Review Article
Biomarkers of head and neck cancer, tools or a gordian knot?

Evangeli S Lampri[1], Georgios Chondrogiannis[2], Elli Ioachim[3], Anna Varouktsi[4], Antigoni Mitselou[5], Aggeliki Galani[6], Evangelos Briassoulis[7], Panagiotis Kanavaros[2], Vasiliki Galani[2]

Departments of [1]Pathology, [2]Anatomy-Histology-Embryology, [5]Forensic Pathology, [7]Hematology, Faculty of Medicine, University of Ioannina, Greece; [3]Department of Pathology, General Hospital "G. Hatzikosta", Ioannina, Greece; [4]Ippokratio Hospital, Thessaloniki, Greece; [6]Department of Environmental and Natural Resources Management, School of Engineering, Agrinio, University of Patras, Greece

Abstract: Head and neck tumors comprise a wide spectrum of heterogeneous neoplasms for which biomarkers are needed to aid in earlier diagnosis, risk assessment and therapy response. Personalized medicine based on predictive markers linked to drug response, it is hoped, will lead to improvements in outcomes and avoidance of unnecessary treatment in carcinoma of the head and neck. Because of the heterogeneity of head and neck tumors, the integration of multiple selected markers in association with the histopathologic features is advocated for risk assessment. Validation of each biomarker in the context of clinical trials will be required before a specific marker can be incorporated into daily practice. Furthermore, we will give evidence that some proteins implicated in cell-cell interaction, such as CD44 may be involved in the multiple mechanism of the development and progression of laryngeal lesions and may help to predict the risk of transformation of the benign or precancerous lesions to cancer.

Keywords: HPV, squamous cell carcinoma, molecular, pharyngeal carcinoma, carcinogenesis, genetic

Introduction

Head and neck cancers are markedly phenotypic and clinically heterogeneous neoplasms of different histogenesis. The most common is epithelial derived head and neck squamous cell carcinoma (HNSCC) with over 500,000 new cases expected worldwide [1]. An estimated 49,260 new cases of HNSCC were diagnosed in the United States in 2010 [2]. Advancement in treatment strategies did not influence significantly the survival rates, which remain poor, as recurrence, distant metastasis, and second primaries are developed in many patients [3].

Pathogenically, the development of these tumors has been associated with the mutagenic role of tobacco carcinogens [4], which can induce specific mutations in different genes. Carcinogens are strongly associated with HNSCC, suggesting a possible implication of pathways related with the cellular response to genotoxic damage; in addition, genes that are implicated in DNA repair processes or metabolism

of carcinogens play their crucial role [5]. Nationality seems to influence the incidence of these tumors, as the Black population has higher rates than Hispanic and Asian population [6]. A role for certain types of human papillomaviruses has also been suggested.

The majority of patients present with late stage disease and the overall 5-year survival rate have not changed significantly over the last three decades, the fact that most of the patients will present with advanced disease increases significantly the morbidity and mortality. The WHO estimates oral cancer as having one of the highest mortality ratios of all malignancies [6]. Early detection in head and neck cancer has been shown to dramatically increase survival rates when compared to detection at later disease stages [7], being the most important variable leading to positive outcomes [8]. Moreover, there are not many screening tools and markers to discriminate the patients who are to be benefited by adjuvant therapy. Despite great advances in the surgical and non-surgical man-

agement of these patients with favorable cure rate, therapies are aggressive and frequently significantly affecting the quality of life with severe short-term toxicity and long term functional impairment, such as speech and swallowing difficulties. Thus, there is a great necessity for better patient selection to avoid unnecessary treatment and minimize long-term toxicity. Fortunately, with the help of clinical trials, there is a great progress in the identification and validation of biomarkers as molecular targeted therapies. To determine which patients will benefit from these treatments requires personalized medicine and the analysis of factors must be specific to the individual and their tumor. The twenty-first century can be characterized as "the age of biomarker discovery" which promises an earlier cancer diagnosis and a more fruitful treatment [1, 9].

Presently, the clinical applications for tumor biomarkers include molecular margin control for surgical resection, tumor aggressiveness prediction for specific modulation of treatment, and identification of targets for gene-and protein-specific targeted therapy. The ultimate goal is the creation of markers for detection and treatment of pre-clinical and pre-malignant disease, avoiding the progression to cancer altogether [3]. A large number of biomarkers have been identified and are currently under study. Underscoring the numerous possibilities is an analysis of over 13,000 genes that found 1,260 with differential expression in HNSCC [3, 10].

The following review try to summarize some of the most relevant biomarkers related to head and neck cancer and discriminate those with the greatest predictive and therapeutic value.

Historical perspective

The search for a biomarker for head and neck cancer started many years ago. One potential biomarker was considered to be carcinoembryonic antigen (CEA) in blood and saliva of 439 HNSCC patients in 1976, but it did not prove to have any prognostic value [11-13]. One year later, Kato and Torigoe [14] described tumor associated antigen (TA-4), a number of proteins with a common antigenic determinant; 148 resulting in the development of SCC-antigen assays [15]. However, except from one study [16], all the others demonstrated that the squa-

mous cell carcinoma antigen (SCC-antigens) role was limited to the possible monitoring of disease [17, 18]. Moreover, many of the biomarkers which proved to be useful in other carcinoma, like carbohydrate antigen (CA-19-9), did not show to have important prognostic significance in head and neck cancer [19].

