Chapter 1

Current Trends in Oral Cancer

Chemoprevention

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Introduction

Head and neck cancer is the sixth most common human cancer worldwide [1]. It represents an important problem in the global public health because despite the efforts and results in the recent years’ research, the 5 years survival rate has not improved significantly [2]. Oral carcinogenesis is a complex multifocal process modulated by endogenous and environmental factors. Smoking and alcohol acting synergistically are the most important risk factors of oral cancer (OSCC) [3]. Among other risk factors, HPV infection is linked to oral and oropharyngeal cancer [4]. Some lesions like leukoplakia, erythroplakia and smoker’s palate are considered to have a high potential for malignant changes [5]. Several conditions such as oral submucous fibrosis, syphilitic glossitis, sideropenic dysphagia, oral lichen planus, discoid lupus erythematosus, and dyskeratosis congenita were associated with higher than normal incidence of OSCC [5]. Genetics and inflammation have also been incriminated in oral carcinogenesis [6]. However, oral potentially malignant disorders and OSCC can occur in the complete absence of any identifiable risk factor. Moreover, increased OSCC incidence has been observed in patients younger than 20 years with no significantly difference being depicted between otherwise healthy patients and patients with well-known predisposing systemic factors such as xeroderma pigmentosum, Fanconi’s anemia, and a history of bone marrow transplant [2]. This underscores incomplete understanding of OSCC etiology and the need to discover
new biomarkers for the early OSCC diagnosis and treatment. Analyzing the mechanistic basis of the process could lead to development molecular tools to manipulate oral carcinogenesis.

**Genetics of Oral Cancer**

For a long time, irreversible damage of DNA sequence such as gene deletions, amplifications and mutations leading to oncogenes activation or tumor suppressor genes inactivation have been considered the major events responsible for cancer onset [7]. The most common genetic alterations during oral carcinogenesis include loss of chromosomal material in: 9p21, 3p21, and 17p13 in dysplastic lesions; 11q13, 13q21, 14q31 in carcinoma in situ; 4q26-28, 6p, 8p, 8q in invasive tumors [8,9,10].

Genetic mutations accumulation act in cancer-associated signaling pathways, causing the acquisition of cancer-related phenotypes: immortality or limitless replicative potential; self-sufficiency in growth signals; insensitivity to anti-growth signals; ability to evade apoptosis; invasion and metastasis; angiogenesis [7].

Telomeres, by protecting chromosomes from degradation and loss of essential genes, contribute to cells immortality [7,11,12]. Telomere length and structure are maintained by the reverse transcriptase telomerase (hTERT) and shelterin, a multiprotein telomere complex. Telomerases are involved in the hyperplastic and dysplastic oral epithelium development [13] and increased expression hTERT is an early event in oral carcinogenesis [14,15].

Loss of growth-inhibitory signals and the acquisition of self-sufficient growth signals through oncogene activation [7,11] are tightly regulated by interactions of the cyclin-dependent kinase (CDK), cyclin, and the product of the retinoblastoma (Rb) gene. The proteins encoded by the tumor suppressor genes p16, p15, p21, and p53 also act as inhibitors of cell-cycle progression [16].

CDKs interact with cyclins forming of complexes required for the cell to pass through specific phases of the replicative sequence. D-type cyclins interact with CDK4 and CDK6 and are necessary for G0/G1 transmission [9]. The cyclin often overexpressed in head and neck carcinoma is Cyclin D1 - a proto-oncogene mapped to 11q13 [17,18].

The Rb gene product is a key regulator of G1/S cell cycle progression being part of a pathway that mediates cellular responses to a variety of signals by controlling the activity of E2F transcription factors. E2F transcription factors regulate numerous genes essential for DNA replication and cell cycle progression [19]. Inhibition of Rb function, reduce Rb control on these factors and contribute to cancer development [20].

p53 is a tumor suppressor gene that plays a role in cell-cycle progression, cellular differentiation, DNA repair, and apoptosis [21,22]. p53 functions as a transcrip-
tion factor that both positively and negatively regulates the expression of a large and disparate group of responsive genes [23] involved in: regulation of glycolysis and autophagy; repair of genotoxic damage; cell survival and regulation of oxidative stress; invasion and motility; cellular senescence; angiogenesis; differentiation; bone remodeling [24]. p53 is the most commonly mutated gene, altered in approximately 50% of head and neck cancers including 25-69% of OSCCs [25]. Reconstitution of the p53 tumor suppressor pathway is one of the most exciting novel concepts for improved cancer therapy [26].

p21, as the critical downstream mediator of the p53 gene, is a tumor suppressor implicated in G1 arrest, apoptosis, senescence and differentiation [22]. Expression of p21 has been found increased in premalignant and malignant oral lesions [9].

The p16 tumor suppressor gene binds to the cyclin-D1 CDK4 and CDK6 to inhibit cellular proliferation by inhibiting Rb phosphorylation and causing cell cycle arrest at the G1 phase. p16 possesses an important role in the oral carcinogenesis, and is mainly inactivated by DNA hypermethylation, rather than gene mutation and allelic deletions [27].

