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REVIEW ARTICLE

# Update of prognostic and predictive biomarkers in oropharyngeal squamous cell carcinoma: a review

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**Abstract** Oropharyngeal squamous cell carcinomas (OSCC) constitute about 5% of all cancers in the western world and the incidence and mortality rates of this tumor have shown little improvement over the last 30 years. Molecular targeted therapy, a promising strategy for the treatment of OSCC and other cancers, requires the understanding of specific molecular events of carcinogenesis and the different pathological, partly interrelated pathways. Extended knowledge of the prognostic or predictive value of molecular biomarkers in oropharyngeal cancer is necessary to allow a better characterization and classification of the tumor, improve the appraisal of clinical outcome and help to specify individual multimodal therapy with increased efficiency. This work affords an updated summary regarding recent data about tissue biomarkers in patients with OSCC, based on the six essential hallmarks of cancer described by Hanahan and Weinberg (Cell 100(1):57–70, 2000) providing the characterization of a malignant cell.

**Keywords** OSCC · Predictive and prognostic biomarker · Hallmarks of cancer · HPV

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## Introduction

Each year in the USA, about 35,700 new cases of oral cavity and oropharyngeal cancer are reported and an estimated 7,600 people die of these diseases [46].Oropharyngeal squamous cell carcinomas (OSCC) constitute about 5% of all cancers in the western world and the incidence and mortality rates of this cancer have shown little improvement over the last 30 years [46, 105]. Histopathologically, squamous cell carcinoma is by far the most common cancer type of the oropharynx and oral cavity, representing more than 90% of all oral cancers. Despite the improved application of multimodal therapy, the survival rate of patients suffering frequently from locoregional and distant recurrences is poor and highlights the need for new approaches concerning early diagnosis and treatment alternatives [15].

### Human papilloma virus in OSCC

Previous molecular biological and epidemiological studies divided OSCC into two biologically different tumor entities, presenting themselves at the same location and with similar histology [113]. Besides a patient's genetic predisposition, it was established that a big part of the etiology of these tumors is related to the consumption of alcohol and nicotine [13]. However, one-third of the patients represented a group that does not feature these risk factors but tested positive for oncogenic human papilloma virus [90]. Human papilloma viruses (HPV) are small DNA viruses causing mainly benign growth, known as papillomas or warts, but the infection with high-risk-types (HR-HPV), basically mucotrop HPV type 16 and 18, were found to be risk factors for carcinogenic degeneration and led to the development of cancer after years of latency. This pathway is found in a range of carcinomas, e.g., cervical carcinoma,

vulva, penis and anal carcinoma, as well as oropharyngeal tumors [22, 36]. These tumors are distinguished from toxin-induced OSCC in multiple biological aspects, including specific molecular and genetic alterations.

The high prevalence of HPV in oropharyngeal carcinomas relates mainly to carcinomas of the tonsils and the tongue base. This might be explainable by their typical cryptic epithelium, which serves as a virus-reservoir. Also, the one- to two-layered cryptic epithelium provides an easier infection of the basal membrane [3]. Further explanations are possible differences in cell differentiation and local interleukin expression [53, 56]. Other tumor locations of the upper aero-digestive mucosa could cause a considerable variation of the prevalence of HPV positivity. Clinically, HPV-positive (HPV+) tumors are characterized by a more favorable prognosis, possibly a result of better response to chemotherapy and radiotherapy due to their distinct biological behavior. It is known that there are considerable genetic aberrations between the two tumor entities [35]: for example, variations of expression of different cell cycle proteins, e.g., p16, and also EGFR or survivin [13]. These different molecular signatures should be unraveled so as to gain more knowledge for future tailored therapies dependent on the HPV status of the tumor.

### Molecular alterations in OSCC

The development and malignant progression of OSCC probably is rarely if ever due to a single dysfunctional gene or pathway. Multiple genetic alterations accumulate during carcinogenesis, involving multiple pathways and downstream genes that have important functions in determining the malignant phenotypes of cancer [37, 55]. In toxin-induced OSCC, carcinogenesis appears to be generated via accumulation of numerous distinct genetic and epigenetic changes. Early common changes are deletions on chromosome 3p and 9p21, which have also been found in premalignant lesions [76]. Loss of heterozygosity (LOH) of chromosome 17p, mutation of the p53 gene, amplification of 11q13 and overexpression of cyclin D1 occur in further stages of carcinogenesis of head and neck squamous cell carcinoma (HNSCC).

## Molecular biomarkers (OSCC)

The effects of multimodal therapy on OSCC tumors vary, but overall more than 50% of multimodally treated patients experience recurrence and the 5-year survival rate of OSCC is not increased by more than 40–50% [40]. It has been noted in the early 1980s that chemosensitive tumors are also likely to be radiosensitive[28] Therefore, induction chemotherapy can help to select patients who are good candidates for radiotherapy and allows early surgery to be performed in cases of insufficient response. Reasons for these varying responses are suspected to be the result of differences in the genetic signature of the otherwise clinically similar tumors [33, 65]. Other selection methods such as molecular biological profiles capable of predicting sensitivity to different therapeutic approaches can not only be time and money saving, but also, more importantly, increase survival. Molecular markers could help to characterize a malignant disease, improve the understanding of variations in the clinical course of individual patients and estimate or even influence the prognosis.

Molecular targeted therapy, a promising strategy for the treatment of OSCC and other cancers, requires understanding of the specific molecular events of carcinogenesis and the different pathological pathways. The amount of literature on molecular markers and potential targets of solid tumors is enormous, but there is still a crucial need for prognostic or predictive markers in OSCC to allow an individual and therefore more effective treatment. The aim of this review is to present an updated summary regarding tissue biomarkers of OSCC, based on the six essential hallmarks of cancer described by Hanahan and Weinberg [42] that provides the characterization of a malignant cell (Fig. 1).

### Self-sufficient growth-stimulatory signalling

In a normal cell, proliferation needs to be regulated by growth signals mediated by soluble and membrane-bound interactions of different signalling proteins. The process of carcinogenesis results from a deregulated, autonomous growth signalling due to gene amplifications, mutations, and imbalances of the level of growth factor receptors and their ligands or autocrine stimulation.

### EGFR/HER-2/neu

Epidermal growth factor receptor (EGFR) is a membrane-bound receptor tyrosine kinase that regulates many cellular functions, including cellular proliferation, differentiation and survival. Normally, EGFR is expressed at low levels on the surface of most cells. Deviant EGFR signalling is a hallmark of carcinogenesis.

There are four receptor types distinguishable: HER1 (human epidermal growth factor receptor 1) or ErbB1, HER-2 (ErbB-2 or the HER-2/neu protooncogene), HER-3 (ErbB3) and HER-4 (ErbB4) [45]. Increase or alteration in ErbB1 and ErbB2 are found in many different cancers, e.g., breast, gastrointestinal, kidney, prostate and lung tumors [97]. After binding their natural ligands, such as EGF and TGF-a, these receptors autophosphorylate, resulting in multiple intracellular signalling cascades, including RAS-RAF-MAPK activity, PI3K/AKT, mTOR, Jak and STAT-pathways,