Only one study occupied with the levels of CEA, CA19-9, SCC-antigen, thymidine kinase (TK), and deoxythymidine 5'-triphosphatase (dTTPase) in 26 patients prior to treatment and over time following treatment [20]. However, none of these markers by itself or in combination proved to have any significant correlation with tumor location, stage, or grade [3].

Micronuclei, chromosomal abnormalities, and ploidy

Micronuclei are chromatid fragments formed during abnormal division of cells with damaged DNA. They correlate with cancer risk and the reduction of micronuclei helps chemoprevention, but inconsistent clinical outcomes have minimized their utility [3, 21-23].

Chromosomal abnormalities have been extensively documented in HNSCC [24-26]. The long list of described chromosomal abnormalities involves many chromosomes, including 3, 4, 7, 8, 9, 11, 13, 17, 18, and 19. The changes include losses and gains of genetic mate specific gene alterations [27, 28]. In addition; there is evidence that the potential for malignant progression may be linked to certain chromosomal alterations. One study examined chromosomal changes at 3p, 4q, 8p, 9p, 11q, 13q, and 17p associated with clinical leukoplakias [29]. Changes at 3p or 9p in combination with any other change were associated with a 33-fold increase in risk for progression to cancer [3].

A chromosomal translocation t (11:19) between CRTC and MAML2 is present in the majority of mucoepidermoid carcinoma (50-80%) [9, 30]. Another recently described translocation t (6; 9) between MYB and NFIB in adenoid cystic carcinoma leads to overexpression of the MYB gene and downstream target genes including c-KIT which shows high-expression in these tumors [31]. Frequent loss of 1p32-p36 has also been identified in adenoid cystic carcinoma and correlated with solid/basaloid morphology and poorer prognosis [32]. These observa-

tions favor potential tumor suppressor genes in this region which are lost and may allow for directed targeted therapy in the future [1].

DNA ploidy has been extensively studied as a marker of prognosis. Anything greater than the normal two (diploid) copies falls under the general category of polyploidy. Finally, if chromosomes are not evenly distributed to the daughter cells the resulting cells are aneuploid. These chromosomal changes are common in HNSCC and seem to have prognostic significance [3, 33, 34]. Based on these results, aneuploidy may prove to be a powerful marker of malignant potential, and poor prognosis [3].

Genetic instability (microsatellite alterations and loss of heterozygosity)

Microsatellites are common genetic elements of the human genome composed of highly polymorphic tandem repeat sequences of DNA. They have been successfully used as markers for tumor formation in a large number of malignancies, including HNSCC [35]. These sequences can be amplified with PCR technology to reveal loss of heterozygosity (LOH) or microsatellite instability. The process greatly enhances tumor suppressor gene mapping and identification of tumor clonality in serum and saliva [3, 35-37]. An early and common genetic event in oral premalignancy includes LOH of 9p21 in dysplasia/hyperplasia (30%) and carcinoma (70-80%) [38]. The detection of 3p and 9p loss by LOH analysis using comparative normal DNA (commonly peripheral blood lymphocytes) is currently being used in a NCI sponsored trial of Erlotinib in the prevention of oral cancer (EPOC trial) [1]. There is study which showed a trend towards increased instability with increased disease state [39]. Tetranucleotide microsatellite instability was recently tested in surgical margins as a predictor for recurrence [40].

Oncogenes and tumor suppressor genes

pP53 (TP53)

Mutations in p53 are one of the most frequent abnormalities in HNSCC. A number of studies have confirmed p53 mutation in 30% to 75% of HNSCC tumors [41, 42], raising the necessity each mutation to be specified. Mutations of p53 are met first in severe dysplasia. p53 overexpression was also correlated strongly with

poor survival in invasive carcinomas (50% incidence) [43-47]. Especially, there was a statistically significant decrease in survival for patients with mutations in the DNA binding regions of p53 compared to those with p53 mutations outside the DNA binding sites [44]. The last finding may explain contrary reports showing a lack of prognostic ability of p53 [45, 46].

NOTCH1

NOTCH1 is the second most commonly mutated gene in HNSCC, with mutation rate of 14 to 15%. NOTCH1 is important in regulating normal cell differentiation, lineage commitment, and embryonic development. It appears to function as a tumor suppressor gene in HNSCC based on the position and characteristics of the mutations and the inactivation of both alleles. Most NOTCH1 mutations observed in HNSCC affect the epidermal growth factor (EGF)-like ligand-binding domain [48-50].

CDKN2A

Alterations of cyclin-dependent kinase inhibitor 2A (CDKN2A/p16INK4A), a tumor suppressor gene located on chromosome 9p21, have long been recognized in HNSCC [51, 52]. In the next generation sequencing studies, CDKN2A mutations were identified in 9 to 12% of all tumors [48-50]. Gene copy number analyses also revealed frequent loss of heterozygosity and deletions of CDKN2A [48]. Moreover, CDKN2A is inactivated by methylation of the 5' CpG region [53].