During oral carcinogenesis, growth signaling can become dysregulated [25]. Epidermal growth factor receptor (EGFR), a member of a membrane-bound receptor tyrosine kinase family is essential for numerous normal cellular processes including activation of the Ras/Raf/mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K), AKT, mammalian target of rapamycin (mTOR), Janus kinase (Jak), signal transducer and activator of transcription (STAT), and protein kinase C (PKC) pathways [28]. EGFR is overexpressed in 80-100% of OSCC [29,30] and increases progressively from oral premalignant lesions to invasive OSCC [16,31]. Furthermore, these mutants showed resistance to chemotherapy and targeting of EGFR.

The MAPK signaling pathway is a potent regulator of survival, cell proliferation and differentiation. The activation of EGFR sets in motion molecules that interact with proteins – most importantly the Ras proteins. Ras is found to be involved in cell growth regulation and the transduction of mitogenic cell signaling from the cell surface to the nucleus [32].

Members of the STAT family are latent cytoplasmic transcription factors activated by extracellular signaling proteins, such as growth factors, cytokines, hormones, and peptides [33]. Activated STAT proteins interact with a variety of DNA loci such as the vascular endothelial growth factor (VEGF), and other genes, responsible for cell cycle and death (cyc-D1, c-Myc and Mcl-1) [34]. Levels of activated STAT3 may be elevated in OSCC through up-regulation of signaling from EGFR, TGF-α, Jak, Src, or interleukin (IL)-6 and its receptor [35]. Constitutive acti-
vation of STAT 3, in turn, can up-regulate transcription of target genes, including cell-cycle regulators, anti-apoptotic genes, and pro-angiogenic factors, resulting in uncontrolled cellular proliferation, anti-apoptotic response, and angiogenesis, all hallmarks of cancer [25].

Another nuclear transcription factor involved in immune responses, inflammation, cell survival, and cancer is the nuclear factor-kappa B (NF-κB). High levels of NF-κB led to high levels of IL-6 and eventually to an increased STAT expression. These results plead for NF-κB implication in OSCC development [36]. Another target-molecule for NF-κB is cyclooxygenase-2 (COX-2), a key regulatory enzyme in the synthesis of prostaglandins (PGs) from arachidonic acid. Overexpression of COX-2 has been associated to OSCC, due to the fact that it inhibits apoptosis, promotes cell proliferation and new vessel growth, and disrupts intracellular junctions [9].

Studies on genetic and cancer biology indicate a prominent role for the phosphatidylinositol 3-kinase (PI3K) pathway in cancer cell growth and survival [37]. PI3K activation initiates many intracellular signaling pathways that regulate functions such as cell metabolism, survival and polarity, and also vesicle trafficking [38]. PI3K– phosphatase and tensin homolog (PTEN)–Protein kinase B (Akt) is an important signaling pathway in cancer, including OSCC [37]. The serine/threonine kinase Akt is the central mediator of the canonical PI3K pathway and mediates multiple cellular processes including: cell survival; proliferation and angiogenesis; metabolism; and protein translation through numerous downstream signaling proteins [39]. Triggering the Akt signaling pathway by amplification of the 3q26 chromosomal region harboring the PI3KCa oncogene (catalytic subunit of PI3K) is considered a frequent event in OSCC resulting in PI3K overexpression and Akt activation [40]. One of the Akt major effectors is mammalian Target of Rapamycin (mTOR). mTOR plays an important role in the complex oral carcinogenesis [41]. PTEN acts as a negative regulator for the PI3K signaling and is the second most commonly mutated tumor suppressor in human cancers [42]. Decreased expression of PTEN is observed in ~30% of OSCCs, and is considered a fine prognostic indicator of poor clinical outcome [9]. PIK3 and PTEN are established cancer genes in OSCC therefore therapies targeting the PI3K signaling network hold great promise in OSCC [38].

Another important signaling pathway in OSCC is that of the Wingless Type 1 family (Wnt) [9]. The Wnt family consists of evolutionarily conserved secreted glycoproteins located at the cellular membrane [43]. In the absence of Wnt signals, beta-catenin connects cadherins to α-catenin and forms a dynamic link to the cytoskeleton contributing to the intracellular junctions and the preservation of tissue architecture [44]. Beta-catenin is phosphorylated by glycogen synthase kinase-3 beta (GSK-3beta), ubiquitized and then degraded by proteosomes. To efficiently ac-
complish phosphorylation, beta-catenin forms a complex with Axin, APC and GSK-3beta protein [45]. In the presence of Wnt signals across cell membranes Wnt receptors bind to Frizzled and low-density lipoprotein receptor-related protein (LRP) 5/6 co-receptors resulting in phosphorylation of LRP and inactivation of GSK3-beta phosphorylation of ß-catenin. Inactivation of GSK3 induces ß-Catenin protein stabilization and binding to transcriptional factors from the lymphoid enhancer factor T-cell factor family [43]. In the final step nuclear β-catenin binds pontin 52-TATA-binding protein and displaces groucho-related gene or cAMP response element-binding protein co repressors from Lymphoid enhancer-binding factor/ T-cell factor resulting in stimulation of transcription of important growth regulatory genes, including cyclin D1 and c-Myc [46]. Ectopic activation of the Wnt signaling pathway is associated with many human tumors, including OSCCs [47]. Different components of Wnt signaling pathway were found overexpressed in and OSCC derived cell lines and nuclear β-catenin localization at the invasive front of carcinomas [48]. Expression of β-catenin is altered in OSCC but it was associated with epigenetic changes rather than mutational alterations [45]. Wnt/β-catenin pathway was involved in progression of dysplasia in oral leukoplakia based on the presence of nuclear expression of β-catenin and other in dysplasia, but not in normal oral epithelium [49]. In another experimental study, β-catenin nucleo-cytoplasmic dislocation was found in oral rat epithelial dysplasia adjacent to early cancer induced by 4-ni-troquinoline 1-oxide [50]. Therefore, small-molecule Wnt pathway antagonists may hold promise in the prevention and treatment of oral tumors [47].

