



Molecular and Signaling Pathways During Oral Carcinogenesis

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Core Message

Oral carcinogenesis is a consequence of multiple alterations in important pathways of a cell's life. These altered pathways provide capabilities to tumorigenesis that include sustained proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming cellular energetics, and evading immune destruction. Several of the genes and proteins involved in these acquired capacities for oral carcinogenesis will be developed in this chapter. Many of them could be useful as diagnostic biomarkers and also as predictors of malignant transformation.

14.1 Introduction

Oral cancer arises from the accumulation of successive genetic and epigenetic alterations in a multistep process over many years, transforming a single cell or a clone of cells of the oral mucosa into a malignant tumor. Although many genes and proteins can be affected by carcinogenic agents, including tobacco or alcohol, a group of them when deregulated are decisive for driving a cell or a clone of cells to transform and form a malignant tumor. These key genes and proteins that are affected belong to several signaling pathways related to cell proliferation, regulation, and differentiation that, when deregulated, predispose to tumor growth and progression. They include some normal genes (termed proto-oncogenes) that when modified (*e.g.* by mutation, gene amplification, or chromosome rearrangements) become oncogenes, leading to persistent cell proliferation. Contrarily, tumor suppressor genes that normally prevent deregulated cell growth but when altered by mutations, deletions, or epigenetic modifications may allow clones of cells to acquire insensitivity to growth inhibition and avoidance of apoptosis [1, 2].

Most oral cancers are squamous cell carcinomas, which means they represent a homogeneous group of tumors arising from lining epithelia comparable with squamous cancers in other locations. This suggests a carcinogenesis model with common characteristics such as Califano's model based on serial and sequential amounts of genetic errors occurring in key oncogenes. Thus tumor suppressor genes would be the basis for transformation of a normal mucosal cell into a cancer [3]. As Hahn and Weinberg had proposed around the beginning of this century for cancer in general, several pathways would be typically affected along the landscape for oral cancer [4, 5]. These altered pathways provide capabilities to tumorigenesis that include sustained proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Additionally, to these features of cancer, more emerging capacities include reprogramming cellular energetics and evading immune destruction [6].

As with other cancers, molecular and genomics studies have thrown new insights into several new aspects of oral carcinogenesis and we can no longer define a linear and unidirectional oral carcinogenesis model driving cells, but

instead what has emerged is a dynamic, personalized, or multidirectional model with heterogeneity [7, 8]. These genetic studies suggested that some subclasses of head and neck cancers can be defined and identified through genetic profiles that have been recently mapped out. One such group of head and neck tumors (especially in the oropharynx) could be clearly related with the presence of HPV infection (HPV + ve) [9, 10]. Comparative genomic hybridization and ploidy status studies have revealed subgroups of HPV -ve tumors with few copy number alterations or with diploid status with a relatively good survival when compared with the rest of HPV -ve tumors [8, 10, 11]. Moreover, other subgroups include patients typically presenting with tobacco-induced tumors with alterations in, for example, the epidermal growth factor receptor (EGFR) pathway that indicate a poor prognosis [7, 10].

This means that the model of carcinogenesis, such as presented in Fig. 14.1, is not similar or uniform for all oral cancers and the identification of the molecular signatures could have important and useful implications for diagnosis, prognosis, and treatment planning of oral cancers. Moreover, each tumor will have its own molecular signature and novel capacity to explore new molecular escapes. The identification of key genes or proteins involved in this tumor escape could be important for the individual characterization of the tumors and could function as biomarkers of the disease not only for early diagnosis but also for predicting prognosis [12].

In the following sections, we will discuss several genes, proteins, and their related pathways most often associated with oral cancer.

Definition

A cancer biomarker refers to a substance that is indicative of the presence of cancer in the body. It could have a diagnostic, predictive or prognostic value.

14.2 Altered Pathways Involved in Oral Carcinogenesis

In physiological states, the cell cycle is controlled by several proteins and genes with activating or suppressing activity which drive cells through the cell cycle, allowing for cell division when needed. Important genes and proteins have a strong capacity to stop and cause inhibition of cell proliferation, especially when DNA errors are detected. Several tumor suppressor genes operate on this principle, such as *RB* and *TP53*, but when affected by mutations or inhibitions such as HPV proteins, they lead to pathways to oral cancer [4].

We present here some commonly altered genes/proteins in oral cancers that have been reported.

Important

A biomarker may be a molecule secreted by a tumor or a specific response of the body to the presence of cancer.