In addition, p16, which is protein product of CDKN2A, is implicated in cell cycle regulation via its interaction with the retinoblastoma (Rb) tumor suppressor. The p16 protein inhibits cyclin-dependent kinases (CDK) 4 and 6, which causes hypophosphorylation of Rb and therefore G1 arrest of the cell cycle 37-39. Although alterations of CDKN2A are common events in early HNSCC development, there are not enough to drive tumorigenesis. The last suggestion finds evidence by the fact that CDKN2A mutations have been reported in benign epithelial lesions with low potential for malignant transformation [50, 54-57].

Genes implicated in cell cycle

Although many papers published to date show a correlation between high proliferative activity

and poor prognosis in head and neck tumors, most of them failed to show any significant association with prognosis for many reasons. One of them is anatomic site. In the last edition of the TNM classification of malignant tumors [58], head and neck tumors include tumors of the maxillary sinus, nasal cavity, ethmoid sinus, oral cavity, lip, salivary gland, thyroid gland, pharynx and larynx, dealing with heterogeneous tumors from different sites, histological types and prognosis [59-63].

Ki67/MIB-1

A high cell proliferation, as expressed by the MIB-1 labeling index, was a significant indicator for treatment failure in a large matched-pair study design of recurrent and non-recurrent oral and oropharyngeal carcinomas initially treated with primary surgery combined with curative post-operative radiation [64]. Also, in another large matched-pair study on recurrent and non-recurrent laryngeal carcinomas, homogeneous for site (glottis), stage (T1 and T2) and treatment (only transoral laser surgery), high index of proliferation, using MIB-1 and PCNA staining, proved to have prognostic significance.

Nucleolar organizer region associated proteins (AgNORs)

In general, a high proliferative activity is associated with poor prognosis in SCC of the oral cavity. Almost 30% of the cases were investigated using AgNOR analysis, although this method is not extensively applied in pathology departments. Piffko et al [65], reported that AgNORs at the invasive front of the tumor is the most significant independent prognostic factor. This study provides a functional background to the clinical relevance of the histopathological tumor front grading [66] and suggests the use of an aggressive surgical approach for those patients with a high AgNOR quantity. However, in a large number of studies, cell proliferative activity in oral SCC does not have any prognostic significance [63].

Cyclin D1

Cyclin D1 overexpression has been reported in SCC in a variety of head and neck subsites including the larynx, hypopharynx and tongue [46, 67-74]. Its prognostic significance

in oropharyngeal SCC has been contradictory [75-80].

Cyclin D1 is a well-known oncogene in a variety of human cancers, regulating cell proliferation through G1 phase. Cyclin D1 forms complexes with CDK4 and CDK6, which phosphorylate the retinoblastoma protein pRb [81]. This causes pRb to release the E2F transcription factor, which then activates the genes which are necessary for cell cycle progression from G1 phase to S phase [82]. Cyclin D1 correlates with lymph node metastases, high grade tumors and poor survival, playing mainly a role in the late phase of tumor progression [71].

HPV-positive cancers are thought to be associated with downregulation of cyclin D1 expression [83, 84]. In hypopharyngeal cancers, cyclin D1 overexpression has been correlated with poor outcome in cases which are not related to HPV [71], but without existing a large amount of evidence suggesting its prognostic significance.

Vaccinia-related kinase 1 (VRK1) protein

The (VRK1) protein belongs to a new family of serine/threonine kinases and phosphorylates several transcription factors, including human p53 [85-88], and can also cooperate with the c-Jun NH2-terminal kinase pathway by phosphorylation of c-Jun [89] and ATF2 [90]. All these proteins phosphorylated by VRK1 have been associated with cellular responses to stress [91-93]. VRK1 contributes to p53 stability by two mechanisms, one of them dependent on Thr18 phosphorylation [90, 94], resulting in its stabilization and favoring its interaction with the transcriptional coactivator p300 which also seems to be implicated in the control of normal proliferation in the absence of cellular stress [94, 95]. The loss of VRK1 also affects the endocytic transport with a phenotype similar to that induced by silencing of mitogen-activated protein kinase [87, 95]. The expression of human VRK1 was determined in normal epithelium, particularly near the basal layer where cellular proliferation takes place, but it is lost as the epithelial cells differentiate. VRK1 was also detected in many lymphocytes within the follicles.

VRK1 was also detected immunohistochemically in HNSCCs, where it was correlated posi-

tively with many proteins related with proliferation, suggesting a possible role in cell cycle regulation in the context of HNSCC [87].