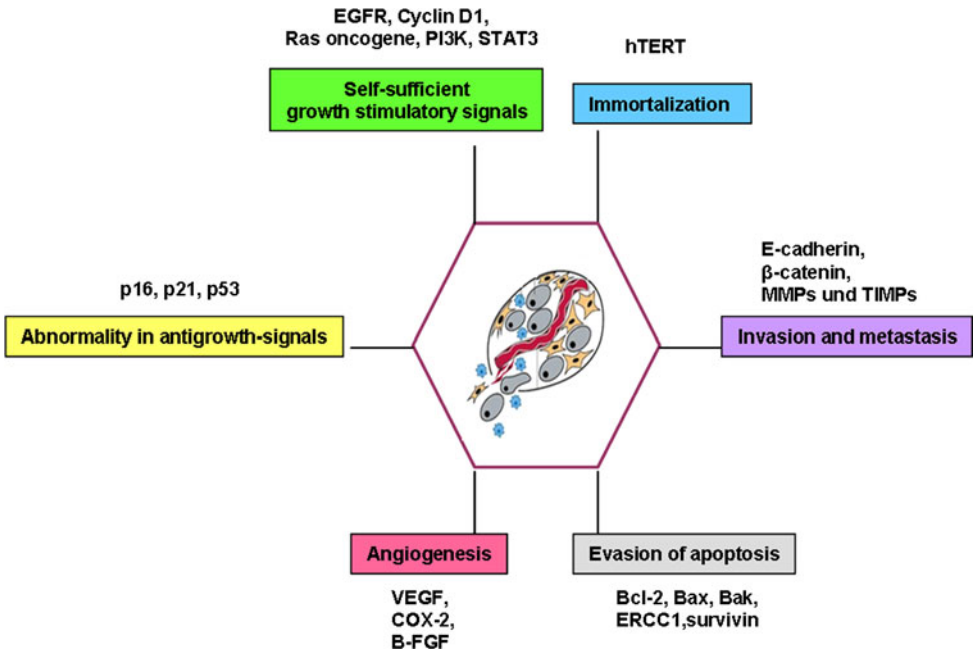


**Fig. 1** Samples of predictive and prognostic biomarkers in oropharyngeal carcinoma according to the six hallmarks of cancer (modified from Hanahan and Weinberg (2000) The hallmarks of cancer. Cell 100(1):57–70)

thus highlighting the central role of EGFR in signal networking with different other oncogenes in cancers [96]. The overwhelming majority (90–100%) of all HNSCC present with EGFR overexpression, which is an independent prognostic marker that correlates with worse prognosis, advanced tumor stage and poor treatment outcome [88, 94].

Concerning the HPV status of a tumor, it has been shown that HPV+/p16+ tumors tend to have decreased EGFR expression and a significantly better prognosis regarding disease-free survival (DFS) as well as overall survival (OS) [93]. Overexpression of EGFR ligands is occasionally found in OSCC and is associated with poor prognosis [123].

Accordingly, various approaches to inhibit EGFR pathway in squamous cell carcinoma (SCC) have been explored, including small molecule tyrosine kinase inhibitors (TKIs) of the cytosolic kinase domain and monoclonal antibodies (mAbs) for the extracellular portions of EGFR [5, 94, 107].

Her2/neu is a well-known epithelial proto-oncogene with sequence homology to EGFR. In gastrointestinal cancers, HER2/neu expression showed convergence with the stage of disease and was inversely correlated with the prognosis of the patients [87]. Cavalot et al. [17] reported that HER-2/neu overexpression was significantly more frequent in lymph node-positive HNSCC patients and in those with capsule rupture and vascular embolization. This finding underlines the important role of HER-2/neu in supporting neoplastic growth and tumor recurrence in HNSCC. In addition, Cavalot et al. showed that HER-2/neu expression and lymph node positivity were the only independent indicators for predicting risk of recurrence (DFS). Their report differs with other studies that showed no correlation between HER-2/neu and the clinical outcome [34, 106]. Discrepancies among studies that use immunohistochemistry (IHC) might be explained by the use of different antibodies, different interpretation of the significance of cytoplasmic staining and the lack of standardized assays.

The role of HER-4 in cancer development is still not clarified, although there have been recent investigations showing that overexpression of HER-4 in breast cancer might be associated with more differentiated histology and a favorable clinical outcome [48, 127]. Studies by Sheu et al. [104] revealed a decreased gene copy number and expression level of HER-4 in OSCC. Accordingly, further investigations are necessary to declare the possible tumor-suppressing function of HER-4.

## Cyclin D1

Cyclin D1 (CCND1) is a proto-oncogene and key regulator protein in the transition from G1 to S phase of the cell cycle [103]. Positively regulated by PIN 1, it increases phosphorylation of the retinoblastoma gene (Rb) and thereby induces the S phase in cells. CCND1 gene amplification is known to be an early event in oral carcinogenesis. Overexpression of CCND1 occurs in 25–75% of oral cancer and is a poor prognostic factor [78, 79]. Interestingly, in HPV-/p16-positive OSCC, CCND1 is found to be downregulated after the inactivation of p53 and pRB by HPV-derived oncoproteins E6 and E7. Conversely, in HPV-negative (HPV−) tumors, increased expression levels of CCND1 were found, possibly due to CCND1 gene amplification, which is associated with worse prognosis [41, 68].

### Ras gene

The *ras* gene family includes proto-oncogenes that are known to participate in cell growth regulation, signal transduction and migration. The members of the human *ras* oncogene family are *H-ras*, *K-ras* and *N-ras* [7]. The *ras* and PI3K-AKT signalling pathways play an important role in carcinogenesis and maintenance of tumor growth, including decreasing the expression of tumor suppressor genes such as p16. RAS–RAF–MAPK cascades are especially active when cancer cells overexpress EGFR, highlighting the critical roles of signal networking among different oncogenes in cancers [95]. Hence, targeting *ras* gene products for cancer treatment is of great interest. Approximately, 30% of all human cancers show mutated *ras* alleles, mostly associated with poor clinical outcome and limited therapeutic options, presenting therefore the most frequently mutated oncogene known [7, 19].

In OSCC, *H-ras* mutations are mainly detected in Asian populations, often associated with advanced stages of the tumor [23, 81]. In Western populations, ras mutations in HNSCC are relatively rare events (<6%) compared to 99% in pancreatic cancer and approximately 40% in colorectal cancer [98, 118, 122]. This difference might be due to their different histological classification, but has to be further investigated.

### PI3K/NF-kB

Phosphoinositol 3-kinase (PI3K) is a heterodimeric lipid kinase, consisting of regulatory subunit p85 and catalytic subunit p110α (PIK3CA), which regulate cellular signalling pathways including cell proliferation, differentiation, adhesion and apoptosis [21]. PI3K can be activated by receptor tyrosine kinases (RTKs), such as EGFR or insulin receptor. Direct positive control is also possible via active *ras*. PTEN, a tumor suppressor, antagonizes PI3K [119]. It has been shown, that PI3KCA is mutated in over 25% of different human cancers, including gastrointestinal, breast, different brain and head and neck cancer [100]. Studies regarding the correlation of PI3K mutations and clinicopathological data in OSCC are still required. Via phosphorylation, PI3K induces plasmaproteins in the cell membrane, for example AKT. Activated AKT supports cell survival by inhibiting apoptosis and by activating nuclear factor-kappa B (NF-*k*B). NF-*k*B is a ubiquitous nuclear transcription factor known to be involved in inflammation and immunoregulation. It is capable of inhibiting apoptosis through the induction of anti-apoptotic proteins, but can also lead to chemoresistance by suppressing the apoptotic potential of some anticancer agents [83].

### STAT3

Signal transducers and activators of transcription (STATs) are cytoplasmic transcription factors relevant in solid tumors. After being activated via extracellular signals, STATs translocate to the nucleus, thereupon regulating the transcription of different target genes involved in cell proliferation, differentiation and apoptosis: all potential activators of oncogenesis [11]. Mutation of STAT3 can lead to malignant transformation by amplified po(sitive activation of downstream pathways, such as the antiapoptotic proteins Bcl-2 and Bcl-xL [64].

Previous studies revealed increased levels of activated STAT3 in different human cancers, including HNSCC [16]. Masuda et al. [77] examined 51 samples of patients with OSCC and revealed a correlation of high STAT3 expression with poor differentiation of the tumor, lymph node metastasis and low survival. At the moment, investigations concerning the association between STAT3 expression, HPV subdivision of OSCC specimen and clinicopathological data are still pending.

## Abnormality in anti-growth signals

Insensitivity of tumor cells to anti-growth signals is mainly achieved through inactivation of tumor-suppressor genes via different mechanisms, including mutation, deletion or promoter hypermethylation. An important part of the regulation of growth-inhibitory signals is taken by cyclin-dependent kinases (CDK), cyclin, the RB gene and the tumor suppressor genes p16, p21, p53 and p15.

