in pooled normal human primary epidermal keratinocytes but not in tumor cell lines, suggesting that p57 might be a key candidate for this protective mechanism. In addition, the EGCG-induced p57 up-regulation is closely associated with cell differentiation in exponentially growing keratinocytes, indicating that this pathway may direct the cells toward differentiation instead of apoptosis.144,145 The evidence from these studies attests to the feasibility that EGCG is a potential candidate for prevention of human oral cancer.

Ginger phenolics

Another phenolic compound, [6]-paradol, derived from ginger root and certain zingiberaceae plants, protected mouse skin from a tumor inducing agent, and showed dose-dependent cytotoxicity in an oral carcinoma cell line (KB), with specific features of caspase-3-mediated apoptosis.146,147 Viable KB cells were reduced in number to less than 50% of untreated control when incubated with 50 μM [6]-paradol for 48 h.146 In addition, an ethanol extract of ginger mediated anti-tumor promoting effects, decreased the number of tumors in a Sencar mice skin tumor model.148 Curcumin, a yellow coloring agent in turmeric (zingiberaceae family) also has been shown to exert anti-carcinogenic effects.124

Chlorogenic acid

Cinnamic acid (found in coffee) yields chlorogenic acid (CGA). CGA is a phenolic compound, which was found to have potential benefits for reducing serum cholesterol and triglycerides.149 It was reported that CGA induced hydrogen peroxide, and showed differential effects in normal vs. oral cancer cells by inducing caspase-3-dependent apoptosis.150 The in vivo effects of CGA are still not known.

Garlic extract

Garlic, in addition to its flavoring property, has been used to treat various illnesses for thousands of years, and its anticancer potential has been investigated.151,152 In a 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis model, water extracts of fresh garlic induced apoptosis of malignant cells and completely prevented the onset of oral carcinoma.153
Narcotics

Narcotics isolated from plants also possess the ability to induce apoptosis in OSCC cells. Codeine, a derivative of the opioid analgesic codeine, was shown to target the mitochondria and cause caspase-3-dependent apoptosis.\textsuperscript{154} The cytotoxicity caused by codeine was selectively higher in oral tumor cells.\textsuperscript{155}

Carotinoids: lycopene and others

There are more than 600 carotenoids in plants, of which approximately 25 are present in human serum (nine are metabolites) and 14 in human tissues.\textsuperscript{156,157} The most common carotenoids in the human diet and plasma are lycopene, lutein, $\beta$-carotene, $\alpha$-carotene, and $\beta$-cryptoxanthin. The prominent carotenoid in serum is the antioxidant red pigment called lycopene. The primary sources of lycopene include tomatoes, apricots, papaya, and other yellow fruits. Consumption of tomato-containing foods is inversely correlated with the incidence of some systemic neoplasms.\textsuperscript{158–160} In particular, lycopene and other carotenoids rich foods also are inversely related to upper aerodigestive tract neoplasms including oral cancer.\textsuperscript{161,162}

Laboratory studies showed that lycopene blocked IGF-1 stimulated proliferation in the breast cancer cell line MCF7 by interfering with IGF-1 signaling.\textsuperscript{163} Lycopene specifically induced a key protein for gap junction formation, connexin 43, and potently inhibited the proliferation of the oral cancer cell line KB-1 in G1 phase. At physiological concentration of 7 $\mu$M, KB-1 cells were inhibited to approximately 10% of control cell numbers.\textsuperscript{164} At a concentration of 20 $\mu$M, lycopene induced apoptosis in malignant T-lymphoblast cells within 24 h.\textsuperscript{165}

Retinoids

Retinoids are the natural and synthetic derivatives of vitamin A. The retinoids in the body originate from retinyl esters, carotenoids, and retinal in diets. The role of retinoids in oral cancer has recently been reviewed.\textsuperscript{166} The available data indicate that retinoids possess a potential for growth inhibition of cancer in vivo and in vitro.\textsuperscript{167} The differentiating agent 13-cis retinoic acid induced a favorable clinical response in oral leukoplakia and a reversal of histologically diagnosed dysplasia; but a relapse occurred following discontinuation of the treatment.\textsuperscript{168,169} It is of note that isotretinoin prevented the development of second primary tumors.\textsuperscript{170} All-trans-retinoid acid (RA) and retinol also exhibited favorable results but relapse was noted.\textsuperscript{171,172} The effects of retinoids are mediated by retinoid acid receptors (RARs) and retinoid X receptors (RXRs) which are ligand-activated transcription factors.\textsuperscript{173,174} Three subtypes, designated as $\alpha$, $\beta$, and $\gamma$ of both RARs and RXRs, have been described, along with several isoforms of each subtype.\textsuperscript{175} A lower expression of RAR-$\beta$ was seen in 60% of oral dysplasia and 65% of head and neck carcinomas compared to 30% in adjacent normal mucosa.\textsuperscript{176,177} The lower expression of RAR was considered as a limiting factor of RA activity on oral lesions; up-regulation of the receptor by isotretinoin produced favorable clinical response.\textsuperscript{177} Furthermore, in head and neck cancer cell lines, it was demonstrated that RAR-$\gamma$ but not RAR-$\beta$ was involved in RA-mediated growth inhibition; however, RAR-$\gamma$ related mechanisms remain unclear.\textsuperscript{178,179} In this regard, some of the novel synthetic retinoids have shown retinoid receptor activity e.g. CD437, which acts in an RAR-$\gamma$ receptor dependent manner and appears to activate and up-regulate transcription factor AP-1 leading to apoptosis in head and neck carcinomas.\textsuperscript{180} Fenretinide, also a synthetic retinoid, induces apoptosis in RA-resistant cell lines\textsuperscript{181} and is under evaluation in clinical trials as a chemopreventive agent in epithelial neoplasms.\textsuperscript{182} Recently, retinoids have been implicated in the induction of cell death in many tumor-derived culture cell systems in both retinoid receptor-dependent and -independent manners.\textsuperscript{183} The continued development of new synthetic drugs to up-regulate RA receptors and/or receptor independent drugs would be valuable.

It appears that exploiting the apoptotic potential of OSCC would lead to contemporary therapies that might be less toxic to normal cells due to their physiologically controlled survival pathways. It is suggested that these newer therapies would also be effective in treatment of epithelial dysplasia. Ideally, the root of cancer control lies in instituting chemoprevention. In addition to the chemotherapeutic and chemopreventive agents, a number of dietary components and micronutrients are emerging with considerable potential for the induction of apoptosis. These agents include green tea constituents (EGCG and others), and a number of other phytochemicals, such as carotenoids (lycopene) and retinoids. The molecular mechanisms of a host of other chemopreventive agents selected via epidemiological studies remain as yet unknown. In conclusion, the emerging and unexplored arena of chemoprevention and therapy via natural prod-
ucts appear to possess a great potential in the control of cancer through non-invasive methods.

Acknowledgements

The authors thank Dr. Gretchen Caughman and Dr. Douglas Dickinson for their critical reading and valuable suggestions.

References