By promoting angiogenesis and suppressing the immune responses, the transforming growth factor (TGF) enhances invasion and metastatic potential in later stages of oral carcinogenesis [51]. In early stages of oral carcinogenesis it suppresses the epithelial tumor progression via its ability to negatively regulate cell proliferation [52].

**Epigenetics of Oral Cancer**

Epigenetics is another important player in multistep oral carcinogenesis. Epigenetics defines all reversible heritable changes in gene expression that are not due to alterations in DNA sequence [53]. Epigenetic changes occur early and at high frequency in different human malignancies and continue developing during each step of carcinogenesis [54].

Both genetic and epigenetic events eventually result in abnormal gene expression, but, if the genetic path to cancer involves mutation of tumor oncogenes and/or suppressor genes resulting in loss or gain of function and abnormal expression, the epigenetic pathway is determined by chromatin structure including DNA methylation, histone modifications, nucleosome remodeling and small non-coding regulatory RNAs [55].

DNA methylation plays an important role in different cellular processes including gene expression, silencing of
transposable elements, and defense against viral sequences [56]. DNA methylation refers to a covalent addition of a methyl group to the 5-carbon (C5) position of cytosine base when it is followed by a guanosine (CpG) in a CpG dinucleotide. The reaction is mediated by enzymes DNA methyltransferases (DNMTs) namely DNMT1, DNMT3A and DNMT3B and DNMT3L [57]. CpG dinucleotides repeat throughout the genome in clusters named CpG islands, most of them localized at the promoter of tumor suppressor genes [58]. Increased methylation of CpG within gene promoters influences genes expression in the same manner as a proper genetic modification does. It can cause transcriptional “silencing” of tumor suppressors involved in cell cycle control, DNA repair, and apoptosis pathways and lead to cancer progression [59]. These methylation patterns occur in all stages of carcinogenesis and could be used as an early diagnostic marker and ideal targets for early intervention [60,61]. At the same time, in precancerous tissues, global DNA hypo/demethylation often occurs and is responsible for chromosomal instability, reactivation of transposable elements (such as retroviral elements), loss of imprinting, and activation of proto-oncogenes [62].

Histones have a structural role and an active function in the regulation of chromatin structure and gene expression. Histone are implicated in cellular processes including gene transcription, DNA repair, recombination and DNA replication, and their deregulation is associated with human malignancies. [63,64] Post-translational modifications of histones occur primarily at the N-terminal tails within each of the four histone complexes (H3, H4, H2A and H2B). The major histone modifications, acetylation, methylation, phosphorylation sumoylation, and ADP ribosylation and generally reversible and modify the tertiary DNA structure [62]. Histone acetylation and methylation are the most common post-translational modifications of histone proteins associated with carcinogenesis [65].

Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) functioning in a dynamic equilibrium. Increased histone acetylation is correlated with gene activation. Little studies were made in the area of histone hypoacetylation that was explained by a decrease in HAT activity. Most of the researches were focused on HDACs’ role in cancer. By removing the negatively charged acetyl groups, HDACs generally act as transcriptional repressors by stabilizing the nucleosomal DNA-histone interaction. HDACs can also bind to various co-repressors to recruit other histone modifiers, thus regulating other chromatin-based processes. Moreover HDACs was also found have a role in deacetylation of non-histone proteins and transcription factors such as E2F, STAT3, p53, Rb, NF-κB, APC etc, which regulate important functions that, in turn, regulate cellular homeostasis (cell-cycle progression, differentiation, and apoptosis) [66].
Histone methylation occurs on lysine and arginine residues and is catalyzed by histone lysine methyltransferase (HMTs) enzymes. Histone methylation may activate or repress gene expression depending on the location of the lysine residue methylated and other histone modifications in the surrounding residues. Methylation on histone H3 lysine 4 (H3K4), H3K36, and H3K79 is linked to active gene expression, whereas di- and trimethylation on H3K9, H3K27, and H4K20 are associated with gene silencing [67].

Although the exact mechanism by which these histone modifying enzymes affect tumorigenesis is not clear, altered expression of histone modifiers caused by mutations disrupt the whole epigenetic regulation mechanisms and result in aberrant gene expression patterns. The disruption of histone modifications has been linked to all the hallmarks of cancer, and it is important to be aware that a precise balance between the enzymes that write, read, and erase histone marks is crucial in preventing tumorigenesis.[68]

miRNAs are the most recent studied regulators of epigenetic gene expression. miRNA are small non-coding RNA molecules (20–22 nucleotides) that are excised from longer (60–110 nucleotides) RNA precursor [69]. They play essential roles in normal biological processes including development, proliferation, differentiation and cell death [69] by regulating gene expression on a post-transcriptional level, inhibiting protein formation by degrada-