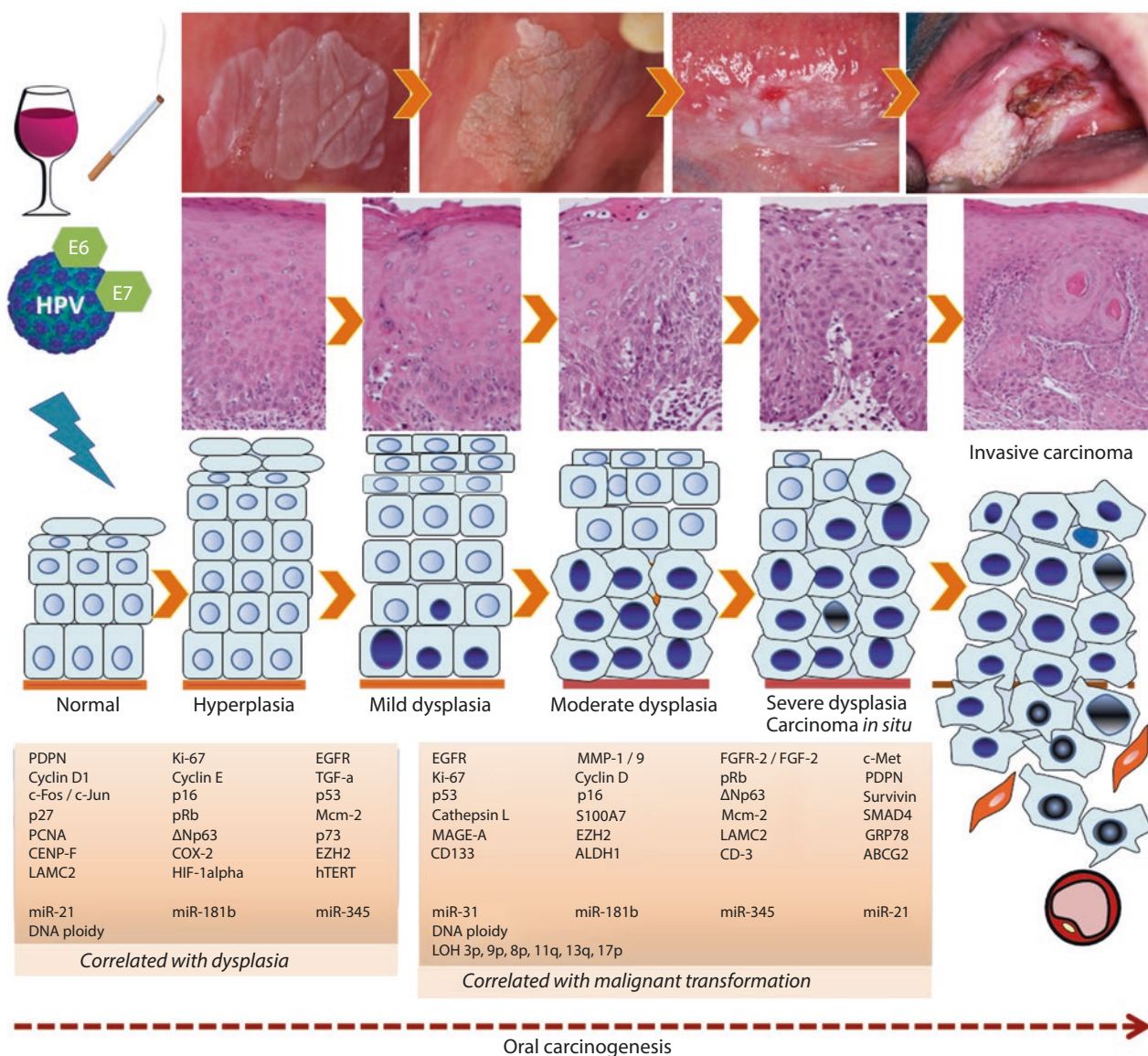


Fig. 14.1 An integrative oral carcinogenesis model depicting changes from a normal cell to a transformed cell indicating possible alterations in clinical, histological, and molecular changes. Several

genes/proteins could be important for the oral carcinogenesis development

14.2.1 Evading Growth Suppressors

— P53

P53, a phosphoprotein of 53 kD, is a transcription factor present in most cells with several significant functions: the regulation of gene transcription, regulation of DNA synthesis, and repair and apoptosis. It is encoded by *TP53*, an onco-suppressor gene located on chromosome 17 (17p13.1) [13].

In the presence of cellular stress (including DNA errors, hypoxia, oxidative damage, or exposure to radiation), P53 levels increase dramatically, initiating a cell protective response. Briefly, an increase in P53 induces transcription of *CDKN1A* gene encoding the P21 protein, responsible for cell

cycle arrest at the G1 phase checkpoint, by inhibition of the complex CDK4/6 – cyclin D1. At this time, non-phosphorylated pRB binds and inhibits E2F family preventing the transcription of factors required for the cell cycle and for other cyclins such as cyclin E and cyclin A to act in consort [14]. This cell cycle arrest allows DNA repair mainly by the activation of the *GADD45* family of genes in association with PCNA, p48/DDB2, ERCC2, and ERC3. Nevertheless, if the DNA injury is too severe, or if p53 DNA repair is not possible, apoptosis occurs. P53 is capable of inducing pro-apoptotic genes such as *BAX*, *PUMA*, *PIG3*, *NOXA*, *TRAIL*, or *PTEN* and can inactivate anti-apoptotic genes such as *BCL2* or *SURVIVIN*. In the case of successful DNA repair, the now active *MDM2* promotes P53 degradation and the break

of the cell cycle [14]. Hence, this protein could be considered as a guardian of the genome [13].

Alterations in P53 protein occurs in several types of tumors and in half of them by mutations in *TP53*. Most of the mutations are located in the DNA binding domain, impairing the P53 binding to target genes [15]. Interestingly, many of the *TP53* mutations observed in head and neck carcinomas affect guanine nucleotide (G) and are caused by tobacco carcinogens [14].

Loss of P53 function can occur by other mechanisms even without any mutations of *TP53* and in the presence of the normal protein, such as its upregulation by amplification or due to polymorphisms of the MDM2, leading to P53 degradation by the ubiquitin proteasome system. Infection of cells with high risk types of human papillomavirus (HPV) can also lead to P53 degradation, brought about by viral E6 protein which binds to P53 leading to ubiquitin proteasome degradation.

Somatic mutations in *TP53* gene with consequent and frequent P53 overexpression represent one of the most reported changes in squamous cell carcinomas of the oral cavity and found in more than 50% of cases and correspond to an early event already present in potentially malignant disorders [16–19]. Overexpression of P53 is associated with poor survival of oral squamous cell carcinomas [15, 19–21].

In addition to *TP53* mutations, other early events in oral carcinogenesis include the loss of chromosome 9p, which is the locus for this gene [22]. *CDKN2A* is a gene located on chromosome 9p21 and encodes *P16*, a tumor suppressor protein that promotes cycle arrest in G1/S check-point, by binding to the complex cyclin D1/CDK4, which inactivates pRb. Loss or inactivation of this gene is frequent (by mutations, methylation, chromosome loss, or homozygous deletion) in early oral carcinogenesis. Many oral carcinomas have reduced expression of P16 and this has been correlated with a poor prognosis [22].

— *pRb*

pRb protein is encoded by the *RB1* gene (3q14.1-q14.2) and the loss of both alleles of this gene leads to retinoblastoma. In the normal cell, pRb is in a hypo-phosphorylated state. When a mitogenic stimulus is transmitted, the transcription of cyclins A, D, E increase dramatically leading to the phosphorylation of pRb. Now, the phosphorylated form of pRb becomes permissive with the transcription of genes involved in DNA replication and cell cycle progression [1]. *RB1* mutations in oral cancer are rare, but pRB protein could be inactivated by other forms such as the action by E7 from HPV [22].

14.2.2 Enabling Replicative Immortality

■ Telomerase and TERT Proteins

Other mechanisms of cell cycle persistency would involve other genes, such as telomerase and TERT proteins. Although the data on these proteins for oral cancer are not obvious yet,

the altered malignant cells could undergo alternative lengthening of telomeres (ALT), a related TERT process of telomere lengthening [22].

Tumor cells could gain the capacity to sustain proliferative signaling using mitogenic signaling pathways by producing growth factors themselves in an autocrine proliferative manner, to induce stromal tumor cells to produce mitogenic factors for tumor cells, or simply by constitutive activation of components of mitogenic pathways.

In oral cancer, the most commonly affected pathways includes EGFR pathway, MAP kinases or PI3K/AKT/mTOR pathways, or even the endpoint of mitotic pathways in the nucleus as cyclins and kinases.

14.2.3 Sustaining Proliferative Signaling

— EGFR

The epidermal growth factor receptor (EGFR) is a transmembrane receptor encoded by the *c-erbB* proto-oncogene (located at 7p12). This glycoprotein is composed of an extracellular ligand-binding part, an intermediate transmembrane region, and an intracellular domain with tyrosine kinase capacity (■ Fig. 14.2).