Epidermal growth factor receptor (EGFR)

Epidermal growth factor receptor (EGFR), is a member of the tyrosine kinase family of receptors, which, in response to extracellular binding with its natural ligands, such as epidermal growth factor and transforming growth factor (TGF)-a, generates an intracellular signaling cascade, resulting in cell proliferation. Cells that acquire the ability to overproduce these ligands or increase the number of EGFRs on their surface can create an autocrine growth pathway, resulting in uncontrolled growth [96, 97]. EGFR activates STAT 1, and 3, phosphatidylinositol 3-kinase (PI3k), the Ras-MAPk/ERk pathway and phospholipase c-c (PLC-c), contributing to cell survival and proliferation in HNSCC [1]. Virtually all HNSCC cases express EGFR [97-104], usually at high levels (2+, 3+).

This very high frequency of EGFR expression in HNSCC greatly limits the usefulness of classifying tumors as simply positive or negative as a marker for selecting treatment. Notably, levels of EGFR expression were also upregulated in normal mucosa from HNSCC patients, suggesting that increased EGFR expression is an early event in the development of HNSCC, and may help to explain the high incidence of synchronous and metachronous disease observed in this type of cancer. Up regulation of this factor occurs early in the progression of dysplasia to HNSCC in the upper aerodigestive tract.

High expression of EGFR is a negative prognostic factor which is often associated with nodal metastases and poor survival in patients with HNSCC [96, 98, 103], because it is implicated in cell motility, alters cell adhesion and promotes angiogenesis [98, 103-105]. Moreover, doses of cetuximab may need to be adjusted in patients with very high levels of EGFR, in order to be more efficacy [106].

EGFR expression is usually assessed immunohistochemically in paraffin embedded tumor samples, facing the commonest problem with this technique which is the variation of results because of the differences in technique, the type of antibody or fixative used, the species, and the storage time of the sample [100]. The

increased EGFR gene copy number, as fluorescent in situ hybridization shows, did not seem to be prognostic factor in patients with HNSCC [97, 101]. Another technique, the automated quantitative analysis of protein expression measures with greater accuracy the total and phosphorylated EGFR expression in tumor (samples) [102].

Mechanisms for EGFR over-expression in HNSCCs show EGFR gene mutations as in lung adenocarcinomas are uncommon and gene amplification is infrequent (< 15%), however a truncated mutant activated EGFRvIII is prevalent (40%) [107].

EGFR variants

A mutant form of EGFR known as EGFRvIII has been detected in up to 40% of HNSCC cases [107]. This truncated, constitutively active receptor is ligand independent and does not bind with antibodies that target the extracellular domain of wild-type EGFR. There are in vitro studies which showed that cells with EGFRvIII expression are less sensitive to the growth-inhibiting effects of cetuximab. Moreover, EGFRvIII is only seen in cells that overexpress wild-type EGFR, that's why there is the suggestion that mutations are a late stage event caused by the rapid proliferation induced by wild-type EGFR overexpression [107]. Other alterations in EGFR include activating mutations, which have been detected in 10% to 30% of patients with HNSCC and predict increased sensitivity to small molecules that target the intracellular portion of EGFR [84, 85]. However, these mutations are present in just 7.3% of Asian patients with HNSCC who have been evaluated for the mutation and in only 1% in the rest population [9, 108-110].

K-RAS

The ras family of genes, H-RAS, K-RAS, and N-RAS, encode a protein that is located in the cytoplasmic side of the plasma membrane. The RAS gene has been identified in approximately one-third of all oral cancers in India and South East Asia [111]. The protein transmits mitogenic signals in response to a variety of physiological stimuli [112]. In HNSCC, the prevalence of K-RAS mutations is low. One study reported a prevalence of 8% [113], but most reports are generally < 5%, or as low as 2%. Although some

evidence suggests that K-RAS mutations may indicate enhanced proliferation and an aggressive disease course in HNSCC [114, 115], their extremely low prevalence will likely preclude a major role in personalized medicine. Until now, there is no clinical benefit to testing for K-RAS mutation status before administering EGFR-targeted therapy to patients with HNSCC. Further evaluation of K-RAS, including the implications of overexpression of wild-type KRAS [116], is needed to better define the potential prognostic and predictive role, if any, of this factor in HNSCC [9].

NF-kB

Biopsy specimens from patients with oral squamous cell carcinoma was found to express higher levels of NF-kB in comparison to normal and dysplastic tissue [117].

Aurora kinase A (AURKA/STK15/BTAK)

Aurora kinase A (AURKA/STK15/BTAK) was analyzed in tumor and adjacent normal mucosa from HNSCC patients by both real-time quantitative reverse transcription-PCR and immunohistochemistry using a tissue microarray. AURKA induces chromosomal instability leading to aneuploidy and transformation. It was found increased expression and the association of AURKA elevation with poor prescience [118].

ERCC1 (excision repair cross-complementation group 1) protein

The ERCC1 plays an important role in repairing DNA damage caused by platinum agents, that's why may help to select the patients who will benefit from platinum-based therapy [119-130]. Patients with low levels of expression had a 4-fold greater chance of achieving an objective response to cisplatin-based chemotherapy and a prolonged survival compared with those with high levels of expression. These findings show that ERCC1 expression levels before treatment are inversely correlated with response and survival after cisplatin-based therapy [9, 129, 130].