### P16 INK4A

P16 INK4A is a tumor suppressor gene located on chromosome 9p21-22 and inhibits phosphorylation of E2F–retinoblastoma complex via binding regulatory cyclin-dependent kinases CDK4 and CDK6 [2]. Phosphorylated Rb (pRb) releases transcription factor E2F, which activates transition of G1 to the S-phase of the cell cycle. P16 INK4A thereby regulates the cell cycle negatively, but can be suppressed by pRb itself [2, 102]. Since E7, a viral regulatory gene of high-risk human papilloma virus (HR-HPV) is capable of inactivating pRb, the repression by pRb of p16 INK4A is therefore missing, which in turn leads to an increased detection of p16 protein in HPV affected cells [126]. Multiple studies significantly demonstrated p16 INK4A as a surrogate marker for HPV-induced carcinoma [54, 93]. In contrast, tumor suppressor gene p16 is found to be inactivated in many other malignant tumors. This includes toxic-induced head and neck carcinomas, in which p16 INK4A inactivation via promoter hypermethylation is apparently

an early event in carcinogenesis [76]. P16 INK4A was revealed as a reliable prognostic marker, even better than direct HPV DNA analyses via PCR. A study of patients with p16 INK4A-positive tumors showed a significant better DFS, compared with p16-negative tumors [54]. Kreimer et al. [58] demonstrated that in rare cases of HPV positivity and p16 INK4A negativity, the affected patients were heavy smokers, which could explain the inactivation of p16 INK4A in these cases. Therefore, a combination of p16 immunohistochemistry and HPV DNA verification via PCR is currently recommended for the identification of HPV-induced oropharyngeal carcinomas [108].

### P53

P53 normally regulates cell cycle progression, cellular differentiation, DNA repair and apoptosis, and is mutated in 50% of all cancers. Following DNA damage, the p53 level increases and causes cell cycle arrest or apoptosis, depending on the cell's ability to self-repair. These reactions can be achieved via p53 induction of kinase inhibitors, which decreases the phosphorylation of Rb. This leads to a larger amount of bound and, therefore, inactive E2F, thereby decreasing initiation of S-phase in cells [66]. Loss of p53 function during carcinogenesis can lead to inappropriate cell growth, increased cell survival and genetic instability [12]. P53 is known to block survivin expression by arresting its promoter. Hence, blockage of p53 in oropharyngeal tumors causes overexpression of survivin due to absence of inhibition [52]. Khan et al. [52] showed a frequent overexpression of survivin and mutated p53 in OSCC as well as in oral premalignant lesions. Alteration and overexpression of these markers in premalignant lesions suggest a role in early stages of oral carcinogenesis [52].

It was shown that HPV+ and HPV− tumors are separable into two different biological tumor entities. Reflecting the different steps of carcinogenesis, tobacco and alcohol-induced tumors commonly show p53 mutations as well as p16 alterations, whereas HPV+ tumors present frequently with a p53 wild type. In the latter case, p53 interacts with the viral E6 protein, which leads to increased ubiquitin-dependent proteolysis of p53. Nevertheless, HPV+ tumors are known to have a favorable prognosis, possibly as a result of less frequent p53 mutations. P53 mutations are associated with field cancerization and therefore correlate with early recurrence and second primary tumors, as well as worse responses to chemotherapy or radiation therapy [82, 93, 116].

### P21

P21 belongs to the group of cyclin-dependent kinase inhibitors that play an essential role in cell cycle control, cellular growth, differentiation and apoptosis. Cell damage stimulates p53 activation, which can cause increased p21 expression and interaction with cyclin/CDK, leading to either cell cycle arrest or apoptosis [131]. In cases of high p21 expression, an improved clinical response to chemotherapy and irradiation was reported by various authors in different types of solid tumors [49, 121, 129]. Several studies revealed that a positive expression of p21 was associated with increased survival dates and, therefore, p21 might represent a predictive marker for response of radiochemotherapy and prognosis in OSCC and other solid tumors [32, 130]. Hafkamp et al. examined 77 oropharyngeal tumors specimens and found that in the 35% of these tumors that were tested HPV positive, there was a strong association with p21 (Cip1/WAF1) expression. P21 overexpression seems to be a significant prognosticator for a favorable prognosis [41].

## Evasion of apoptosis

Malignancies are characterized by increased survival and resistance to apoptosis. Apoptosis belongs to the essential cellular regulation processes and contains a number of important components, including the family of caspases. These cysteine proteases regulate the initiation and accomplishment of programmed cell death in altered and consumed cells that constitute danger to the multicellular organism. Caspases induce proteolysis of important proteins, finally causing dissolution of the nucleus with DNA degradation and alterations of the cell membrane [75]. P53 is activated by extrinsic or intrinsic damage of the cell and is involved in regulations of the apoptotic cascade. Since p53 is often found mutated in tumor cells, it partly explains decreased apoptosis in malignancies [91]. The Bcl-2 family is also a group of apoptosis-regulatory factors. They can be classified into pro-apoptotic proteins, (Bax and Bak) and anti-apoptotic proteins (Bcl-2 and Bcl-xL). Alterations or mutations of their expression as well as disequilibrium of their ratio can lead to deterioration of the cell [124].

### Bcl-2, Bax

Bcl-2, which stands for B-cell-lymphoma-2, plays an important role in the regulation of apoptosis and prolonged cell survival [59] and has been linked to prognosis in several solid tumors [39]. In a study by Zhang et al. [134] analyzing the expression of Bcl-2 and Bax proteins via IHC in specimens from 110 patients with OSCC, the ratio of Bcl-2/Bax was found to be an independent marker of prognosis. Camisasca et al. [14] demonstrated in 53 OSCC patients treated with curative surgery at a single institution that the expression of Bcl-2 family proteins was strongly associated

with the clinical outcome of those patients. Similar results could be revealed by Lo Muzio et al. [70] suggesting that decreased Bcl-2 expression represents a more aggressive biological behavior in OSCC. Wilson et al. examined the histological material from 400 patients via IHC staining. Their results showed an association between Bcl-2, histological dedifferentiation and a more advanced disease, but yet a lower locoregional recurrence rate and an improved survival [125].

### Survivin

Survivin is a member of the inhibitor of apoptosis family (IAP) and a regulator of cell division. Five different splicing variants are known so far. Survivin is rarely detectable in normal differentiated tissue, but highly expressed in most of human malignant lesions [132].

It binds and disables caspase-3 and caspase-7, both important enzymes of the apoptosis process, thereby enabling inhibition of apoptosis in the cell. Survivin is frequently expressed in OSCC and its expression has been reported to correlate with worse prognosis, disseminated disease and resistance to therapy [1]. Interestingly, there seems to be a connection between survivin expression and the detection of oncogenic HPV in OSCC [69]. It is already known that there is convergence between survivin expression and increase of dysplasia at the cervix, a disease that is also linked to HR-HPV infection [6, 9]. Preuss et al. [89] demonstrated a significantly inverse association between cytoplasmic survivin expression and HPV-associated OSCC. The localization of survivin was found to be dynamic, since IHC showed cytoplasmic as well as nuclear positivity, the latter possibly presenting the participation of survivin in controlling cell proliferation [67]. HR-HPV-correlated oncogenesis seems to influence active nuclear import of survivin, since a nuclear expression of survivin, which is associated with poor OS, was significantly found in HPV tumors [89]. Due to its crucial role in carcinogenesis, its absence in normal tissue and potential possibilities as a molecular target, survivin has notable therapeutic and prognostic interest.

### ERCC1

In a broad sense, avoidance of apoptosis can be achieved by increased DNA repair processes. Platinum chemotherapeutic agents are used against many different cancers, especially germ cell tumors, ovarian, cervical and bladder cancer, small cell and non-small cell lung cancer, colorectal cancer and head and neck cancer [63]. Platinum based chemotherapy induces apoptosis by forming DNA adducts, which leads to inter- and intrastrand cross-linking. Limitations of the efficiency of platinum chemotherapeutics are based on the development of resistance. The nucleotide excision repair pathway (NER) is an important feature to recognize and remove DNA cross-links [92]. After excision of the modified DNA nucleotides, DNA polymerase restores the missing parts [44]. The excision repair cross-complementation group 1 (ERCC1) is a crucial enzyme involved in the NER pathway. Interestingly, alterations in DNA damage repair proteins can cause chemotherapeutic treatment failure and continued viability of tumor cells in many human cancers [80]. The determination of parameters to identify patients who would benefit from a platinum based chemotherapy regimen is an important goal. Analysis of ERCC1 expression levels in different tumors, including gastrointestinal [8], ovarian and lung cancer [71], exposed an inverse correlation to treatment response and survival outcome.