Chemoprevention of Oral Cancer

The potential adverse effects from the treatment for head and neck squamous cell carcinoma (HNSCC) are numerous. Radical surgery can result in disfigurement & functional impairment, but even the organ-preserving treatment methods of radiation and chemotherapy may result in a host of negative side effects, some permanent. Common side effects of radiotherapy include mucositis, oral candidiasis, loss of taste and xerostomia, which may be permanent due to the damaging effect of radiation on the salivary glands. Osteoradionecrosis of bones within the radiation field (most commonly the mandible) may occur as a result of damage to the bone vasculature and osteocytes and is one of the most serious complications of radiotherapy. The toxic effects of platinum-based chemotherapeutic agents can also contribute to the development of oral cancer due to their ability to bind to DNA and alter its structure, leading to the formation of DNA adducts which can act as mutagens and initiators of cancer. The use of chemopreventive agents such as aspirin, flavonoids, and polyphenols has been shown to inhibit the expression of certain genes associated with tumor development and to induce cell death in cancer cells.

miRNAs are also promising as chemopreventive agents. miRNAs can regulate the expression of genes involved in the development and progression of oral cancer, such as those encoding for cellular proliferation, survival, and invasion. The downregulation of miRNAs has been linked to the development of oral cancer, and the upregulation of miRNAs has been shown to inhibit the growth and progression of oral cancer cells. miRNAs can also be used as biomarkers for the detection and monitoring of oral cancer, as their expression levels can be measured in saliva and oral swabs, providing a non-invasive means of screening for the disease.

In conclusion, the development of chemopreventive strategies is an exciting area of research in oral cancer prevention, with the potential to significantly reduce the incidence and impact of this disease.
therapy are dose dependent and include renal, otologic, and bone marrow suppressive sequelae) [73].

OSCC develops as a group of genetically altered clones of cells cluster in multifocal patches, that evolve into synchronous and metachronous tumors [74]. These oral multifocal cancers in individual sites explain the high probability of OSCC recurrences even after complete surgical removal of the lesions. [75]. Beside surgery, chemotherapy using including ifosfamide, 5-fluorouracil, taxane and methotrexate, represents the first treatment approach but patients eventually develop resistance to these agents Therefore preventive strategies could considerably improve the OSCC management [76,77].

Prevention of OSCC could be divided into three stages: primary, secondary, and tertiary prevention [78]. The primary prevention refers to the avoidance of smoking of tobacco products and alcohol consumption [79]. Secondary cancer prevention refers to early diagnostic and treatment of patients with oral premalignant lesions and screening the population at risk and asymptomatic [80]. Tertiary cancer prevention refers to the prevention and early detection of recurrence, second primary tumors in individuals who have been treated for cancer or its precursors detect and reduction of complications [81,82].

Using specific natural and synthetic agents is directed toward secondary chemoprevention that has evolved as a promising strategy to inhibit, suppress or control the incidence of carcinogenesis by [83-86].

<table>
<thead>
<tr>
<th>Drug</th>
<th>Associated drugs</th>
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<tr>
<td>Curcumin</td>
<td>Dysplastic leukoplakia derived cell lines MSK-1, MSK-2, and MSK-3.</td>
<td>Decreased DNA damage, inhibition of cell growth, reduced percentage of cells in S phase was, accumulated cells in G1 phase.</td>
<td>Tca8113 telomerase-negative oral squamous cell line derived clones HCT116R, SW480R their corresponding isogenic 5-FU-chemoresistant clones.</td>
<td>Induction of growth and differentiation of oral keratinocytes.</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>HNSCC cell line SCC-25</td>
<td>Inhibition of tumor cells growth and proliferation [144].</td>
<td>HNSCC cell lines CAL27 and UM-SCC1.</td>
<td>Enhancement of growth suppression [128].</td>
</tr>
<tr>
<td>Teas and tea</td>
<td>Curcumin 5-fluorouracil, and rapamycin.</td>
<td>Inhibition of cell growth, apoptosis, and angiogenesis.</td>
<td>HNSCC cell lines Restoration of the epigenetic regulation balance [124].</td>
<td>Restoration of the epigenetic regulation balance [124].</td>
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<tr>
<td>Genistein</td>
<td>erbB2 negative breast cancer cell lines Tu177, Tu212, and 886LN.</td>
<td>Decreased DNA damage, inhibition of cell growth, reduced percentage of cells in S phase was, accumulated cells in G1 phase.</td>
<td>Squamous cell carcinoma line SCC-9 [103].</td>
<td>Decreased: oral visible tumor incidence, number of visible tumors, dysplastic lesions an papillomas, tumor volume, decreased the proliferation index in hyperplasia, dysplasia and papillomas, apoptotic index in dysplasia and SCC.</td>
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<tr>
<td>Cisplatin</td>
<td>HNSCC cell lines YD-10B, MDA 686LN, JMAR.</td>
<td>Inhibition of tumor cells proliferation and motility [120].</td>
<td>HNSCC cell line YCU-H891 and YCU-N861.</td>
<td>Increased proportion of cells in the G1 phase of the cell cycle and induced apoptosis via a mitochondrial pathway [97].</td>
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<tr>
<td>Radiotherapy</td>
<td>HNSCC cell lines SCC-1, SCC-9, KB, A431.</td>
<td>CD-1 athymic nude mice.</td>
<td>HNSCC cell line SCC-9.</td>
<td>Induction of apoptosis [141].</td>
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| Chemopreventive agents can be divided into two general classes: blocking agents - prevent carcinogenic compounds from reaching or reacting with critical target sites by preventing the metabolic activation of carcinogens or tumor promoters via enhancing detoxification systems and by trapping reactive carcinogens; and suppressing agents - prevent the evolution of the neoplastic process in cells that would otherwise become malignant [87]. The mechanism of chemoprotective activities might correlate and balance between phase I + phase II enzymes levels,
and influence cellular macromolecules, transporters, release of carcinogens, or DNA adducts and DNA repair [87].