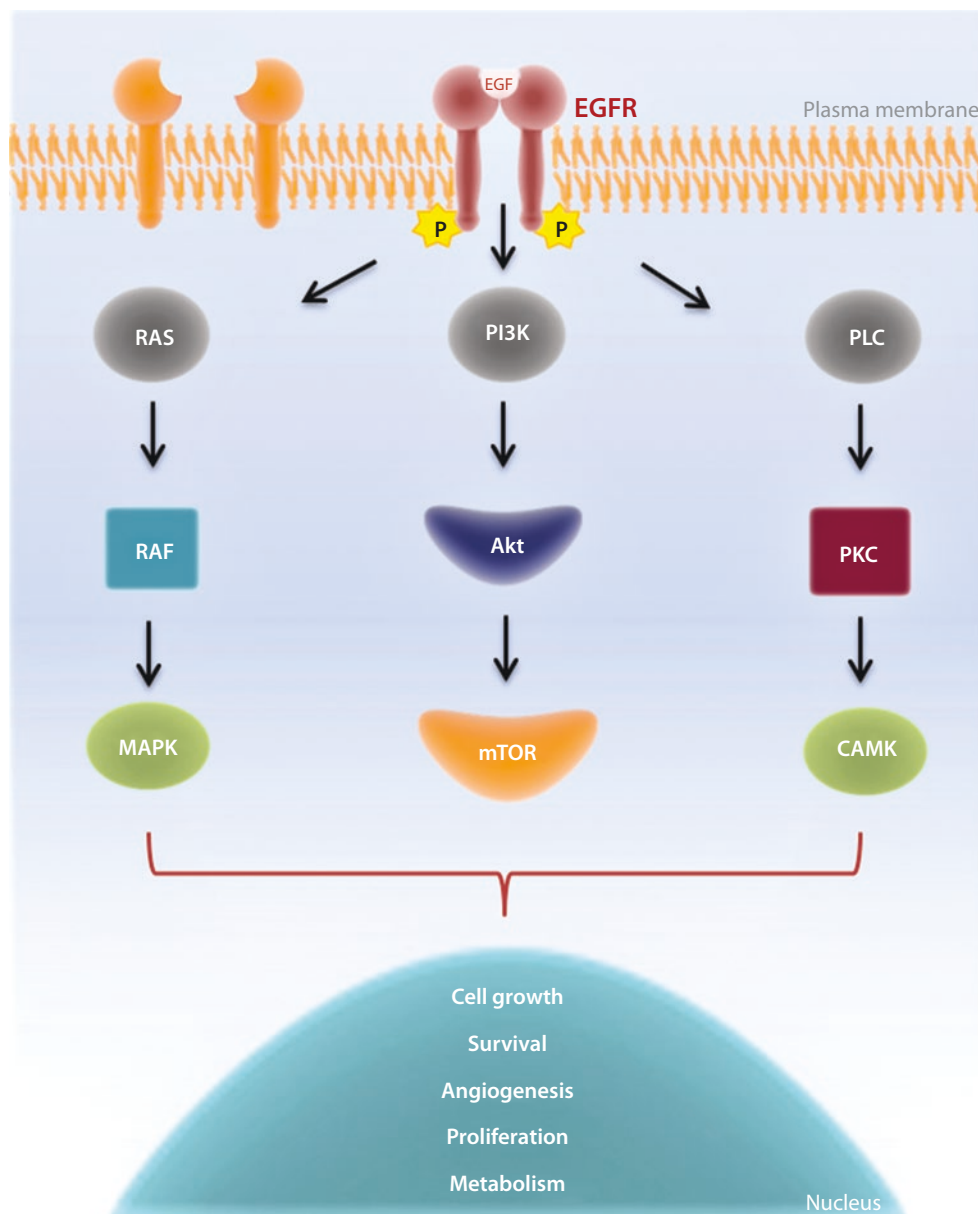
When a ligand such as epidermal growth factor (EGF), transforming growth factor alpha (TGF- α), epiregulin (ER), or amphiregulin (RA) binds to EGFR, a homo- or heterodimer is formed with one or more members of the ErbB family (such as ErbB2/HER-2, ErbB3/HER-3, or ErbB4/HER-4) leading to the phosphorylation of cytoplasmic tyrosine residues mainly at positions 992, 1068, 1086, 1148, or 1173 [23]. The activated tyrosine kinase domain induces the transduction of mitogenic and survival signals by mitogen-activated protein kinase (MAPK) pathway, phosphatidylinositol 3-kinase (PI3K)/Akt pathway, or phospholipase C γ (PLC γ 1) [24].

EGFR is involved in cell proliferation and survival not only in a normal cell but also promotes tumor growth and resistance to apoptosis, promotion of cell motility, alteration and reduction of adhesion molecules such as E-cadherin, stimulating metalloproteinases (MMP-9), or even in the process of angiogenesis by regulating VEGF [24].

EGFR overexpression has been reported in several cancers and interestingly in more than 90% of head and neck squamous cell carcinomas (HNSCC) and has been associated with aggressive disease and poor prognosis [7, 25–27]. Some studies have shown that both membranous and cytoplasmic expression of EGFR could have an adverse influence in the overall survival of patients with oral squamous cell carcinoma [28].

EGFR overexpression can be caused by several mechanisms. *EGFR* gene amplification has been reported in 10–30% of head and neck cancers [24]. *EGFR* mutations have been described with a fewer frequency (1–7%) and could include point mutations (e.g., exon 21 (L858R)), deletions in exons 2–7 that result in the *EGFRv III* variant, lacking the extracellular binding domain but with active constituent [29–33] and

Fig. 14.2 A schematic resume of EGFR pathway is shown including the dimerization and phosphorylation of the tyrosine kinase receptor EGFR and subsequent activation of several pathways resulting in cell growth, proliferation, and survival



by other mechanisms such as an autocrine expression with EGF and TGF- α , albeit unusual in head and neck cancers [34].

EGFR is one of the popular molecular targets for therapeutic agents against head and neck cancers due to the high overexpression rate of this receptor found in these cancers. The inhibition of this receptor can be achieved using monoclonal antibodies, tyrosine kinase inhibitors (TKIs), ligand-toxin conjugates, or immunoconjugates [35]. Monoclonal antibodies, such as Cetuximab, block the binding of the growth factor to the external domain of EGFR impairing the activation of the receptor. This anti-EGFR drug has shown to increase the overall survival of HNSCC patients presenting in advanced stages as part of combination therapies with radiation and chemotherapy [36]. EGFR can be inactivated also using tyrosine kinase inhibitors, small molecules which inhibit the tyrosine kinase activity of this receptor. TKIs include gefitinib, erlotinib, and lapatinib [37] (see for details ► Chapter 27).

— *c-MET*

Another tyrosine-protein kinase receptor that has been shown to be involved in oral cancer is the mesenchymal-epithelial transition factor (*c-MET*), a receptor for the hepatocyte growth factor (HGF) encoded by the proto-oncogene *MET* [38]. Mutations and gene amplifications of *MET* have been found in several cancers, including oral cancers [22]. *c-MET* has been found overexpressed in tumor cells and also in carcinoma-associated fibroblasts (CAFs) promoting cell growth, motility, and lymphangiogenesis in oral squamous cell carcinomas (OSCC) via PI3K/AKT, ERK1/2, and NF- κ B pathways [38, 39].

— *TGF β /SMAD Pathway*

Signaling through TGF β /SMAD pathway can be involved in oral tumorigenesis [40]. Transforming growth factor- β

(TGF- β) is a receptor with inhibitory growth control regulation function [39]. This receptor can phosphorylate SMAD2 and SMAD3 proteins, and then together they can activate SMAD4 protein that will regulate the expression of target genes such as p15, p21, or p57 [22]. Interestingly, mutations have been found on SMAD2, SMAD3, and SMAD4 proteins in OSCC [22, 41]. Numerous studies showed that TGF- β /SMAD signaling pathway is associated with tumor progression and worsening of prognosis of OSCC [41, 42].