Olaussen et al. examined 761 metastatic lung cancer specimen immunohistochemically. They reported a significant survival benefit in patients with low ERCC1 levels who were treated with platinum chemotherapeutic agents, compared to their counterparts with increased ERCC1 expression [84]. Recently, a prospective randomized trial of 444 patients with progressed non-small cell lung cancer, using ERCC1 expression to assign platinum-based chemotherapy, was performed. It was shown that ERCC1 mRNA expression levels could significantly help to predict the response to docetaxel and cisplatin chemotherapy, although the survival rates were not significantly different [20].

In HNSCC, relatively few studies have been carried out to evaluate the role of ERCC1 expression as a predictive marker. Jun et al. examined 45 patients with locally advanced HNSCC who received concurrent cisplatin-based chemotherapy. Their results supported an inverse association of ERCC1 expression and prolonged survival [47]. Another study showed concordance with Jun's results by analyzing 96 patients who underwent an induction chemotherapy regimen for progressed HNSCC via IHC [43]. A recent study by Koh et al. reports contrary results. They examined 85 specimens of patients with HNSCC receiving platinum-containing induction chemotherapy via IHC. ERCC1 expression did not correlate with treatment response or prognosis in their retrospective setting [57]. Further investigations to approve the clinical relevance of ERCC1 expression as a predictor of treatment response in oropharyngeal or other head and neck carcinomas are therefore still necessary.

## Immortalization

A normal cell can replicate itself limited by the length of its telomeres, which affect the life span of a cell. Telomerase is an enzyme that can elongate telomeric DNA by reverse transcription. hTERT, which stands for human telomerase reverse transcriptase, is a subunit of the telomerase, which

is found to be increased in over 90% of all cancers. Expression of hTERT enables a malignant cell to escape limited replication and become immortal [111, 120]. Recent studies revealed that hTERT expression in OSCC correlates with prognostic values. Chen et al. for example, examined 82 specimens of OSCC, 116 specimens of oral epithelial dysplasia and 21 specimens of normal oral mucosa via immunohistochemistry. They demonstrated that hTERT expression was an early event in oral carcinogenesis and significantly associated with the progression, recurrence and prognosis of OSCC in Taiwan [18].

## Angiogenesis

Sufficient blood supply is an important factor enabling a malignant tumor to self-maintain and grow. Therefore, pro-angiogenetic factors play an important role in the regulation of pathological angiogenesis and metastasis [42].

### VEGF

VEGF is not only responsible for increased angiogenesis, but stimulates also vessel permeability, endothelial cell growth and proliferation. There are six different VEGF family members differentiable (placental growth factor, VEGF A–E) and three VEGF receptors (VEGFR 1–3). VEGF and other angiogenic mediators, such as basic fibroblast growth factor (b-FGF), transmit their signal by binding tyrosine kinase receptors on the cell surface [31].

At the time of diagnosis, oropharyngeal cancers mainly present in advanced tumor stages, already exhibiting positive regional lymph nodes. VEGF-C and -D and VEGFR-3 are known to be involved in lymphangiongenesis signalling pathway, therefore presenting an interesting target for cancer therapy [74, 112]. There are contradictory findings concerning VEGF expression in OSCC and its prognostic value. Sappayatosok et al. examined 66 OSCC paraffin-embedded specimens with IHC regarding their expression of pro-inflammatory and angiogenetic proteins, including VEGF and COX-2. They demonstrated a correlation of VEGF with tumor grading, tumor staging and angiogenesis. [101]. A study by Smith et al. of 77 patients with OSCC treated with surgery and postoperative radiotherapy revealed increased VEGF expression as the most significant predictor of poor disease-free and overall survival with important prognostic value [109]. Tanigaki et al. studied the expression of VEGF-A and -C, and VEGRF- 1 and - 3 in 73 patients with OSCC by IHC. Multivariate analysis demonstrated that lymph node metastasis and VEGF-C expression were exclusive, independent factors influencing the overall survival rate and correlated with locoregional recurrence and distant failure [115]. However, Salven et al. [99] examined 156 specimens of OSCC patients treated with multimodal therapy and reported no correlation between VEGF expression and QS. The latter results have been confirmed by Brocic et al., who also rejected VEGF as a clinical parameter for prognosis and outcome [10]. Fei et al. [30] analyzed 85 SCC of the tonsil and could not reveal any associations between VEGF and HPV status, gender, patient age, TNM stage or EGFR expression either.

### COX-2

Cyclooxygenase-2 (COX-2) is a proinflammatory enzyme that takes part in the conversion of arachidonic acid to prostaglandins and thromboxanes, and participates in cell proliferation, tumor angiogenesis and apoptosis. Unlike the other isoform COX-1, COX-2 is commonly not present in normal tissue, but has an increased expression in several human cancers, including OSCC [38, 118]. COX-2 synthesis is stimulated during inflammatory events by cytokines and growth factors, but can be also regulated by tumor promoters and transcription factors.

In esophageal carcinoma, Takatori et al. [114] demonstrated in 228 patients a positive correlation between an increased COX-2 expression and the depth of tumor invasion, tumor stage and prognosis. In OSCC, Sappayatosok et al. [101] examined 66 OSCC samples and found an association of COX-2 expression with the degree of dysplasia as well as the lymph node status, but no significant correlation with tumor grading, staging or survival. Pannone et al. demonstrated in 22 patients with OSCC via real-time RT-PCR that a high COX-2 expression was associated with a worse DFS. A correlation with QS, tumor stage and grade could not be shown [85]. At this time, studies analyzing the relation of HPV status, COX-2 expression and clinicopathological parameters in OSCC still have not been done. In colorectal malignancies, around 80% show increased COX-2 expression, which presumably enhances tumor proliferation, angiogenesis and metastasis. The chemopreventive effect of NSAID in colorectal cancer, including the treatment of familial adenomatous polyposis (FAP), has been already established [110].

Nonsteroidal anti-inflammatory drugs, especially selective COX-2-inhibitors present an important possibility of preventing and targeting several cancers in early and progressed stages, despite the cardiovascular risk that has been reported.

## Invasion and metastasis

### Cadherins

The capability of malignant cells to infiltrate surrounding tissue differentiates them from normal cells. Cell–cell and

cell–matrix interactions are important regulators for normal cellular functioning. There are different specialized molecules and their corresponding receptors, including cadherins and the associated catenins. Cadherins are cell adhesion molecules that play a significant role in maintaining epithelial integrity, as wll as epithelial–mesenchymal transition [117]. Alterations in E-cadherin expression or their subcellular location are a well-known step of carcinogenesis. Inactivation of E-cadherin can be accomplished by promoter hypermethylation and inhibition of transcription via repressors such as Snail, Slug or SIP1 [24, 86]. A low expression of E-cadherin is associated with increased motility, invasion and metastasis of tumor cells [4]. Diniz-Freitas et al. assessed 47 cases of OSCC to judge the relation of E-cadherin expression and clinicopathological parameters by using IHC. Their results showed that less or absent E-cadherin expression correlated with more invasive tumor characteristics, positive cervical lymph nodes and worse prognosis concerning disease-free and overall-survival time [27]. Kaur et al. [50] confirmed these results in a study of 37 OSCC samples and 10 metastasized cervical lymph nodes. On the contrary, Mahomed et al. [73] who examined 30 cases of OSCC, partly with nodal metastasis, reported that a weak expression of E-cadherin and its associated protein, beta-catenin, correlated with a lower degree of differentiation but did not show significant association with nodal metastasis.

### Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are extracellular proteases, capable of destroying the surroundings of a tumor cell and allowing tumor invasion and metastasis. The family of MMPs contains different members, distinguishable into collagenases, gelatinases, stromelysins, membrane-type MMPs and new MMPs [61].