An ideal chemopreventive agent should have: a known mechanism of action; high efficacy against multiple sites; little or no toxic effects; capability of oral administration; human acceptance [88].

New research in the field of molecular determinants in carcinogenesis is highly promising in detecting alterations earlier than they are seen under a microscope and clinically detectable on one hand and development of safe and effective agents to target these determinants [88,89]. Epigenetics and genetics cooperate in cancer initiation and progression, intertwine and take advantage of each other during tumorigenesis. Alterations in epigenetic mechanisms can lead to genetic mutations, and genetic mutations in epigenetic regulators lead to an altered epigenome [68]. Therefore, targeting genetic as well as epigenetic mechanisms is a promising approach for cancer prevention and/or therapy [90]. The fact that epigenetic changes unlike genetic changes are reversible and represent an opportunity of developing new effective chemopreventive strategies [63].

A wide range of compounds has been investigated as possible chemopreventive therapies. Based on extended duration of treatment required for OSCC prevention natural products represent a group of compounds fit for contribution to chemoprevention due to their decreased dose-limiting toxicity profile. Genetic and epigenetic studies for determining molecular targets of natural product anticancer agents revealed that some common natural products, in particular polyphenols, have protective effects against OSCC [such as epigallocatechin-3-gallate have anticancer properties [91,92,93].

**Chemopreventive Activity of Some Polyphenols**

Many studies indicate polyphenols as potent natural agents in modulating carcinogenesis pathways and therefore contribute in developing novel possible therapeutic strategies in chemoprevention and treatment of OSCC.

**EGCG**

Several studies have been demonstrated that tea polyphenol and (−)-EGCG could inhibit cell proliferation and induce cell death in a variety of cancer cells including head and neck tumor. EGCG modulates many proteins and genes involved in OSCC development and progression [94].

In a human human tongue squamous carcinoma cell line tea polyphenols reduced hTERT activity in a time and dose dependent manner, disabling telomerase activity and inhibiting unlimited cancer cell proliferation [95].

In another study on cell lines derived from dysplastic leukoplakia and squamous cell carcinoma, EGCG showed antiproliferative effect by blocking cell division in G1 due
to the blockage of growth factor binding to the receptor and the suppression of mitogenic signal transduction [96]. In the same experiment curcumin blocked cells in S/G2M [96]. In combination, EGCG and curcumin exhibited synergistic chemopreventive effects [96].

Administration of EGCG on two human HNSCC cell lines increased the proportion of cells in the G1 phase of the cell cycle and induced apoptosis explained by the decrease in the cyclin D1 protein, the increase in the p21Cip1 and p27Kip1 proteins. EGCG also caused a decrease in the Bcl-2 and Bcl-XL proteins, an increase in the Bax protein, and activation of caspase 9, suggesting a mitochondrial pathway responsible for the induction of apoptosis. EGCG inhibited phosphorylation of the EGFR, signal transducer and activator of STAT3, and extracellular regulated kinase (ERK) proteins and also inhibited basal and transforming growth factor-α-stimulated c-fos and cyclin D1 promoter activity [97]. p21WAF1 upregulation was found responsible for EGCG-mediated growth arrest which may have facilitated caspase 3-mediated apoptosis in oral carcinoma cells [98].

In vivo studies showed that green tea and its constituents suppressed the cell proliferation, induced apoptosis and inhibited angiogenesis and invasion of oral carcinoma [99, 100]. Selectively induced apoptosis in oral carcinoma cells and not normal cells was correlated with the induction of p57, a cell cycle regulator, suggesting the involvement of a p57 mediated pathway in normal epithelial cells and a apoptotic pathway in tumoral cells as a mechanism of green tea chemoprevention [98].

Dietary administration of black tea polyphenols prior to DMBA exposure in a hamster buccal pouch carcinogenesis carcinomas model reduced the incidence preneoplastic lesions. The inhibitory effect of black tea polyphenols was associated with reduced expressions of p21, cyclin D1, GST-P, NF-κB, cytokeratins, VEGF, and Bcl-2, and increased expressions of Bax, cytochrome C, caspase-3, caspase-9, and PARP. Decreased expression of cyclin D1 was explained by a yet unclear effect of p21 on it [101].

During the course of green tea administration smoking-induced DNA damage was decreased, cell growth was inhibited, and the percentage of cells in S phase was reduced, cells accumulated in G1 phase (cyclin D1 positive), DNA content became more diploid and p53, caspase-3, and TUNEL, markers of apoptosis, were increased [102].

EGCG showed inhibitory effects on cell migration, motility, spread, complete inhibition on the invasion of OSCC cells via the reduced expression of matrix metalloproteinase-2(MMP-2) and urokinase type plasminogen activator [103].