— PI3K /AKT and mTOR Pathways

PI3K/AKT/PTEN/mTOR has become a recognized important dysregulated signaling pathway in head and neck cancer. This multiple role pathway can influence proliferation and cellular survival, as well as cell motility, migration, and glucose metabolism [43]. Several genes and proteins are involved in this pathway, such as PI3K proteins, mTOR complex, AKT protein, and PTEN.

The phosphatidyl inositol 3-Kinase (PI3K) is composed of the subunits p100 and p87 which works as a heterodimer coupled to tyrosine kinase receptors such as EGFR. When the receptor is activated, p100 promotes the phosphorylation of PIP2 into PIP3. This attracts the PDK1 and phosphorylates the AKT protein a serine/threonine kinase, which in turn stimulates several proto-oncogenes and suppresses other tumor oncosuppressor genes, resulting in cell proliferation and inhibition of apoptosis [22]. The p110 subunit is encoded by PIK3CA, on locus 3q26. Interestingly, this locus or the gene have been found to be amplified or to have activating mutations, in some head and neck cancers. AKT can also be phosphorylated by activation of the mTOR complex [22].

One of the main targets of the PI3K/AKT signaling pathway is the mammalian target of rapamycin (mTOR), a serine-threonine protein kinase that makes part of two different protein complexes – mTOR complex 1 (rapamycin sensitive) and mTOR complex 2 with multiple actions, including cell proliferation and survival, cell motility, protein synthesis, or insulin receptors regulation (■ Fig. 14.3). In the presence of nutrient or oxygen stimuli or other factors such as insulin, growth factors, ATP, or toxins of tobacco, mTOR becomes phosphorylated and activates the eukaryotic translation factor 4E (eIF4E), the p70 ribosomal S6 kinase (p70S6 kinase), and elongation factor 2 (eEF2) that will modulate protein biosynthesis. pS6 protein is one of the targets of mTORC1 and is inhibited by rapamycin and is used as a marker for the mTORC1 pathway [44].

mTOR has been found to be overexpressed and is related with poor overall survival in several cancers including oral cancer [45, 46]. Importantly, mTOR has been used as a molecular target for anticancer therapy including everolimus, temsirolimus, and ridaforolimus [47]. Interestingly, some authors have reported on the anti-cancer effect of metformin by inhibition of mTOR activity [48].

Another protein involved in this pathway is PTEN. *PTEN* gene (phosphatase and tensin homolog deleted on chromosome TEN) is a tumor suppressor gene, located at 10q23.3, that

encodes a protein phosphatase with lipid and protein phosphatase activity. The AKT activation is normally turned off by *PTEN* gene that promotes the switch of phosphatidylinositol (3,4,5)-triphosphate (PIP-3) to PIP2. Loss of the PTEN function is reported in ~10% of head and neck cancers. This may lead to increasing levels of PIP-3, resulting in a hyperactivation of AKT and unrestricted activity of mTOR [46].

— RAS and MAPK Pathway

The classical cellular signal transducers include a family of proteins with ~21 kDa protein with guanosine triphosphate (GTP) activity known as RAS proteins. They were named because they were discovered from the genome of murine leukemia virus (rat sarcoma virus) in rodent sarcomas. Currently, three genes characterize the RAS gene family: *HRAS* (Harvey sarcoma virus-associated oncogene), *KRAS* (Kirsten sarcoma virus), and *NRAS* (neuroblastoma-derived sarcoma virus) [49].

RAS becomes active after the phosphorylation of a tyrosine kinases receptor (rtks) thought to result in Grb2-SOS complexes and G proteins (guanine nucleotide-binding proteins). Active RAS leads to the stimulation of several pathways, including mitogen-activated protein kinase (MAPK) by RAF1 kinase, of both MEK1/2 or the phosphatidylinositol-3-kinase (RAS)/AKT, resulting in cell growth and differentiation [49].

RAS is commonly mutated in several cancers including oral cancer, especially as point mutations. Some mutations occur in codons 12, 13, or 61, and the RAS protein becomes permanently activated with a continuous cell growth. Although mutations can appear in all three isoforms of the RAS gene, most of them appear in *HRAS* (0–55%) especially in South Asian populations [49, 50]. Other mechanisms of RAS overexpression can be related to gene amplification.

— Cyclins and Mitotic Checkpoint

Cyclins correspond to several forms of proteins divided into two groups based on their function: the G1 cyclins (C, D, E), regulating the passage of cells through the G1 phase and their entry into the S phase, and the mitotic cyclins (A, B) [2, 51]. Cyclins have no phosphorylation capacity, so they work together with several kinases (CDK) during the passage of the cell cycle. The cyclin D1, encoded by *CCND1* proto-oncogene (11q13), is the great opener or activator of the cell cycle. After activation, by second messengers such as proteins from MAPK the pathway, this cyclin binds and activates proteins CDK4 and CDK6, leading to phosphorylation of pRb, driving the cell cycle from the G1 to the S-phase. Other cyclins conduct the completion of the rest of the phases of cell cycle. Cyclin A (*CCNA2* gene 4q25-q31) is required for DNA synthesis during the S phase and progression through the G2/M transition. Cyclin E (*CCNE1* gene in 19q12) is expressed in the middle of G1 and ends at the beginning of the S phase, and cyclin B1 (*CCNB1* gene in 5q12) is crucial to drive cells into mitosis phase.