Oropharyngeal cancer is known to spread quickly in its locoregional surrounding and 50% of the patients present with regional metastasis at the time of diagnosis. Several different MMPs, such as MMP-2, -3,-10 and -11, have been reported to take part in the progression of oral cancer [60]. MMP-2 expression was found to have predictive value concerning tumor metastasis in oral carcinomas [51]. Kusukawa et al. [62] revealed in 65 patients with stage I and II of oral carcinoma that an increased expression of MMP-3 is associated with tumor size, thickness, mode of invasion and lymph node metastasis. Lü et al. examined MMP-1 mRNA expression of 30 OSCC patients in paired tumor and non-tumor specimens via RT-PCR. They found high MMP-1 mRNA expression to be associated with advanced tumor stages and positive cervical lymph node invasion and, therefore, reasoned MMP-1 as a potential biomarker for diagnostic and prognostic evaluation of OSCC [72]. Yorioka et al. examined 44 samples of primary OSCC. Their techniques included microdissection and measurement of the activity of secreted MMPs via gelatin zymography and densitometry. Yorioka et al. [133] revealed an increased activity of MMP-2 and -9, which correlated with a shorter DFS.

Tissue inhibitors of metalloproteinase (TIMP) are antagonists of MMPs, but unexpectedly show a poor prognostic effect. They can suppress angiogenesis, tumor invasion and metastasis in vivo, but especially TIMP-2 seems to have a dual function and is capable of activating MMP-2 and independently regulating cell growth and survival [26]. A study of TIMP expression in OSCC by De Vicente et al. [25] revealed that TIMP-2 expression correlated significantly with tumor stage, local recurrence and survival outcome, while TIMP-1 showed no relation to clinicopathological parameters. A complete understanding of the pathophysiological features of MMPs and TIMPs is still lacking. Our knowledge on the influence of HPV infection on MMP expression, and therefore progression of carcinogenesis in OSCC, is still incomplete and needs further investigations.

## Conclusion

In recent years, important progress in the understanding of oropharyngeal carcinogenesis has been achieved. Extended knowledge of the prognostic or predictive value of molecular biomarkers in oropharyngeal cancer provides more insight into carcinogenesis and can, therefore, improve and specify diagnosis, tumor classifications, therapy and appraisal of clinical outcome in OSCC.

Declaration of HPV status in OSCC, using immunohistochemical staining of p16 and PCR of HPV-DNA, for example, presents a reliable prognostic and predictive marker. Recently published studies indicate that HPV/p16 associated carcinomas present a better response to induction chemotherapy and a better prognosis after definite radiochemotherapy [29, 128]. These results indicated that the possibility of a reliable selection of patients for individualized therapy depended on the HPV status of their tumors. However, more prospective, possibly collaborative studies incorporating a reliable quality of samples from a uniform anatomic site, standardization of the analysis methods and synchronic curative protocols are warranted to further improve the clinical validation of biomarkers and their use for tailored treatment strategies in patients with OSCC.

## References

1. Altieri DC (2003) Validating survivin as a cancer therapeutic target. Nat Rev Cancer 3:46–54

2. Andl T, Kahn T, Pfuhl A, Nicola T, Erber R, Conradt C, Klein W, Helbig M, Dietz A, Weidauer H, Bosch FX (1998) Etiological involvement of oncogenic human papillomavirus in tonsillar squamous cell carcinomas lacking retinoblastoma cell cycle control. Cancer Res 58:5–13
3. Begum S, Cao D, Gillison M, Zahurak M, Westra WH (2005) Tissue distribution of human papillomavirus 16 DNA integration in patients with tonsillar carcinoma. Clin Cancer Res 11:5694–5699
4. Birchmeier W, Behrens J (1994) Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim Biophys Acta 1198:11–26
5. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, Jones CU, Sur R, Raben D, Jassem J, Ove R, Kies MS, Baselga J, Youssoufian H, Amellal N, Rowinsky EK, Ang KK (2006) Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 354:567–578
6. Borbely AA, Murvai M, Konya J, Beck Z, Gergely L, Li F, Veress G (2006) Effects of human papillomavirus type 16 oncoproteins on survivin gene expression. J Gen Virol 87:287–294
7. Bos JL (1989) ras oncogenes in human cancer: a review. Cancer Res 49:4682–4689
8. Brabender J, Vallbohmer D, Grimminger P, Hoffmann AC, Ling F, Lurje G, Bollschweiler E, Schneider PM, Holscher AH, Metzger R (2008) ERCC1 RNA expression in peripheral blood predicts minor histopathological response to neoadjuvant radiochemotherapy in patients with locally advanced cancer of the esophagus. J Gastrointest Surg 12:1815–1821
9. Branca M, Ciotti M, Giorgi C, Santini D, Di Bonito L, Costa S, Benedetto A, Bonifacio D, Di Bonito P, Paba P, Accardi L, Syrjanen S, Favalli C, Syrjanen K (2008) Predicting high-risk human papillomavirus infection, progression of cervical intraepithelial neoplasia, and prognosis of cervical cancer with a panel of 13 biomarkers tested in multivariate modeling. Int J Gynecol Pathol 27:265–273
10. Brocic M, Kozomara R, Cerovic S, Jovic N, Vukelic-Markovic S, Stosic S (2009) Clinical significance of vascular endothelial growth factor expression in patients with carcinoma of the mouth floor and tongue. Vojnosanit Pregl 66:440–448
11. Bromberg J, Darnell JE Jr (2000) The role of STATs in transcriptional control and their impact on cellular function. Oncogene 19:2468–2473
12. Caamano J, Zhang SY, Rosvold EA, Bauer B, Klein-Szanto AJ (1993) p53 alterations in human squamous cell carcinomas and carcinoma cell lines. Am J Pathol 142:1131–1139
13. Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, Corio R, Lee D, Greenberg B, Koch W, Sidransky D (1996) Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res 56:2488–2492
14. Camisasca DR, Honorato J, Bernardo V, da Silva LE, da Fonseca EC, de Faria PA, Dias FL, Lourenco Sde Q (2009) Expression of Bcl-2 family proteins and associated clinicopathologic factors predict survival outcome in patients with oral squamous cell carcinoma. Oral Oncol 45:225–233
15. Carvalho AL, Nishimoto IN, Califano JA, Kowalski LP (2005) Trends in incidence and prognosis for head and neck cancer in the United States: a site-specific analysis of the SEER database. Int J Cancer 114:806–816
16. Catlett-Falcone R, Dalton WS, Jove R (1999) STAT proteins as novel targets for cancer therapy. Signal transducer an activator of transcription. Curr Opin Oncol 11:490–496
17. Cavalot A, Martone T, Roggero N, Brondino G, Pagano M, Cortesina G (2007) Prognostic impact of HER-2/neu expression on squamous head and neck carcinomas. Head Neck 29:655–664
18. Chen HH, Yu CH, Wang JT, Liu BY, Wang YP, Sun A, Tsai TC, Chiang CP (2007) Expression of human telomerase reverse transcriptase (hTERT) protein is significantly associated with the progression, recurrence and prognosis of oral squamous cell carcinoma in Taiwan. Oral Oncol 43:122–129
19. Chin L, Tam A, Pomerantz J, Wong M, Holash J, Bardeesy N, Shen Q, O'Hagan R, Pantginis J, Zhou H, Horner JW 2nd, Cordon-Cardo C, Yancopoulos GD, DePinho RA (1999) Essential role for oncogenic Ras in tumour maintenance. Nature 400:468–472
20. Cobo M, Isla D, Massuti B, Montes A, Sanchez JM, Provencio M, Vinolas N, Paz-Ares L, Lopez-Vivanco G, Munoz MA, Felip E, Alberola V, Camps C, Domine M, Sanchez JJ, Sanchez-Ronco M, Danenberg K, Taron M, Gandara D, Rosell R (2007) Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small-cell lung cancer. J Clin Oncol 25:2747–2754
21. Cully M, You H, Levine AJ, Mak TW (2006) Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat Rev Cancer 6:184–192
22. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML (2007) Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 356:1944–1956
23. Das N, Majumder J, DasGupta UB (2000) ras gene mutations in oral cancer in eastern India. Oral Oncol 36:76–80
24. De Craene B, Gilbert B, Stove C, Bruyneel E, van Roy F, Berx G (2005) The transcription factor snail induces tumor cell invasion through modulation of the epithelial cell differentiation program. Cancer Res 65:6237–6244
25. de Vicente JC, Fresno MF, Villalain L, Vega JA, Lopez Arranz JS (2005) Immunoexpression and prognostic significance of TIMP-1 and -2 in oral squamous cell carcinoma. Oral Oncol 41:568–579
26. DeClerck YA, Perez N, Shimada H, Boone TC, Langley KE, Taylor SM (1992) Inhibition of invasion and metastasis in cells transfected with an inhibitor of metalloproteinases. Cancer Res 52:701–708
27. Diniz-Freitas M, Garcia-Caballero T, Antunez-Lopez J, Gandara-Rey JM, Garcia-Garcia A (2006) Reduced E-cadherin expression is an indicator of unfavourable prognosis in oral squamous cell carcinoma. Oral Oncol 42:190–200
28. Ensley JF, Jacobs JR, Weaver A, Kinzie J, Crissman J, Kish JA, Cummings G, Al-Sarraf M (1984) Correlation between response to cisplatinum combination chemotherapy and subsequent radiotherapy in previously untreated patients with advanced squamous cell cancers of the head and neck. Cancer 54:811–814
29. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML (2008) Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 100:261–269
30. Fei J, Hong A, Dobbins TA, Jones D, Lee CS, Loo C, Al-Ghamdi M, Harnett GB, Clark J, O'Brien CJ, Rose B (2009) Prognostic significance of vascular endothelial growth factor in squamous cell carcinomas of the tonsil in relation to human papillomavirus status and epidermal growth factor receptor. Ann Surg Oncol 16:2908–2917
31. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. Nat Med 9:669–676
32. Fillies T, Woltering M, Brandt B, Van Diest JP, Werkmeister R, Joos U, Buerger H (2007) Cell cycle regulating proteins p21 and p27 in prognosis of oral squamous cell carcinomas. Oncol Rep 17:355–359
33. Forastiere AA (1996) Another look at induction chemotherapy for organ preservation in patients with head and neck cancer. J Natl Cancer Inst 88:855–856
34. Giatromanolaki A, Koukourakis MI, Sivridis E, Fountzilas G (2000) c-erbB-2 oncoprotein is overexpressed in poorly vascularised