Several drugs are concomitantly tested in OSCC chemoprevention. The combined effect of their association with natural compounds was studied. EGCG was found to markedly enhance the growth-inhibitory effects of 5-fluorouracil [97]. In vitro studies with five head and
neck cancer cell lines revealed that synergistic cell growth inhibition by EGCG and erlotinib was associated with significantly greater inhibition of pEGFR and pAkt, increased activation of caspases -9, -3 and PARP compared to the inhibition induced by EGCG or erlotinib alone [104]. In a more recent work, simultaneous treatment with EGCG and erlotinib, an inhibitor of EGFR-tyrosine kinase, strongly induced cell cycle arrest and apoptosis via p53-dependent induction of p21, p27, and p53-dependent inhibition of NF-κB and its antiapoptotic target, Bcl-2 in HNSCC cells [105]. Clinical administration of Doxorubicin, the most effective antitumor antibiotics, is limited due to its multidrug resistance. Tea polyphenols and EGCG were found to modulate the multidrug resistance that could influence activity of this anti-neoplastic drug by enhancing its intracellular concentration [106-91].

The beneficial effects of polyphenols in cancer treatment can be linked to their ability to modulate, in a reversible manner, epigenetic mechanisms involved in tumorigenesis leading to gene expression activation or silencing. [106].

Dietary polyphenols were found capable of inhibiting DNMT-mediated DNA methylation in vitro with varying potencies and efficacies [107,108].

The modulating effects of several tea catechins and bioflavonoids were evaluated on DNA methylation catalyzed by prokaryotic SssI DNA methyltransferase (DNMT) and human DNMT1. Each tea polyphenols, with EGCG being the more potent, inhibited SssI DNMT- and DNMT1-mediated DNA methylation in a concentration-dependent manner [109].

Recent studies have also demonstrated tRECK methylation being associated with enhanced metastasis and invasion in human cancers and with a poor prognosis in OSCC [110]. Treatment of OSCC cells with EGCG partially reversed the hypermethylation status of the RECK gene and significantly enhanced the expression level of RECK mRNA. Inhibition of MMP-2 and MMP-9 levels was also observed in these cells after treatment with EGCG. EGCG inhibited cancer cell invasion through reversal of hypermethylation status of RECK and downregulation of MMP-2 and MMP-9 [111].

These findings about EGCG activity on oral carcinogenesis suggest that this naturally occurring compound may be useful, alone or in combination with other agents, in the chemoprevention and/or treatment of OSCC.

Curcumin

Curcumin, the major yellow pigment in turmeric and curry, has been widely used as a spice, food preservative, coloring agent and an additive in cosmetic and drug preparations [112]. Curcumin is able to exert anticancer activities as it regulates several key molecular signaling pathways involved in mutagenesis, apoptosis, tumorigenesis, cell cycle regulation and metastasis [112, 113, 114].
Its anti-cancer effects are through complex mechanisms targeting many levels of regulation in the processes of cellular growth and apoptosis [112].

In oral carcinomas cell lines curcumin suppressed the growth of tumoral cells \textit{in vitro} and reduces tumor volume \textit{in vivo} through the inhibition of the antiapoptotic transcription factor NF-κB [115]. In a more recent study curcumin suppressed the activation of NF-κB through inhibition of IκK and an Akt-independent mechanism. That consecutively resulted in suppression of many NF-κB-regulated genes involved in tumorigenesis including TNF, COX-2, cyclin D1, c-myc, MMP-9 and interleukins [116].

A reduction in tumor burden by dietary turmeric was observed in DMBA induced hamster buccal pouch carcinogenesis. Turmeric modulated DMBA-induced p21ras, MAP kinases and AP-1/NF-kB pathway to alter cellular responses during oral carcinogenesis; diminished the DMBA-induced mRNA expression of proto-oncogenes (c-jun, c-fos) and NF-κB, leading to decreased protein levels and in further attenuation of DMBA-induced AP-1/ NF-kB DNA-binding in the buccal pouch nuclear extracts; decreased cell proliferation (diminished PCNA and Bcl2 expression); enhanced apoptosis (increased expression of Bax, caspase-3 and apoptotic index); decreased inflammation (levels of COX-2, the downstream target of AP-1/ NF-kB, and PGE2); aberrant expression of differentiation markers, the cytokeratins (1, 5, 8, and 18) [117].

In a 4-nitroquinolone-1-oxide oral carcinogenesis experimental model curcumin markedly decreased the expression of PCNA, Bcl-2, SOCS1 e -3 and STAT3. Curcumin also decreased the cellular atypia under microscopic analysis and the expression of the genes associated with EMT [118]. In a research on OSCC cell lines curcumin suppressed proliferation of tumoral cells via constitutive and IL-6 induced STAT3 phosphorylation [119].

Curcumin is involved in cell cycle control and stimulation of apoptosis via upregulation of p16 and p53. In addition, curcumin modulated autophagy and inhibited tumor angiogenesis and metastasis via suppression of VEGF, COX-2, MMPs and ICAMs [112].

Curcumin has proven effective in reducing oral squamous cell motility via suppression of ERK and NF-κB activations [120]. It also suppressed HNSCC proliferation and migration by inhibiting nicotine-induced activation of the mammalian target of rapamycin pathway (MTOR) pathway [121].

In a clinical study curcumin ameliorated the symptoms in patients diagnosed with oral lichen planus [122].

Recent research has been made on curcumin capacity to epigenetically regulate the expression of important genes by inhibition of DNMTs, regulation of histone modifications via regulation of HATs and HDACs and regulation of miRNA [123].