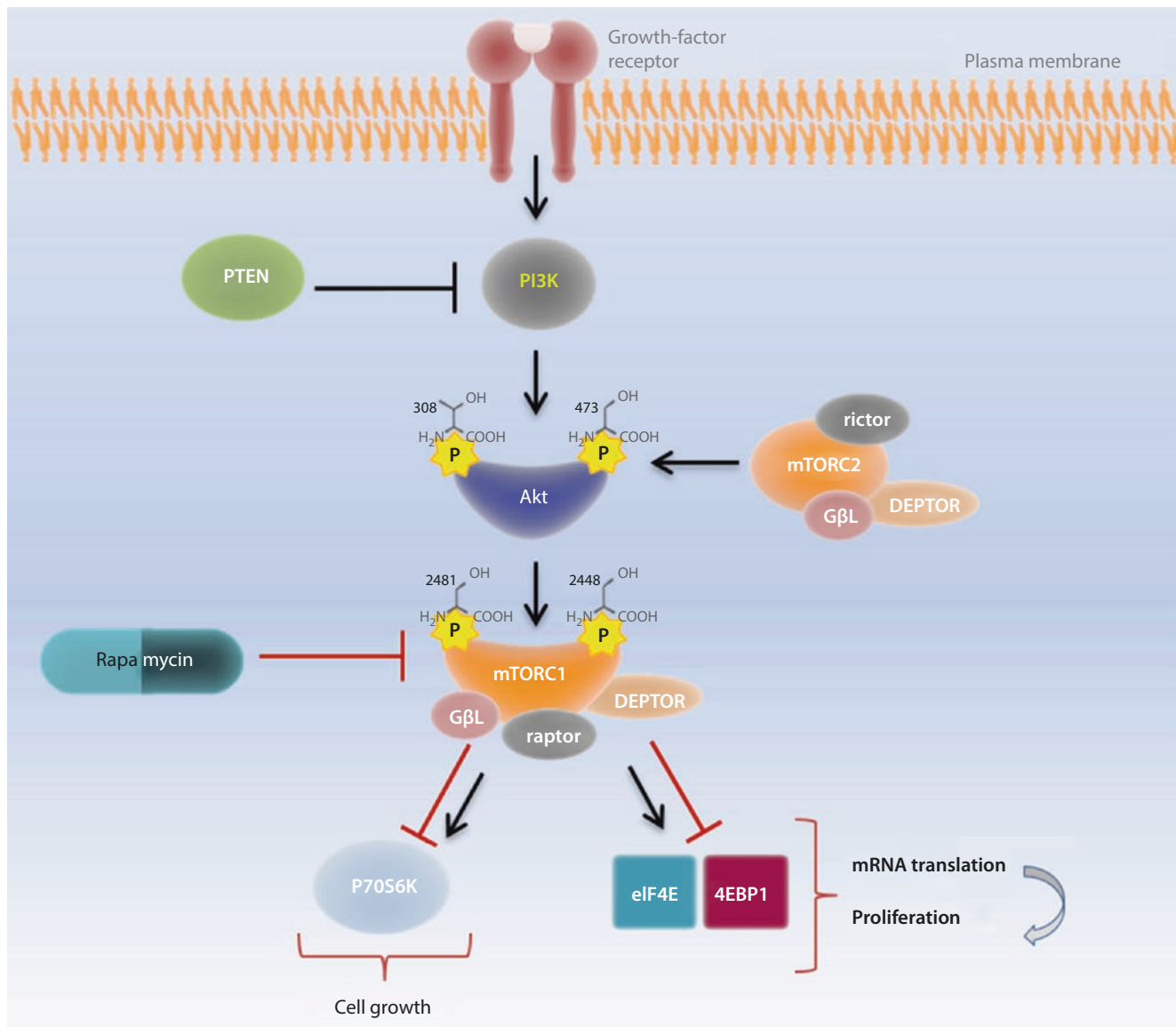


Fig. 14.3 Representation of the AKT/mTOR pathway from a membrane receptor activation to cell growth and proliferation. The inhibition effect of rapamycin is illustrated

CCND1 amplification and Cyclin D1 overexpression have been reported to be a frequent event in several tumors, including head and neck cancers, and are related to poor survival [20, 52–55]. Cyclin E and cyclin B1 overexpression may lead to accelerated G1/S transition or even to premature entry into mitosis, contributing to increased chromosomal instability [56] and abnormal cell proliferation. Cyclin A, E, or B1 overexpression have been found to be adverse prognostic factors in oral potentially malignant disorders and oral malignancies [51, 56–60].

Genetic instability is one of the hallmarks of cancer and is a known process for tumor development. Spindle assembly checkpoint (SAC) is one of the most important checkpoints that controls cell division and prevents genetic instability. During this phase, when errors in the attachment of chromosomes are detected, there is formation of an

inhibitory complex, called mitotic checkpoint complex (MCC), composed by mitotic checkpoint proteins such as Mad2, Bub3, or BubR1 [61]. This complex prevents the function of other proteins such as CDC20 that normally lead to activation of the anaphase-promoting complex/cyclosome (APC/C), and the 26S proteasome degradation of securin and cyclin B, preventing premature anaphase and aneuploidy due to deregulation of chromosomal alignment and separation (Fig. 14.4, adapted from Teixeira et al. 2014) [61]. After normal sister chromatid separation and anaphase onset, the destruction of securin and cyclin B promotes the exit from mitosis and the beginning of interphase [61–63].

Among the proteins mentioned, CDC20 and BubR1 have been found overexpressed and related to reduced survival rates in OSCC [64, 65].

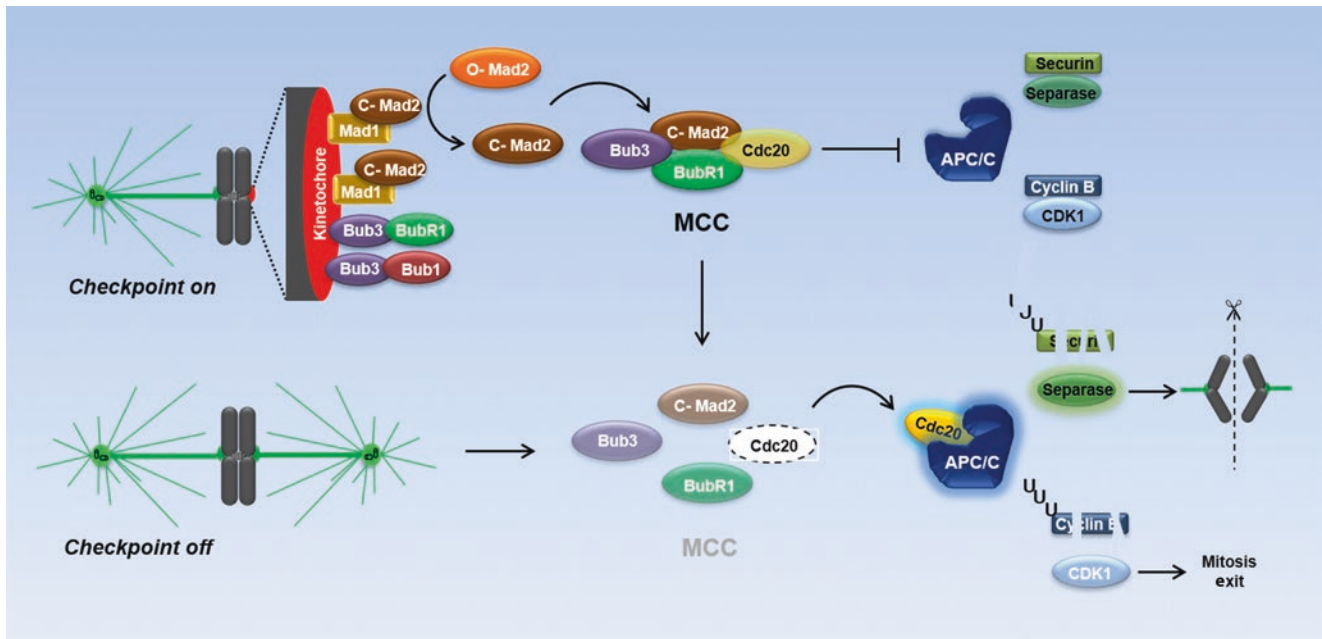


Fig. 14.4 Signaling pathway of the spindle assembly checkpoint (SAC). An inappropriate attached kinetochore activates the spindle assembly checkpoint via an association of Mad2, Bub3, Bub1, and Cdc20 [61]

Wingless-Related Integration Site (WNT)

WNT signaling pathway is an important signaling pathway composed of several proteins including WNT ligands, the protein AXIN, and APC. During a non-activated phase a complex including AXIN, APC, and glycogen synthase kinase 3 β sequesters β -catenin leading to their proteasomal degradation. By contrast, in the presence of a WNT ligand this complex is attached to the cell membrane leaving β -catenin free into cytoplasm and nucleus with the activation of several Wnt target genes, resulting in cell proliferation, tumor growth, and a stem cell phenotype [66].