squamous cell carcinomas of the head and neck, but is not associated with response to cytotoxic therapy or survival. Anticancer Res 20:997–1004
35. Gillison ML (2004) Human papillomavirus-associated head and neck cancer is a distinct epidemiologic, clinical, and molecular entity. Semin Oncol 31:744–754
36. Gillison ML, Lowy DR (2004) A causal role for human papillomavirus in head and neck cancer. Lancet 363:1488–1489
37. Gollin SM (2001) Chromosomal alterations in squamous cell carcinomas of the head and neck: window to the biology of disease. Head Neck 23:238–253
38. Goulart Filho JA, Nonaka CF, da Costa Miguel MC, de Almeida Freitas R, Galvao HC (2009) Immunoexpression of cyclooxygenase-2 and p53 in oral squamous cell carcinoma. Am J Otolaryngol 30:89–94
39. Grimminger PP, Schneider PM, Metzger R, Vallbohmer D, Danenberg KD, Danenberg PV, Holscher AH, Brabender J (2010) The prognostic role of Bcl-2 mRNA expression in curatively resected non-small cell lung cancer (NSCLC). Lung Cancer [Epub ahaed of print]
40. Gupta S, Kong W, Peng Y, Miao Q, Mackillop WJ (2009) Temporal trends in the incidence and survival of cancers of the upper aerodigestive tract in Ontario and the United States. Int J Cancer 125:2159–2165
41. Hafkamp HC, Mooren JJ, Claessen SM, Klingenberg B, Voogd AC, Bot FJ, Klussmann JP, Hopman AH, Manni JJ, Kremer B, Ramaekers FC, Speel EJ (2009) P21 Cip1/WAF1 expression is strongly associated with HPV-positive tonsillar carcinoma and a favorable prognosis. Mod Pathol 22:686–698
42. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100(1):57–70
43. Handra-Luca A, Hernandez J, Mountzios G, Taranchon E, Lacau-St-Guily J, Soria JC, Fouret P (2007) Excision repair cross complementation group 1 immunohistochemical expression predicts objective response and cancer-specific survival in patients treated by Cisplatin-based induction chemotherapy for locally advanced head and neck squamous cell carcinoma. Clin Cancer Res 13:3855–3859
44. Hoeijmakers JH (1993) Nucleotide excision repair II: from yeast to mammals. Trends Genet 9:211–217
45. Hynes NE, Lane HA (2005) ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 5:341–354
46. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ (2009) Cancer statistics, 2009. CA Cancer J Clin 59:225–249
47. Jun HJ, Ahn MJ, Kim HS, Yi SY, Han J, Lee SK, Ahn YC, Jeong HS, Son YI, Baek JH, Park K (2008) ERCC1 expression as a predictive marker of squamous cell carcinoma of the head and neck treated with cisplatin-based concurrent chemoradiation. Br J Cancer 99:167–172
48. Junttila TT, Sundvall M, Lundin M, Lundin J, Tanner M, Harkonen P, Joensuu H, Isola J, Elenius K (2005) Cleavable ErbB4 isoform in estrogen receptor-regulated growth of breast cancer cells. Cancer Res 65:1384–1393
49. Kapranos N, Stathopoulos GP, Manolopoulos L, Kokka E, Papadimitriou C, Bibas A, Yiotakis J, Adamopoulos G (2001) p53, p21 and p27 protein expression in head and neck cancer and their prognostic value. Anticancer Res 21:521–528
50. Kaur G, Carnelio S, Rao N, Rao L (2009) Expression of E-cadherin in primary oral squamous cell carcinoma and metastatic lymph nodes: an immunohistochemical study. Indian J Dent Res 20:71–76
51. Kawamata H, Uchida D, Hamano H, Kimura-Yanagawa T, Nakashiro KI, Hino S, Omotehara F, Yoshida H, Sato M (1998) Active-MMP2 in cancer cell nests of oral cancer patients: correlation with lymph node metastasis. Int J Oncol 13:699–704
52. Khan Z, Tiwari RP, Mulherkar R, Sah NK, Prasad GB, Shrivastava BR, Bisen PS (2009) Detection of survivin and p53 in human oral cancer: correlation with clinicopathologic findings. Head Neck 31:1039–1048
53. Kim SH, Koo BS, Kang S, Park K, Kim H, Lee KR, Lee MJ, Kim JM, Choi EC, Cho NH (2007) HPV integration begins in the tonsillar crypt and leads to the alteration of p16, EGFR and c-myc during tumor formation. Int J Cancer 120:1418–1425
54. Klussmann JP, Gultekin E, Weissenborn SJ, Wieland U, Dries V, Dienes HP, Eckel HE, Pfister HJ, Fuchs PG (2003) Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. Am J Pathol 162:747–753
55. Klussmann JP, Mooren JJ, Lehnen M, Claessen SM, Stenner M, Huebbers CU, Weissenborn SJ, Wedemeyer I, Preuss SF, Straetmans JM, Manni JJ, Hopman AH, Speel EJ (2009) Genetic signatures of HPV-related and unrelated oropharyngeal carcinoma and their prognostic implications. Clin Cancer Res 15:1779–1786
56. Klussmann JP, Weissenborn SJ, Wieland U, Dries V, Eckel HE, Pfister HJ, Fuchs PG (2003) Human papillomavirus-positive tonsillar carcinomas: a different tumor entity? Med Microbiol Immunol 192:129–132
57. Koh Y, Kim TM, Jeon YK, Kwon TK, Hah JH, Lee SH, Kim DW, Wu HG, Rhee CS, Sung MW, Kim CW, Kim KH, Heo DS (2009) Class III beta-tubulin, but not ERCC1, is a strong predictive and prognostic marker in locally advanced head and neck squamous cell carcinoma. Ann Oncol 20:1414–1419
58. Kreimer AR, Clifford GM, Boyle P, Franceschi S (2005) Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 14:467–475
59. Kroemer G (1997) The proto-oncogene Bcl-2 and its role in regulating apoptosis. Nat Med 3:614–620
60. Kurahara S, Shinohara M, Ikebe T, Nakamura S, Beppu M, Hiraki A, Takeuchi H, Shirasuna K (1999) Expression of MMPS, MT-MMP, and TIMPs in squamous cell carcinoma of the oral cavity: correlations with tumor invasion and metastasis. Head Neck 21:627–638
61. Kuropkat C, Plehn S, Herz U, Dunne AA, Renz H, Werner JA (2002) Tumor marker potential of serum matrix metalloproteinases in patients with head and neck cancer. Anticancer Res 22:2221–2227
62. Kusukawa J, Sasaguri Y, Morimatsu M, Kameyama T (1995) Expression of matrix metalloproteinase-3 in stage I and II squamous cell carcinoma of the oral cavity. J Oral Maxillofac Surg 53:530–534
63. Lebwohl D, Canetta R (1998) Clinical development of platinum complexes in cancer therapy: an historical perspective and an update. Eur J Cancer 34:1522–1534
64. Leeman RJ, Lui VW, Grandis JR (2006) STAT3 as a therapeutic target in head and neck cancer. Expert Opin Biol Ther 6:231–241
65. Lefebvre JL (2006) Laryngeal preservation in head and neck cancer: multidisciplinary approach. Lancet Oncol 7:747–755
66. Levine AJ, Momand J, Finlay CA (1991) The p53 tumour suppressor gene. Nature 351:453–456
67. Li F, Yang J, Ramnath N, Javle MM, Tan D (2005) Nuclear or cytoplasmic expression of survivin: what is the significance? Int J Cancer 114:509–512
68. Li Y, Nichols MA, Shay JW, Xiong Y (1994) Transcriptional repression of the D-type cyclin-dependent kinase inhibitor p16 by the retinoblastoma susceptibility gene product pRb. Cancer Res 54:6078–6082
69. Lo Muzio L, Campisi G, Giovannelli L, Ammatuna P, Greco I, Staibano S, Pannone G, De Rosa G, Di Liberto C, D'Angelo M