Curcumin was found to reestablish the balance between HAT and (?) HDAC 1, 3, 4, 5, 8 activities to se-
lectively activate or inactivate the expression of genes involved in cancer death and progression, respectively [124]. It was more effective than other well-known HDAC inhibitors like valproic acid and sodium butyrate [125].

Curcumin modulates miRNAs (miR-15a, miR-16, miR-21, miR-22, miR-26, miR-101, miR-146, miR-200, miR-203, and let-7) and their multiple target genes [124]. In an OSCC line curcumin inhibited proliferation by regulating miR-9 expression [126]. In the same experiment delivering anti-miR-9 resulted in downregulation of miR-9 and reactivation of Wnt/β-catenin signaling that was inhibited by curcumin [15]. In esophageal cancer cells significantly down regulated miR-21 and miR-34a expression while upregulating let-7a miRNA expression [127].

Promising data resulted from a study in which OSCC cell growth was suppressed by a combination between curcumin and suboptimal concentrations of cisplatin, both in vitro and in vivo [128]. The therapeutic agents acted in different ways: curcumin via inhibition of cytoplasmic and nuclear IkK, leading to inhibition of NF-κB activity in an Akt-independent mechanism; and cisplatin via increased expression of p16 and p53. Different mechanisms plead for potential use of sub therapeutic effective doses with minimal toxic effects of cisplatin [128].

Curcumin is an adjuvant in radiation therapy as well. In different HNSCC cell lines combination of curcumin and radiotherapy had an increased growth suppressive effect compared with administration of each of them alone [129]. Curcumin acted by enhancing cells susceptibility to the cytotoxic effect of radiotherapy by arresting the carcinoma cells in the G2/M phase of the cell cycle. Curcumin also decreased COX-2 expression and inhibited EGFR phosphorylation in carcinoma cells [129].

In another study, in an in vitro and in a mouse orthotopic model, dietary curcumin combined with fractionated radiation resulted in tumor doubling time and highly significant increase in time survival of the animals respectively [130] supporting the theory of additive effects of curcumin and radiotherapy.

**Genistein**

Genistein, a natural isoflavonoid found in soybeans, exerts important anti-carcinogenic effects via multiple molecular mechanisms implicating cell cycle, cell apoptotic processes, angiogenesis, invasion, and metastasis [131].

In an in vitro study genistein inhibited proliferation of a OSCC cell line and induced programmed cell death (apoptosis) by causing cell cycle arrest at the S/G2-M phase [132]. Antiproliferative activity of genistein was also revealed in another in vitro research - curcumin down-regulated Cdk1, and cyclinB1, and up-regulated the Cdk inhibitor p21WAF1, that leaded to induction of cell cycle arrest and apoptosis [133]. In the same study it was observed the up-regulation of Bax, with modest down-regulation of Bcl-2 protein expression, modifying the bal-
ance between pro- and anti-apoptosis molecules in favor of pro-apoptosis [133]. Down-regulation and degradation of Cdc25C, a marker of cell proliferation, was also reported indicating the antiproliferative capacity of genistein in HNSCC cells [133].

Genistein was reported to down-regulate the secretion of both MMP-2 and MMP-9 in a dose- and time-dependent manner and to inhibit the tumor cell invasion [134]. Genistein reduced DNA binding activity of NF-κB that could bind to the promoter sequence of MMP-9, transactivated the expression of MMP-9 and activate telomerase catalytic subunit hTERT [134]. It also induced the inhibition of Akt that led to the inhibition in the phosphorylation of hTERT and inhibition of nuclear translocation of hTERT [134]. Downregulation of 14-3-3, proteins acting as molecular chaperon in translocating telomerase from cytoplasmic compartment to nuclear compartment [135] also inhibited nuclear translocation of hTERT [134].

Genistein was found to inhibit cell growth with regard to concentration but induction of apoptosis was not observed [136]. By decreasing ERK phosphorylation and lowering of Akt and pAkt expression genistein suppressed cell proliferation in a concentration-related manner [137].

In another in vitro study genistein exhibited a dose-dependent inhibition of DNA methyltransferase activity and a reversal of the methylation status [136]. This activity was associated with reactivation of RARβ (retinoic acid receptor beta), p16, and MGMT (O6-methylguanine methyltransferase) genes [137,138]. These results suggest the reactivation of methylation-silenced genes, partially through a direct inhibition of DNA methyltransferase a possible mechanism in genistein chemoprevention [136,137].

Combination of genistein with different therapeutic agents was studied. Additive therapeutic effects of genistein combination with other DNMT inhibitor, decitabine, or a HDAC inhibitor, trichostatin A, in reactivation of methylation-silenced genes were observed [137].

The inactivation of NF-κB, important factor contributing to cisplatin resistance, by genistein overcome this resistance, and resulted in resulting cell growth inhibition and induction of apoptosis. The authors propose the use of genistein in developing the treatment strategy of patients diagnosed with cisplatin-resistant tumors of SCC [138].

In an OSCC-bearing nude mice model genistein reduced the microvessel density and in vitro inhibited VEGF mRNA expression [139]. In the same study genistein inhibited cancer cells invasion by reducing the gelatinolytic activity in vitro [139].