Deregulation of the Wnt/ β -catenin pathway lead to the carcinogenesis of many types of human cancers. Recently, other proteins were associated with this pathway including the Fat1 protein, a cadherin-like protein, and the NOCTH, a tumor suppressor gene, in oral cancer. FAT1 mutations in multiple cancer types suggests that FAT1 is a major cause of Wnt pathway activation in several human cancers. The inactivation of FAT1 by mutation has been reported to increase the Wnt signaling and tumor progression carrying an adverse prognosis in patients with head and neck cancers) [67].

NOTCH

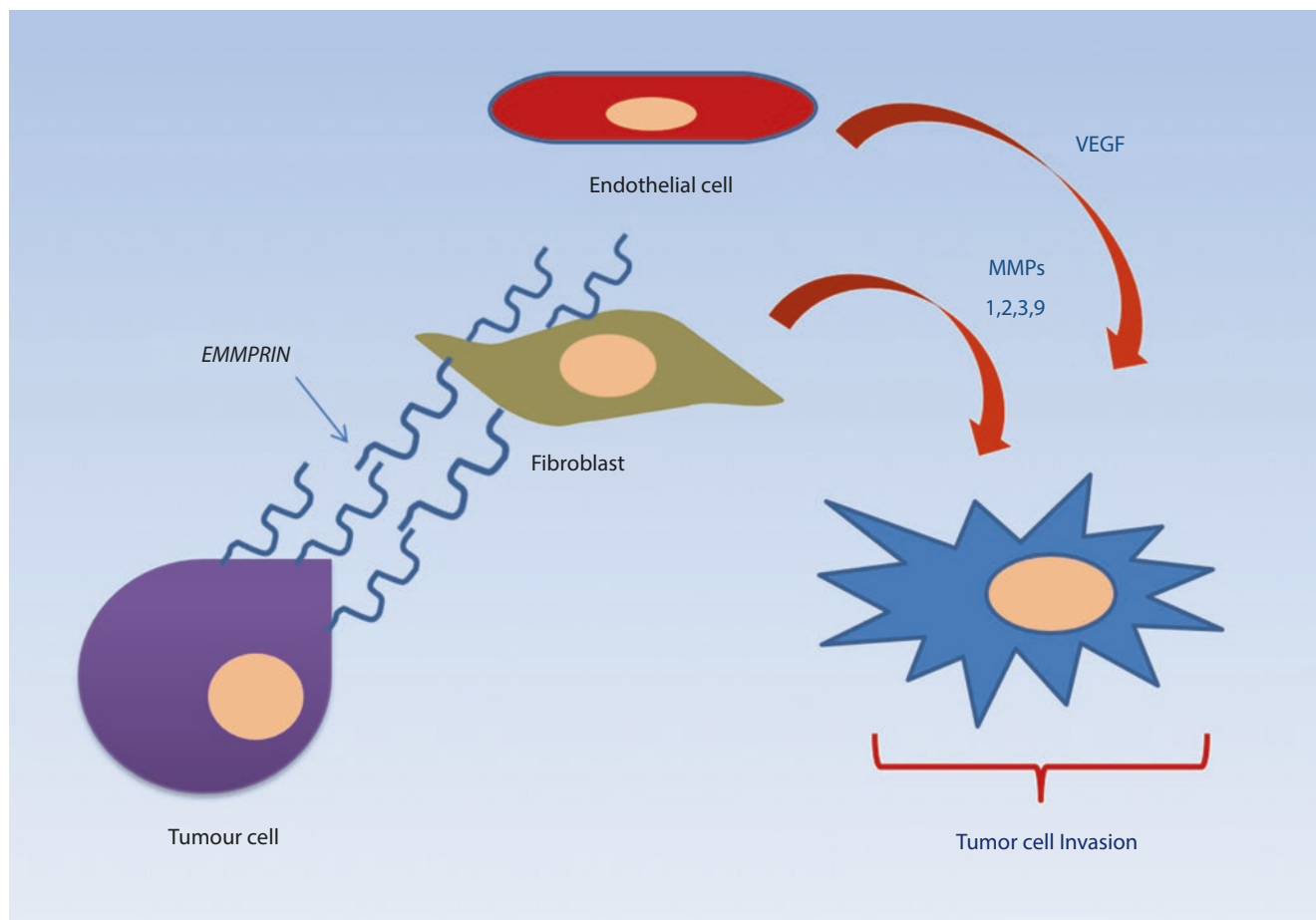
NOTCH signaling is an evolutionarily conserved pathway already present in unicellular eukaryotic cells and in multicellular organisms, regulating cell proliferation, apoptosis, and differentiation. In humans, the NOTCH family is composed of four receptors (NOTCH1–4) and five ligands (JAGGED1 and 2, and DLL1, 3, and 4) [68]. The binding of the ligand to NOTCH receptor leads to the proteolytic release of the NOTCH intracellular domain (NIC) by secretases and

its translocation to the nucleus, starting the transcription of the NOTCH target genes [69].

Dysregulation of NOTCH pathway has been reported in several cancers, including non-small cell lung cancer, ovarian carcinomas, colon cancer, pancreatic cancer, osteosarcoma, T-cell acute lymphoblastic leukemia, and head and neck carcinoma [70, 71]. *NOTCH-1* has been reported as the second most frequently mutated gene in head and neck carcinoma after *TP53* [72]. This was reported especially in a Chinese population and was related to the use of high alcohol-containing beverages in China [70]. *NOTCH-1* mutations were also found in oral potentially malignant disorders, suggesting a role for NOTCH receptor in early stages of oral carcinogenesis and OSCC progression [73, 74]. Molecular therapies directed to NOTCH pathway could be interesting, such as the γ -secretase inhibitor (GSI), a pharmacological agent, which is capable of blocking NOTCH activation, preventing the in vitro growth of OSCC cells and resulting in the delay of tumorigenesis [75].

14.2.4 Invasion and Angiogenesis

Many oral cancers show an invasive phenotype and are capable of metastasis, especially to the regional lymph nodes. Regional spread occurs in more than one-third of the cases [76]. Invasion and metastatic dissemination are sequential processes in which, with acquired capacities, tumor cells escape from their tissue of origin, enter the stroma, and travel to distant sites. As part of such acquired capabilities, tumor cells must lose their surface adhesion molecules, which bind them to their own tissue, must be capable of migrating into and through the connective tissue and must be capable of



■ **Fig. 14.5** The stimulation effect of EMMPRIN on fibroblast and endothelial cells resulting in MMP or VEGF production promoting tumor cell invasion

entering lymphatic or vascular channels to escape to other locations.

A group of molecules play a key role in intercellular adhesion of keratinocytes in the oral epithelium.

— Cadherins, Claudins, and Occludins

Cadherins are a family of junctional cell-surface glycoproteins commonly represented by E-cadherin, a 120-kDa transmembrane glycoprotein encoded by the *CDH1* gene located on chromosome 16q22.1. E-cadherin is also involved in the transduction of signals controlling various cellular events, including polarity, differentiation, cell growth, and cell migration [77]. Reduced expression of E-Cadherin has been found in oral cancers and was related to tumor progression, dissemination, and poor prognosis [77]. Another group of adhesion molecules belongs to tight junctions. These form intercellular junctional complexes located at the apical side of the lateral membranous surface cells and are important in maintaining cell polarity. This group of proteins includes claudins and occludins. Their deregulation has been reported in a variety of cancers, including oral squamous cell carcinomas, and is related with poor survival rates [78, 79].