(2004) HPV DNA and survivin expression in epithelial oral carcinogenesis: a relationship? Oral Oncol 40:736–741
70. Lo Muzio L, Falaschini S, Farina A, Rubini C, Pezzetti F, Campisi G, De Rosa G, Capogreco M, Carinci F (2005) Bcl-2 as prognostic factor in head and neck squamous cell carcinoma. Oncol Res 15:249–255
71. Lord RV, Brabender J, Gandara D, Alberola V, Camps C, Domine M, Cardenal F, Sanchez JM, Gumerlock PH, Taron M, Sanchez JJ, Danenberg KD, Danenberg PV, Rosell R (2002) Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. Clin Cancer Res 8:2286–2291
72. Lu XZ, Zou YG, Yin XM, Chen WT, Zhang CP (2008) Expression of MMP1 mRNA in oral squamous cell carcinoma and paired normal tissues. Nan Fang Yi Ke Da Xue Xue Bao 28:1362–1364
73. Mahomed F, Altini M, Meer S (2007) Altered E-cadherin/beta-catenin expression in oral squamous carcinoma with and without nodal metastasis. Oral Dis 13:386–392
74. Mandriota SJ, Jussila L, Jeltsch M, Compagni A, Baetens D, Prevo R, Banerji S, Huarte J, Montesano R, Jackson DG, Orci L, Alitalo K, Christofori G, Pepper MS (2001) Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. EMBO J 20:672–682
75. Manning FC, Patierno SR (1996) Apoptosis: inhibitor or instigator of carcinogenesis? Cancer Invest 14:455–465
76. Mao L, Hong WK, Papadimitrakopoulou VA (2004) Focus on head and neck cancer. Cancer Cell 5:311–316
77. Masuda M, Suzui M, Yasumatu R, Nakashima T, Kuratomi Y, Azuma K, Tomita K, Komiyama S, Weinstein IB (2002) Constitutive activation of signal transducers and activators of transcription 3 correlates with cyclin D1 overexpression and may provide a novel prognostic marker in head and neck squamous cell carcinoma. Cancer Res 62:3351–3355
78. Mineta H, Miura K, Takebayashi S, Ueda Y, Misawa K, Harada H, Wennerberg J, Dictor M (2000) Cyclin D1 overexpression correlates with poor prognosis in patients with tongue squamous cell carcinoma. Oral Oncol 36:194–198
79. Miyamoto R, Uzawa N, Nagaoka S, Hirata Y, Amagasa T (2003) Prognostic significance of cyclin D1 amplification and overexpression in oral squamous cell carcinomas. Oral Oncol 39:610–618
80. Murray D, Rosenberg E (1996) The importance of the ERCC1/ERCC4[XPF] complex for hypoxic-cell radioresistance does not appear to derive from its participation in the nucleotide excision repair pathway. Mutat Res 364:217–226
81. Murugan AK, Hong NT, Cuc TT, Hung NC, Munirajan AK, Ikeda MA, Tsuchida N (2009) Detection of two novel mutations and relatively high incidence of H-RAS mutations in Vietnamese oral cancer. Oral Oncol 45:e161–e166
82. Nagpal JK, Patnaik S, Das BR (2002) Prevalence of high-risk human papilloma virus types and its association with P53 codon 72 polymorphism in tobacco addicted oral squamous cell carcinoma (OSCC) patients of Eastern India. Int J Cancer 97:649–653
83. Nakanishi C, Toi M (2005) Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. Nat Rev Cancer 5:297–309
84. Olaussen KA, Dunant A, Fouret P, Brambilla E, Andre F, Haddad V, Taranchon E, Filipits M, Pirker R, Popper HH, Stahel R, Sabatier L, Pignon JP, Tursz T, Le Chevalier T, Soria JC (2006) DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 355:983–991
85. Pannone G, Sanguedolce F, De Maria S, Farina E, Lo Muzio L, Serpico R, Emanuelli M, Rubini C, De Rosa G, Staibano S, Macchia L, Bufo P (2007) Cyclooxygenase isozymes in oral squamous cell carcinoma: a real-time RT-PCR study with clinic pathological correlations. Int J Immunopathol Pharmacol 20:317–324
86. Peinado H, Portillo F, Cano A (2004) Transcriptional regulation of cadherins during development and carcinogenesis. Int J Dev Biol 48:365–375
87. Polkowski W, van Sandick JW, Offerhaus GJ, ten Kate FJ, Mulder J, Obertop H, van Lanschot JJ (1999) Prognostic value of Lauren classification and c-erbB-2 oncogene overexpression in adenocarcinoma of the esophagus and gastroesophageal junction. Ann Surg Oncol 6:290–297
88. Preuss SF, Weinell A, Molitor M, Semrau R, Stenner M, Drebber U, Wedemeyer I, Hoffmann TK, Guntinas-Lichius O, Klussmann JP (2008) Survivin and epidermal growth factor receptor expression in surgically treated oropharyngeal squamous cell carcinoma. Head Neck 30:1318–1324
89. Preuss SF, Weinell A, Molitor M, Stenner M, Semrau R, Drebber U, Weissenborn SJ, Speel EJ, Wittekindt C, Guntinas-Lichius O, Hoffmann TK, Eslick GD, Klussmann JP (2008) Nuclear survivin expression is associated with HPV-independent carcinogenesis and is an indicator of poor prognosis in oropharyngeal cancer. Br J Cancer 98:627–632
90. Ragin CC, Taioli E (2007) Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: review and meta-analysis. Int J Cancer 121:1813–1820
91. Ravi D, Ramadas K, Mathew BS, Nalinakumari KR, Nair MK, Pillai MR (1999) De novo programmed cell death in oral cancer. Histopathology 34:241–249
92. Reed E (2005) ERCC1 and clinical resistance to platinum-based therapy. Clin Cancer Res 11:6100–6102
93. Reimers N, Kasper HU, Weissenborn SJ, Stutzer H, Preuss SF, Hoffmann TK, Speel EJ, Dienes HP, Pfister HJ, Guntinas-Lichius O, Klussmann JP (2007) Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. Int J Cancer 120:1731–1738
94. Reuter CW, Morgan MA, Eckardt A (2007) Targeting EGF-receptor-signalling in squamous cell carcinomas of the head and neck. Br J Cancer 96:408–416
95. Roberts PJ, Der CJ (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 26:3291–3310
96. Rogers SJ, Harrington KJ, Rhys-Evans P, OC P, Eccles SA (2005) Biological significance of c-erbB family oncogenes in head and neck cancer. Cancer Metastasis Rev 24:47–69
97. Roskoski R Jr (2004) The ErbB/HER receptor protein-tyrosine kinases and cancer. Biochem Biophys Res Commun 319:1–11
98. Rozenblum E, Schutte M, Goggins M, Hahn SA, Panzer S, Zahurak M, Goodman SN, Sohn TA, Hruban RH, Yeo CJ, Kern SE (1997) Tumor-suppressive pathways in pancreatic carcinoma. Cancer Res 57:1731–1734
99. Salven P, Heikkila P, Anttonen A, Kajanti M, Joensuu H (1997) Vascular endothelial growth factor in squamous cell head and neck carcinoma: expression and prognostic significance. Mod Pathol 10:1128–1133
100. Samuels Y, Velculescu VE (2004) Oncogenic mutations of PIK3CA in human cancers. Cell Cycle 3:1221–1224
101. Sappayatosok K, Maneerat Y, Swasdison S, Viriyavejakul P, Dhanuthai K, Zwang J, Chaisri U (2009) Expression of pro-inflammatory protein, iNOS, VEGF and COX-2 in oral squamous cell carcinoma (OSCC), relationship with angiogenesis and their clinico-pathological correlation. Med Oral Patol Oral Cir Bucal 14:E319–E324
102. Serrano M, Hannon GJ, Beach D (1993) A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 366:704–707
103. Sherr CJ, Roberts JM (1995) Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev 9:1149–1163