In head and neck cancer was found high expression of ERCC1. From a practical perspective, it appears that high ERCC1 expression in HNSCC tumors may suggest a low probability of benefiting from platinum therapy, so these patients may be benefit more by alternative approaches [9, 131-134]. For *ERCC1*, there are numerous studies indicating that low mRNA or protein expression is associated with a better prognosis in HNSCC. However, it is not established that *ERCC1* expression is regulated at the transcriptional level. The main problem is that a nonspecific antibody was used to measure protein level. Studies manage to validate the utility of these biomarkers (*ERCC1* mRNA levels or 8F1 immunohistochemical signal) for predicting clinical outcomes, but they do not demonstrate that DNA repair levels are altered in tumors [135].

Base excision repair pathway (XRCC1)

XRCC1 reduced expression or activity results in increased genomic instability and sensitivity to DNA damaging agents. High XRCC1 expression was correlated with resistance to radiotherapy in HNSCC [135, 136].

Angiogenic factors

Overexpression of vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) play their vital role in angiogenesis of SCC. EGFRvIII leads to autoactivation and upregulated VEGF and tissue factor (TF) further augmenting this pathway [137]. Hypoxia is also a strong driving force in tumor angiogenesis, through the overexpression of hypoxia inducible factor-1 (HIF-1), at the top of a cascade of inducible proangiogenic proteins contributing to angiogenesis in HNSCC. Another marker of tumor hypoxia is lysyl oxidase, which may predict distant metastasis [1].

Structural related biomarkers

Epithelial to mesenchymal transition (EMT)

Another mechanism contributing to cell migration and metastases in SCC is epithelial to mesenchymal transition (EMT), a process by which carcinoma cells loose adhesion factors (i.e. E-cadherin). This process is highlighted by the spectrum/progression of SCC from well differentiated tumors to poorly differentiate and ultimately sarcomatoid carcinoma with single cell invasion and morphologic features of mesenchymal cells [138]. Moreover, Src is a strong inducer of EMT abolishing loss of cell-cell adhesion and E-cadherin and associates with SCC progression and aggressive feature [1, 138].

b-Tubulin

Isotypes Taxanes exert their anticancer activity by binding to b-tubulin polymers and inhibiting microtubule depolymerization and mitotic process. As preclinical models showed, high expression levels of the isotype III of b-tubulin have been associated with resistance to paclitaxel in several cell types [9, 139, 140]. In HNSCC, docetaxel has become part of the standard induction regimen in combination with cisplatin and 5-fluorouracil. The expression of b-tubulin II measured by IHC (samples were scored by percentage of stained cells and intensity of staining; positive results were those above the median score and negative those below) may be associated with outcomes. Patients with low expression of b-tubulin II had better prognosis, regardless the treatment of docetaxel, as well as the benefit from the addition of docetaxel seemed to be greater [141]. As the use of taxanes in HNSCC grows, this marker may assume a greater role in therapeutic decision making [9].

cd44

CD44 is an integral membrane glycoprotein that has diverse functions in cell-cell and cell-substrate interactions. It has been suggested that it may be a determinant of metastatic and invasive behavior in carcinomas. CD44 expression may be involved in the multiple mechanism of the development and progression of laryngeal lesions and may help to predict the risk of transformation of the benign or precancerous lesions to cancer [142].

Viral etiologies

Epstein barr virus (EBV)

In 1970, EBV was identified in SCCs arising in the nasopharynx which maybe the earliest tumor in the head and neck with a known biomarker. EBV can be detected in tumor tissue by in situ hybridization for EBV encoded RNAs (EBER) or by the less sensitive method of immunohistochemistry [1].

Human papilloma virus (HPV)

Approximately 30 years after EBV's association with nasopharyngeal carcinoma, human papilloma virus (HPV), especially type 16, proved to be etiologic and prognostic factor in oropharyngeal carcinomas [143]. HPV-positive HNSCC is associated with a younger age of onset than in smokers, and it was proved that HPV is a reliable prognostic marker for improved local control and overall survival at 5 years of 79% for HPV + versus 20% for HPV negative patients [144]. Evaluation for HPV 16 may be performed on archival sections of biopsy tissue or FNA (i.e. neck nodes) by in situ hybridization or PCR amplification of viral related genes (E6, E7).

HPV-positive tumors in HNSCC are almost always oropharyngeal. However, prevalence of the reported HPV infection varies widely, because of the different method of detection applied. About 35% of overall head and neck tumors have been reported to be HPV positive, as detected by PCR [145], with cancers of the tonsils (43.6%) and the base of the tongue (38.4%) to be the most often detected sites [146]. One of the largest and most recent analyses of patients with oropharynx cancer from RTOG (Radiation Therapy Oncology Group) 0129 demonstrated a 64% incidence of HPV-positive tumors, greater than previously appreciated, using in situ hybridization techniques [147]. The prevalence of HPV-related HNSCC may also have geographical differences. While a world-wide survey of oropharynx cancer found that the prevalence of HPV infection was 18%, another report indicated the prevalence was 38% and notably higher in North America (47%) than in Europe (28%) [148].