Tumor cells and specially tumor microenvironment (TME) cells, such as tumor-associated fibroblasts, can produce factors that stimulate the production of collagenases such as matrix metalloproteinase (MMPs). There are several types of MMPs and related proteins such as extracellular matrix metalloproteinase inducer (EMMPRIN), which increase their expression and function or decreases such as TIMPs. EMMPRIN and MMP-9 have been related to tumor progression and invasion in oral cancers [80, 81].

— EMMPRIN and MMP-9

One of these molecules is the EMMPRIN, also known as CD147. It is a highly glycosylated transmembrane protein that has shown a strong capacity to induce the expression of matrix metalloproteinases. EMMPRIN also contributes to cell adhesion modulation, tumor growth, invasion, and angiogenesis. Overexpression of EMMPRIN was found in OSCC, with an autocrine and paracrine positive effect for MMP production enhancing tumor invasion and dissemination [80] (■ Fig. 14.5).

Matrix metalloproteinase 9 (MMP-9), also known as gelatinase-B or type IV collagenase, is a 92-kDa zinc-dependent endopeptidase, involved in the degradation of the extracel-

lular matrix. Overexpression of MMP-9 could promote the degradation of the basement membrane and the extracellular matrix, in particular collagen IV, contributing to tumor invasion. Overexpression of MMP-9 has been associated with the lymph node and distant metastasis in oral squamous cell carcinomas and is related to adverse overall survival [81].

Neovascularization has been considered an important hallmark of tumorigenesis and tumor dissemination. Since the work of Folkman, it is well known that solid tumors cannot exceed 1–2 mm³ without the existence of a new blood supply formed from the adjacent connective tissue vessels [80, 82]. Several molecules participate in this angiogenic process, including vascular endothelial growth factor (VEGF) and its receptors VEGFRs [83].

— VEGF and VEGFRs

VEGF is a heparin glycoprotein produced by tumor cells, and also by peritumoral endothelial cells and inflammatory cells such as macrophages in the presence of hypoxic-inducing factors (HIF). VEGF can increase vascular permeability, stimulate production of proteases, migration, proliferation, and differentiation of endothelial cells and capillary tube formation, increasing vascular support within tumor cells [83, 84]. The majority of solid cancers overexpress this factor and this is associated with a higher risk of recurrence, metastasis, and poor survival [83].

VEGF effects are mediated by vascular endothelial growth factor receptors (VEGFR) composed of 3 tyrosine kinase receptors including VEGFR-1 (Flt-1), VEGFR-2 (KDR / flk-1), and VEGFR-3 (flt-4) [83–85].

VEGFR-1 and VEGFR-2 are located on the vascular endothelial cells and macrophages, while VEGFR-3 is found mostly in the endothelium of lymphatic vessels. Interestingly, all can be found in the cells of several tumors, including head and neck cancers. Each receptor could contribute differently to tumorigenesis. VEGFR-1 has been related to the infiltration of macrophages and increases in MMP-9 in lung tissues before the appearance of lung metastasis. VEGFR-2 has been related to the recruitment of hematopoietic and endothelial precursor cells from bone marrow, while VEGFR-3 is mostly involved in tumoral lymphangiogenesis, contributing to its metastatic effect [83–86].

Targeting tumoral angiogenesis is an attractive therapeutic approach. Several antibodies and selective inhibitors have been studied and validated and some are already in clinical use. Bevacizumab is a VEGF antibody approved for anti-angiogenic therapy for several types of cancers, while vandetanib, sorafenib, and sunitinib are tyrosine kinase inhibitors [85, 87] (see ► Chapter 27).

— Podoplanin

Podoplanin is a transmembrane glycoprotein encoded by the *PDPN* gene and was named after its discovery in kidney podocytes [88, 89]. It is a classic marker of lymphatic endothelium but not blood vessel endothelium. Some recent

reports gave visibility to this protein as a predictive marker of malignant transformation in oral leukoplakia. Podoplanin is normally not expressed in normal oral epithelium and when expressed, sometimes in cluster points, it indicates that it could be a stem cell marker, or even in a diffuse pattern it may represent a sign of an increased risk of malignant transformation. Moreover, podoplanin could promote cell motility and migration of tumor cells to the invasive front of the tumors, many times working along with metalloproteins such as MMP-9 [90–92]. As a lymphatic endothelium marker, podoplanin has been related with lymph node metastasis [93, 94]. Overexpression of podoplanin has been reported in cancers of the lung, breast, skin, larynx, uterine cervix, esophagus, germ cell tumors, as well as head and neck cancers including oral cancers and is related with poor prognosis [81, 95–98].

Interestingly, molecular therapies against PDPN have been evaluated, including antibodies against PDPN with promising results in preclinical studies [99].

Eyecatcher

Podoplanin has been recently reported to have both prognostic significance in oral cancer and heightened malignant transformation of oral leukoplakia.

14.2.5 Reprogramming Cellular Energetics and Evading Immune Destruction

Malignant cells can recruit and corrupt adjacent non-transformed cells and become involved in interactions that create the tumor microenvironment (TME). Such interactions modify the tumor stroma and, ultimately, promote regulation of energy availability, angiogenesis, and tumor metastasis [6, 100, 101]. Moreover, in addition to the fibroblasts, cells of tumor vasculature and lymphatics, the non-transformed cells include cells of the immune system, suggesting a relation between tumor cells and the immune system [6].

Proliferative tumors have also developed energy pathways responsible for sustained tumor growth and survival in adverse conditions. This is obtained essentially by glycolysis, even in an aerobic environment, a condition known as “Warburg effect” that results in lactic acid secretion in the stroma [101, 102]. To compensate the missing energetic efficiency of aerobic energy, tumor cells use glucose transporters such as GLUT1, which increase glucose transportation to the cytoplasm. In the presence of hypoxia, HIF1 and HIF2 also upregulate glycolysis. As a consequence, high concentration of lactic acid is produced, which is exported out of the cells by monocarboxylate transporters (MCTs). Deregulated expression of MCT1 and MCT4 has been reported in many cancers including oral cancers and has been correlated with poor prognosis [6, 103].

For many years there have been reports of infiltration of immune cells in the tumor microenvironment but without

any known significance. Nowadays, it is believed that cancers, such as oral cancer, can avoid their identification by immune cells, escaping any host defense mechanisms. A hypothesis for this could be the related remodulation of tumor cells in order to eliminate some high immunogenic clones, a process called immunoeediting. Deficiencies in the CD8+ and CD4+ T lymphocytes and NK cells had been related with increased tumor incidence [6]. Other explanations could include the capacity of tumor cells to produce immunosuppressive factors. In particular, special attention has been put on some molecules that can control the function of T-cells, programmed death protein one (PD-1) and its ligands, programmed death ligand one and two (PD-L1, PD-L2). PD-1 and PD-L1 are immune-checkpoint proteins that primarily function to limit the effector function of T-cells in peripheral tissues during inflammatory responses and limit autoimmunity. These are considered as one of the immune evasion mechanisms for cancer. Recently, strategies to help improve the efficacy of the immune system against cancer represent an important breakthrough in cancer treatment. In humans, clinical trials with anti-programmed death (PD)-1/PD-ligand 1 (L1) monoclonal antibodies have shown objective clinical activity of these agents (e.g., nivolumab, pembrolizumab) in several malignancies, including melanoma, non-small-cell lung cancer, bladder cancer, and squamous cell head and neck cancer [104–106].