104. Sheu JJ, Hua CH, Wan L, Lin YJ, Lai MT, Tseng HC, Jinawath N, Tsai MH, Chang NW, Lin CF, Lin CC, Hsieh LJ, Wang TL, Shih Ie M, Tsai FJ (2009) Functional genomic analysis identified epidermal growth factor receptor activation as the most common genetic event in oral squamous cell carcinoma. Cancer Res 69:2568–2576
105. Shiboski CH, Schmidt BL, Jordan RC (2005) Tongue and tonsil carcinoma: increasing trends in the US population ages 20–44 years. Cancer 103:1843–1849
106. Shiga H, Rasmussen AA, Johnston PG, Langmacher M, Baylor A, Lee M, Cullen KJ (2000) Prognostic value of c-erbB2 and other markers in patients treated with chemotherapy for recurrent head and neck cancer. Head Neck 22:599–608
107. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344:783–792
108. Smeets SJ, Hesselink AT, Speel EJ, Haesevoets A, Snijders PJ, Pawlita M, Meijer CJ, Braakhuis BJ, Leemans CR, Brakenhoff RH (2007) A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. Int J Cancer 121:2465–2472
109. Smith BD, Smith GL, Carter D, Sasaki CT, Haffty BG (2000) Prognostic significance of vascular endothelial growth factor protein levels in oral and oropharyngeal squamous cell carcinoma. J Clin Oncol 18:2046–2052
110. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK, Levin B (2000) The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 342:1946–1952
111. Stewart SA, Weinberg RA (2000) Telomerase and human tumorigenesis. Semin Cancer Biol 10:399–406
112. Su JL, Yang PC, Shih JY, Yang CY, Wei LH, Hsieh CY, Chou CH, Jeng YM, Wang MY, Chang KJ, Hung MC, Kuo ML (2006) The VEGF-C/Flt-4 axis promotes invasion and metastasis of cancer cells. Cancer Cell 9:209–223
113. Syrjanen S (2007) Human papillomaviruses in head and neck carcinomas. N Engl J Med 356:1993–1995
114. Takatori H, Natsugoe S, Okumura H, Matsumoto M, Uchikado Y, Setoyama T, Sasaki K, Tamotsu K, Owaki T, Ishigami S, Aikou T (2008) Cyclooxygenase-2 expression is related to prognosis in patients with esophageal squamous cell carcinoma. Eur J Surg Oncol 34:397–402
115. Tanigaki Y, Nagashima Y, Kitamura Y, Matsuda H, Mikami Y, Tsukuda M (2004) The expression of vascular endothelial growth factor-A and -C, and receptors 1 and 3: correlation with lymph node metastasis and prognosis in tongue squamous cell carcinoma. Int J Mol Med 14:389–395
116. Temam S, Flahault A, Perie S, Monceaux G, Coulet F, Callard P, Bernaudin JF, St Guily JL, Fouret P (2000) p53 gene status as a predictor of tumor response to induction chemotherapy of patients with locoregionally advanced squamous cell carcinomas of the head and neck. J Clin Oncol 18:385–394
117. Thiery JP, Sleeman JP (2006) Complex networks orchestrate epithelial–mesenchymal transitions. Nat Rev Mol Cell Biol 7:131–142
118. Vallbohmer D, Brabender J, Metzger R, Holscher AH (2009) Genetics in the pathogenesis of esophageal cancer: possible predictive and prognostic factors. J Gastrointest Surg
119. Vivanco I, Palaskas N, Tran C, Finn SP, Getz G, Kennedy NJ, Jiao J, Rose J, Xie W, Loda M, Golub T, Mellinghoff IK, Davis RJ, Wu H, Sawyers CL (2007) Identification of the JNK signaling pathway as a functional target of the tumor suppressor PTEN. Cancer Cell 11:555–569
120. Vonderheide RH, Hahn WC, Schultze JL, Nadler LM (1999) The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. Immunity 10:673–679
121. Waldman T, Lengauer C, Kinzler KW, Vogelstein B (1996) Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. Nature 381:713–716
122. Weber A, Langhanki L, Sommerer F, Markwarth A, Wittekind C, Tannapfel A (2003) Mutations of the BRAF gene in squamous cell carcinoma of the head and neck. Oncogene 22:4757–4759
123. Werkmeister R, Brandt B, Joos U (2000) Clinical relevance of erbB-1 and -2 oncogenes in oral carcinomas. Oral Oncol 36:100–105
124. Williams GT (1991) Programmed cell death: apoptosis and oncogenesis. Cell 65:1097–1098
125. Wilson GD, Saunders MI, Dische S, Richman PI, Daley FM, Bentzen SM (2001) bcl-2 expression in head and neck cancer: an enigmatic prognostic marker. Int J Radiat Oncol Biol Phys 49:435–441
126. Wittekindt C, Gultekin E, Weissenborn SJ, Dienes HP, Pfister HJ, Klussmann JP (2005) Expression of p16 protein is associated with human papillomavirus status in tonsillar carcinomas and has implications on survival. Adv Otorhinolaryngol 62:72–80
127. Witton CJ, Reeves JR, Going JJ, Cooke TG, Bartlett JM (2003) Expression of the HER1–4 family of receptor tyrosine kinases in breast cancer. J Pathol 200:290–297
128. Worden FP, Kumar B, Lee JS, Wolf GT, Cordell KG, Taylor JM, Urba SG, Eisbruch A, Teknos TN, Chepeha DB, Prince ME, Tsien CI, D'Silva NJ, Yang K, Kurnit DM, Mason HL, Miller TH, Wallace NE, Bradford CR, Carey TE (2008) Chemoselection as a strategy for organ preservation in advanced oropharynx cancer: response and survival positively associated with HPV16 copy number. J Clin Oncol 26:3138–3146
129. Wouters BG, Giaccia AJ, Denko NC, Brown JM (1997) Loss of p21Waf1/Cip1 sensitizes tumors to radiation by an apoptosis-independent mechanism. Cancer Res 57:4703–4706
130. Xie X, Clausen OP, Boysen M (2002) Prognostic significance of p21WAF1/CIP1 expression in tongue squamous cell carcinomas. Arch Otolaryngol Head Neck Surg 128:897–902
131. Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D (1993) p21 is a universal inhibitor of cyclin kinases. Nature 366:701–704
132. Yamamoto T, Tanigawa N (2001) The role of survivin as a new target of diagnosis and treatment in human cancer. Med Electron Microsc 34:207–212
133. Yorioka CW, Coletta RD, Alves F, Nishimoto IN, Kowalski LP, Graner E (2002) Matrix metalloproteinase-2 and -9 activities correlate with the disease-free survival of oral squamous cell carcinoma patients. Int J Oncol 20:189–194
134. Zhang M, Zhang P, Zhang C, Sun J, Wang L, Li J, Tian Z, Chen W (2009) Prognostic significance of Bcl-2 and Bax protein expression in the patients with oral squamous cell carcinoma. J Oral Pathol Med 38:307–313