The prevalence of types of HNSCC potentially related to HPV infection appears to be increasing in the United States [145]. Especially, in younger-age cohorts, the HPV-related tumors (e.g., base of tongue, tonsil, oropharynx) increased the last years, in contrast to the incidence of unrelated to HPV cancers (e.g., tongue, gum, lip, other oral cancers) which has decreased. The previous findings may be in part the result of changes in both sexual behavior and smoking. Of those head and neck tumors that contain HPV DNA, up to 90% present with the oncogenic variant HPV-16 [9, 149-150]. Compared with tumors of the oral cavity and oropharynx, squamous cell carcinoma of tonsils has been most strongly and consistently associated with HPV-16 infection [150]. In an epidemiological study [156], the prevalence of seropositivity of the oncogenic HPV-16 was nearly twice as high

in HNSCC patients as in controls (12% vs. 7%). HPV-positive oropharyngeal squamous cell carcinoma has distinct risk factors and molecular profiles, as they seem to be related with certain types of sexual habits and not smoking or alcohol use. In a nested case control study involving 130 consecutive patients with oropharynx cancer, the risk of oropharynx cancer was related to sexual behavior [66]. As far as it concerns the molecular profile, HPV-16 has been suggested to immortalize epithelial cells of both cervical and oral origin in vitro [151, 152]. HPV contributes to tumorigenesis through proteins E6 and E7 which inactivates p53 and the retinoblastoma protein pRb. The loss of the pRb negative feedback loop causes increased p16 protein expression [1, 153, 154]. p16 then inhibits cyclinD1-CDK4/6 (cyclin-dependent kinase 4/6) complexes, which regulate the progress from the G1 phase to the S phase of the cell cycle. In that way, HPV-positive tumors are associated with downregulation of cyclin D1 expression [83, 84]. And express wild-type p53 tumor suppressor genes.

p16 over-expression by immunohistochemical assessment may be used as a substitute marker of HPV [144]. HPV may also be detected in saliva promising a screening or monitoring role in oropharyngeal cancer [155]. Positivity of HPV-16 in serum proved to be an independent predictor of oropharynx cancer, after adjusting for age, sex, and alcohol use.

HPV status is not only a risk factor for developing HNSCC, but also define a distinct subtype of HNSCC tumors, promising a more personalized treatment. There are studies which showed HPV-positive tumors have better response and survival after treatment, maybe because these tumors maintain an apoptotic response to radiation and chemotherapy [143]. Alternatively, these patients tend to be younger and healthier compared with other head and neck cancer patients, with fewer smoking related comorbidities; however, it is important to note that in multivariate analyses of clinical trials, the prognostic effect of HPV is not driven by demographics alone [9].

The impact of HPV status on response and survival was evaluated by the Eastern Cooperative Oncology Group (ECOG) in a prospective multi-center phase 2 study of chemoradiation as organ-preserving therapy in 96 patients with resectable stage III/IV laryngeal or oropharynx cancer, using in situ hybridization. HPV DNA was detected in 40% of tumor samples; 63% of oropharyngeal tumors were HPV positive, but none of the laryngeal tumors had HPV DNA. Most positive cases had the subtype HPV-16 (95%). Patients with HPV-positive tumors had a higher response rate after induction chemotherapy and after chemoradiation. Overall survival (OS) was significantly longer in HPV-positive patients than in HPV-negative patients (alive at 2 years: 95% vs. 62%). The lower death and progression risk for HPV-positive patients was still present after adjusting for age, disease stage, and performance status [9].

Recent retrospective studies of large phase 3 trials have solidified the clinical importance of HPV as a prognostic marker for HNSCC. The phase 3 TROG (Trans Tasman Oncology Group) 02.02 (HeadSTART) trial, which randomized patients with stage III/IV HNSCC to receive cisplatin/radiotherapy-tirapazamine. At the 2-year mark, HPV-positive tumors were associated with better OS versus HPV negative tumors as well as improved failure-free survival. In addition, patients with p16-positive tumors had improved 2-year OS and improved 2-year FFS. Patients with both HPV and p16- positive tumors had increased OS and FFS rates compared with double-negative patients [9, 157].

Similarly, analysis from the RTOG 0129 study, randomized patients to receive standard fractionation 70 grays (Gy) + cisplatin or accelerated fractionation with concomitant impulse 72 Gy + cisplatin, correlated HPV status with patient prognosis. OS, PFS, and locoregional failure were all significantly superior among HPV-positive patients at 2 years and locoregional failure. Interestingly, heavy smoking status (> 20 pack-years) seemed to negate partially the benefit conferred by an HPV-positive tumor status; compared with HPV-positive/< 20 pack the year [156].

A retrospective analysis [142] of 646 evaluable samples showed that HPV-positive patients (identified by the surrogate marker p16) had a 5-year survival rate of 49% compared with 19.6% for those who were HPV negative and showed a strong prognostic correlation with HPV status [9, 158].