Other immune cells that could be of importance in tumor microenvironment (TME) are the tumor-associated macrophages (TAMs). In this context, macrophages contribute to tumor progression through wound-healing and tissue-repair mechanisms that allow cancerous tissues to repair damages caused by low oxygen tension and acidic pH that result from metabolic reprogramming of tumor cells and vascular abnormalities of the tumor. TAMs belong to the monocyte-macrophage lineage and, according to the stimulus, there are two main phenotypes of macrophages: the pro-inflammatory (anti-tumoral) M1 and the immunosuppressive (pro-tumoral) M2 macrophages [107]. Soluble tumor-derived factors initiate the polarization of macrophages into M2 macrophages, leading to the expression of molecules that support angiogenesis, immunosuppression, tumor growth, and metastasis. M2 macrophage phenotypes have been identified in oral cancer and were related with more aggressive tumors [108].

14.3 HPV+ve Pathways for Oral Cancer

A new group of head and neck cancers, referred to as HPV +ve cancers, are now identified and related with the identification of human papillomavirus in tumor cells. They are present in cancers located mainly in the oropharynx, especially in the tonsillar crypt epithelium (47%). By contrast, HPV-related SCC in oral cavity correspond only to 3.9% of all tumors [109]. There are more than 200 genotypes of HPV, and some are related with tumor carcinogenesis – these are

the high-risk HPVs. HPV-16 type is the most common genotype found in these tumors. The virus contains a circular double-stranded DNA with several areas such as E6 and E7 oncogenes. These proteins, E6 and E7 can bind and inhibit two proteins p53 and pRb. In these HPV +ve tumors no mutations are found on these tumor suppressor genes, but their function is inhibited by E6 and E7 viral proteins which represent an early event in oral carcinogenesis [6]. The same is observed in the *CDKN2A*, the gene encoding p16, without mutations or deletions on HPV +ve cancer but overexpressed in these tumors. In the view of this, p16 overexpression is a surrogate marker of HPV-16 infection of tumor cells in oral cancers. Interestingly, genetic studies have shown that PI3K pathway genes are commonly altered in HPV +ve cancers with mutations or amplifications of the gene *PIK3CA* [10, 109]. Knowledge of these molecular characteristics is important in the selection of a treatment plan for patients with HPV +ve tumors as they have a more favorable prognosis than tumors that harbor *TP53* mutations or p16 loss, generally HPV -ve tumors. Recent genomic analysis has identified two subgroups of HPV +ve tumors – one having a mesenchymal and immunological signature (HPV-IMU), and the other having a keratinocyte differentiation and oxidative stress genes signature (HPV-KRT) [110].

! Warning

Some of the biomarkers published in the literature are neither necessary nor sufficient for the evolution of a cancer.

14.4 Prognostic Biomarkers

The genes and proteins involved in the multiple pathways most often disrupted in oral cancer permit the possible use of such alterations as biomarkers of prognosis. We present in Table 14.1 several genes or proteins that have been reported as usefulness prognostic biomarkers in oral cancers [108, 109, 111–115].

14.5 Conclusion

Oral carcinogenesis is a multistep process where many biological pathways could be affected. These pathways are not always similar or common to all patients. Subgroups of head and neck cancers have been identified, including patients with HPV infection (HPV +ve), tumors with aneuploidy status, tumors with few copy number alterations, and some pathways have been linked to some subclasses of tumors. This highlights the importance of the molecular knowledge of the biological tumoral phenotype for each patient and they could function as biomarkers of disease, not only for early diagnosis but also for predicting prognosis as presented in this chapter. The significant markers include p53, EGFR, p16, cyclin A, or Akt/mTOR pathways.

Table 14.1 Most common protein markers reported as prognostic markers in oral cancer

Marker	Gene	Most important function
EGFR	<i>EGFR</i>	Positive regulation of cell proliferation
Ki-67	<i>MKI67</i>	Cell cycle, cell proliferation
Cyclin D1	<i>CCND1</i>	Cell cycle, cell division
Cyclin A	<i>CCNA2</i>	Cell cycle, cell division
Ras	<i>RAS</i>	Signal transduction of cell proliferation
p-mTOR	<i>MTOR</i>	Signal transduction of cell proliferation
Myc	<i>MYC</i>	Positive regulation of cell proliferation
BubR1	<i>BUBR1</i>	Cell cycle, cell proliferation
Cdc 20	<i>CDC20</i>	Cell cycle, positive regulation of cell proliferation
p53	<i>TP53</i>	Cell cycle, cell cycle arrest
p16	<i>CDKN2A</i>	Cell cycle, cell cycle arrest
p21	<i>CDKN1A</i>	Cell cycle, cell cycle arrest
pRB	<i>RB1</i>	Cell cycle, cell cycle arrest
p63	<i>TP63</i>	Stem cell and positive regulation of cell proliferation
CD44	<i>CD44</i>	Cell adhesion and stem cell marker
Cd147	<i>BSG</i>	Metalloproteinase inducer
E-cadherin	<i>CDH1</i>	Cell adhesion
β-catenin	<i>CTNNB1</i>	Cell adhesion
Mucin-4	<i>MUC4</i>	Cell adhesion
Versican	<i>VCAN</i>	Cell adhesion
Cortactin	<i>CTTN</i>	Cell motility and focal adhesion assembly
MMP-11	<i>MMP1</i>	Proteolysis
MMP-2	<i>MMP2</i>	Angiogenesis, response to hypoxia and proteolysis
MMP-9	<i>MMP9</i>	Proteolysis
Podoplanin	<i>PDPN</i>	Lymphangiogenesis
VEGF	<i>VEGF</i>	Angiogenesis
CD34	<i>CD34</i>	Angiogenesis
CD31	<i>CD31</i>	Angiogenesis
HMOX1	<i>HMOX1</i>	Angiogenesis
PTK2	<i>PTK2</i>	Angiogenesis
CXCL8	<i>CXCL8</i>	Angiogenesis, movement of cell or subcellular component
HIF-1 α	<i>HIF1A</i>	Angiogenesis, response to hypoxia
LSD1	<i>LSD1</i>	Cell differentiation and stem cell maintenance
GLUT-1	<i>GLUT-1</i>	Metabolism marker
SLC2A1	<i>SLC2A1</i>	Glucose transport
STAT3	<i>STAT3</i>	Transcription factor of cell proliferation
Snail	<i>SNAI1</i>	Transcription factor of epithelial-mesenchymal transition
Bcl-2	<i>BCL-2</i>	Apoptotic process

■ **Table 14.1** (continued)

Marker	Gene	Most important function
Bax	<i>BAX</i>	Apoptotic process
MAP1LC3A	<i>MAP1LC3A</i>	Microtubule-associated protein; autophagy
FAS	<i>FAS</i>	Apoptotic process
FASLG	<i>FASLG</i>	Apoptotic process
Aldehyde dehydrogenase 1 A1	<i>ALDH1A1</i>	Ethanol oxidation
S100-A2	<i>S100-A2</i>	S100 calcium binding protein A2; endothelial cell migration

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