In the light of these findings, genistein may be useful as an adjunctive treatment modality for OSCC, on combination therapy with other anti-angiogenic agents or chemotherapeutics due to its effects on reversing chemoresistance could be of value.
Quercetin

Quercetin is the major flavonoid compound commonly found in cranberries, blueberries, apples, and onions. It possesses a wide spectrum of biopharmacological effects such as anticarcinogenic and antiproliferative properties that seem to be promising in developing effective chemopreventive and chemotherapeutic strategies [140].

Studying the effects of quercetin on cell growth and necrosis/apoptosis and cell cycle regulation in OSCC cells showed that quercetin induced dose- and time-dependent, irreversible inhibition of cell growth and cellular DNA synthesis [2]. Initially, quercetin induced a stress response, resulting in necrosis of the oral epithelial cells. Prolonged exposure of the surviving cells to quercetin caused apoptosis, presumably mediated by inhibition of thymidylate synthase, a key S-phase enzyme [141].

A research on molecular signaling pathways of quercetin it induced inhibition of migration and invasion in human OSCC cells. Quercetin had showed a significant inhibitory effect on cell migration and invasion of oral carcinoma cells. It also inhibited MMP-9 and MMP-2, important enzymes in invasion. In addition, the level of protein expression associated to migration and invasion was evaluated: MMP-2, -7, -9 and -10 and VEGF (Figure 5A), NF-κB p65, iNOS, COX-2 and uPA (Figure 5B), PI3K, IKB-α, IKB-α/β, p-IKKα/β (Figure 3C), FAK, SOS1, GRB2, MEKK3 and MEKK7 (Figure D), ERK1/2, p-ERK1/2, JNK1/2, p38, p-p38, c-JUN and p-c-JUN and the results leaded to the involvement of signal pathways for quercetin inhibition of migration and invasion via inhibition of MMP-2 and MMP-9 via down-regulation of PKC, blocking of MAPK and PI3K signaling pathways and both COX-2 and NF-κB [142].

Other findings were in accord with these results. Quercetin inhibited cell growth and invasion/migration via cell cycle arrest accompanied by mitochondria-mediated apoptosis [140].

In an EGFR-overexpressing OSCC cells quercetin induced growth arrest through activation of Forkhead box protein O1 (FOXO1), an Akt downstream effector. FOXO1 knockdown attenuated quercetin-induced p21 and Fas ligand expression and subsequent G2 arrest and apoptosis, respectively. Quercetin induced growth arrest through activation of FOXO1 transcription factor in EGFR-overexpressing OSCC cells [143].

A combination of quercetin with resveratrol resulted in a gradual and significant increase in the inhibitory effect of quercetin on cell growth and DNA synthesis [144].

Resveratrol

Resveratrol is a pleiotropic phytochemical belonging to the stilbene family, significantly present in grape products and red wine. The targeted molecular pathways of resveratrol involve signaling pathways related to extracellular growth factors and receptor tyrosine kinases; forma-
tion of multiprotein complexes and cell metabolism; cell proliferation and genome instability; cytoplasmic tyrosine kinase signaling (cytokine, integrin, and developmental pathways); signal transduction by the transforming growth factor-β super-family; apoptosis and inflammation; and immune surveillance and hormone signaling [145]. Resveratrol also serves as an adjuvant in multidrug resistance to 5-fluorouracil and cisplatin [145].

Examination of the cell cycle in vitro showed that resveratrol induced cell cycle arrest in the G2/M phase and enhanced the expression of phospho-cdc2 (Tyr 15), cyclin A2, and cyclin B1 in the OSCC cells and markedly increased the percentage of apoptotic cell. Resveratrol was considered an antiproliferative agent of OSCC through the induction of apoptosis and G2/M phase cell cycle arrest [146].

Resveratrol and curcumin compared to curcumin alone increased the PARP-1 cleavage, the Bax/Bcl-2 ratio, the inhibition of ERK1 and ERK2 phosphorylation, and the expression of LC3 II, simultaneously with the formation of autophagic vacuoles. The combination of these agents induced cytoplasmic NF-κB accumulation. Resveratrol and curcumin administrations were safe in BALB/c mice and reduced the growth of transplanted salivary gland cancer cells more efficiently than curcumin alone. This combination was more effective in inhibiting in vivo and in vitro cancer growth than the treatment with curcumin [147].

Resveratrol inhibited HIF-1α and its downstream target gene and VEGF, evidence that support the antiangiogenic effects of resveratrol in the setting of in vitro hypoxia or the equivalent of in vivo intratumoral hypoxia. [148].

By acting on diverse mechanisms resveratrol appears as a promising, multi-target, anticancer agent, relevant in both cancer prevention and treatment.

**Conclusion**

Although intensively studied, oral carcinogenesis remains an important global public health problem because this disease’s prognostic has not improved significantly.

Exploring the genetic and epigenetic molecular events in oral carcinogenesis could improve OSCC diagnostic, screening and treatment opportunities.

The classical therapeutic approach is often problematic therefore chemoprevention, especially by using natural agents, became very attractive. Several natural chemopreventive compounds have been studied and among them polyphenols have proved to modulate genetic and epigenetic events and play a pivotal role in chemoprevention. In addition, they showed efficacy in adjunctive treatments on combination with classical chemotherapeutics by increasing tumoral cells radiosensitivity or reversing their chemoresistance. Still, further studies are needed in order to clarify their exact mechanism of chemoprevention and develop targeted individual treatment strategies.
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