Together, these studies have definitively established HPV as a meaningful prognostic marker

for oropharyngeal HNSCC, and are sufficient to warrant accounting for HPV status in the design and analysis of future clinical trials using combined or sequential chemotherapy and radiation [9].

As the prevalence of HPV infection and HPV related HNSCC increases [145, 149, 159], questions regarding screening are raised. Fortunately, only basic laboratory techniques required to screen serum samples for HPV-16, reliably [160]. However, the value of screening high-risk patients for HPV infection has not been demonstrated and remains unknown the best way to treat HPV-related premalignant lesions detected by screening. Furthermore, it is not clear yet whether identifying patients at high risk of contracting HPV may improve early detection of HNSCC by raising patient and physician awareness and increasing monitoring. Moreover, the rising incidence of HPV infection raises other issues, such as vaccination strategies. There is a great need for continued retrospective analyses and epidemiological studies in order the treatment strategies of HNSCC patients according to HPV status to be clarified [9].

Epigenetic modifications

Epigenetic modifications, consisting of aberrant DNA methylation, histone modifications and miRNAs, intervening in regulation of gene expression, induce HNSCC tumorigenesis and perhaps play a more central role in the evolution and progression of this disease [161].

Analysis of DNA extracted from oral SCC tissues and oral premalignant lesions (OPLs) found they exhibit more frequent and higher levels of DNA methylation compared with healthy or corresponding normal tissue from neoplastic tissues [162]. Smoking, a major risk factor for the development of OSCC, has been linked to nonspecific global hypomethylation [163, 164]. In contrast to smokers, patients who drink heavily have an increased risk for CpG hypermethylation of multiple oral SCC-related genes. Chronic inflammation of the oral mucosa is another risk factor that can potentially modify the methylation status of various genes in SCC tumors [165]. The occurrence of multiple CpG methylation sites in a panel of tumor-related genes in OSCC was highly associated with cancer stage and may correlate with

lymph node metastasis [166]. To date, most oral cancer-related publications focused on CpG methylation of APC, Survivin, E-cadherin, MGMT, MLH1, p14ARF, p15INK4B, p16INK4A, RARβ and RASSF genes [161, 162].

Post-translational modifications of histones are frequently observed in oral cancer. These epigenetic alterations occur primarily at the N-terminal tails within each of the four histone complexes (H3, H4, H2A and H2B). Various modifications include methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation and sumoylation of specific residues within these histone tails, modifying the tertiary DNA structure. There is a study, where the patterns of histone and DNA methylation were positively correlated in normal, OPL and OSCC tissues [162], and in another study, the pattern of H3K4 histone methylation was associated with OSCC malignancy. Moreover, H3K4me2 histones, transcriptionally inactive, are more frequent in oral SCC compared with normal tissues [167]. Interestingly, a similar pattern of histone methylation was detected in OPLs, such as leukoplakia, resembling more like OSCC tissues than with normal tissues. That's why in many instances, leukoplakia needs to aware great surveillance as a premalignant condition for the development of OSCC and treated accordingly.

Similar to histone methylation during oral carcinogenesis, histone deacetylation, plays its own important role. It has been demonstrated that histone deacetylation, catalyzed by various histone deacetylase 2 (HDAC2) improves the stability of the HIF-1α protein, which may enhance invasion and migration in OSCC [168]. Likewise, expression of HDAC6 was upregulated in oral SCC and was found to be stage-specific; the higher the stage, the greater the activity [169]. In addition to deacetylating histones, HDAC6 was revealed to be capable of deacetylating α-tubulin, thus promoting microtubule-dependent cell motility [170].

Interestingly, Arif et al. discovered that histone H3, primarily H3K14, is hyperacetylated in oral SCC [171]. These investigators discovered that in the KB oral cancer cell line, increased H3 acetylation was nitric oxide-dependent [171].

The poly (ADP-ribose) polymerase (PARP) family of enzymes is responsible for the post-transla-

tional covalent transfer of ADP-ribose to proteins, as well as formation of polymers of poly (ADP-ribose). Actively proliferating OSCC has greater activity of PARP-1, DNA synthesis rates and ADP ribosylation [172], a finding that is ascribed to histones rather than nonhistone chromosomal proteins. The addition of poly (ADP-ribose) to histones loosens the chromatin structure, facilitating DNA repair through large protein complexes such as chromatin assembly factor (CAF)-1, which integrate H3K56-acetylated histones into the chromatin. Worst prognosis in terms of survival and metastasizing behavior may be predicted in PARP-1high and CAF-1/p60high OSCC tissues [173]. Taken together, DNA methylation, acetylation and/or poly (ADP)-ribosylation may all be important factors in the development of OSCC [161].

miRNAs are the most recent entrants into the category of epigenetic gene expression regulators. miRNAs represent small RNA molecules with important regulatory functions, while each miRNA can target multiple mRNAs or gene targets. MiRNAs inhibit protein formation by degradation or repression of translation of the mRNA transcript. MiRNAs are introduced recently in research area, but increased rapidly [161].

Conclusion

There are a growing number of molecular markers that may potentially be used as either prognostic or predictive tools in the treatment of head and neck cancer. The understanding of these biological markers is not as well defined as in other tumor types, such as breast or lung cancer, but recent advances have brought us closer to providing personalized medicine for these patients. Most patients diagnosed with head and neck cancer present with locally advanced disease, for which a combined modality treatment approach with curative intent is usually prescribed. By tailoring treatment to the specific genetic or molecular profile of a tumor in an individual patient, response and survival outcomes could be improved through refined treatment decisions, treatment-related costs, complications reduced, and unnecessary interventions avoided. Such refinements may also facilitate the development of newer, more effective therapies.

To accelerate the validation of available biomarkers and the discovery of new ones, and to address questions emerging from clinical studies, it will be important to associate relevant preclinical models to clinical studies. The development of biomarkers of angiogenesis represents a unique opportunity for clinical oncologists and laboratory scientists to join forces to address relevant questions and design meaningful studies. Despite the great increasing number of biomarkers, further studies will cut the gordian knot and show which biomarkers are the best tools for head and neck cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Evangeli S Lampri, Department of Pathology, Faculty of Medicine, University of Ioannina, Greece. E-mail: evangeli.lampri@gmail.com

References

[1]   Williams MD. Integration of biomarkers including molecular targeted therapies in head and neck cancer. Head Neck Pathol 2010; 4: 62-69.

[2]   Jemal A, Siegel R, Xu J. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.

[3]   Ward BB. Biomarkers for Head and Neck Cancer. SPOMS. Volume 13. 3; 1-48.

[4]   Diaz EM, Sturges EM, Laramore GE, Sabichi AL, Clayman G. Neoplasms of the Head and Neck. In: Kufe DW, Pollock RE, Weichselbaum RR, editors. Cancer medicine, Vol. 2. Hamilton (Ontario, Canada): American Cancer Society BC Dekker Inc.; 2003. pp. 1325-72.

[5]   Crowe DL, Hacia JG, Hsieh CL, Sinha UK, Rice H. Molecular pathology of head and neck cancer. Histol Histopathol 2002; 17: 909-14.

[6]   Ferlay J, Bray F, Pisani P. GLOBOCAN, Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0, Lyon: 2000, IARC Press; 2001.

[7]   Ries LA, Miller, Hankey FB. SEER cancer statistics review, 1973-1991: tables and graphs. Bethesda, Md.: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute; 1994. NIH Publication 94-2789.

[8]   Mashberg A and Samit AM. Early diagnosis of asymptomatic oral and oropharyngeal squamous cancers. CA Cancer J Clin 1995; 45: 328, 1995.

[9]   Langer CJ. Exploring Biomarkers in Head and Neck Cancer. Cancer 2012; 118: 3882-92.

[10]   Chin D, Boyle GM, Williams RM, Ferguson K, Pandeya N, Pedley J, Campbell CM, Theile DR,

Parsons PG, Coman WB. Novel markers for poor prognosis in head and neck cancer. Int J Cancer 2005; 113: 789.

[11] Al-Sarraf M, Chu C, Lai L. Multiple tumor markers in monitoring patients with epidermoid cancer of the head and neck. Proceeding from the american association of cancer research. AACR Abstracts; 1981.

[12] Airoldi M, Piantino P, Pacchioni D, Mastromatteo V, Pedani F, Gandolfo S. Gastrointestinal cancer associated antigen (GICA) in oral carcinoma. Oral Surg Oral Med Oral Pathol 1986; 61: 263.

[13] Schneider M, Demard F, Chauvel P. Carcinoembryonic antigen determinations in head and neck cancer. In: Krebs B, Lalanne C and Schneider M, editors. Clinical Application of Carcinoembryonic Antigen Assay: Proceeding of a Symposium. Amsterdam: Excerpta Medica; 1978. pp. 384.

[14] Kato H and Torigoe T. Radioimmunoassay for tumor-antigen of human cervical squamous cell carcinoma: Correlation of circulating levels with disease progress. Cancer 1977; 43: 585.

[15] In: Dato H, deBruijn H, Ebert W, editors. SCC Antigen in the Management of Squamous Cell Carcinoma. Princeton: Excerpta Medica; 1987. pp. 54-61.

[16] Johnson J, Wagner R, Eibling D. Radioimmunoassay for SCC Antigen In the Diagnosis of Squamous Cell Carcinoma of the Head and Neck: A preliminary Report. In: Kato H, deBruijn H, Ebert W, editors. SCC Antigen in the Management of Squamous Cell Carcinoma. Princeton: Excerpta Medica; 1987. pp. 112-23.

[17] Fischbach W, Meyer T and Barthel K. Squamous cell carcinoma antigen in the diagnosis and treatment follow-up of oral and facial squamous cell carcinoma. Cancer 1990; 65: 1321.

[18] Ebling C, Johnson JT, Wagner RL, Su S. SCC-RIA in the diagnosis of squamous cell carcinoma of the head and neck. Laryngoscope 1989; 99: 117.

[19] Gustafsson H, Franzén L, Grankvist K, Anniko M, Henriksson R. Glycoprotein tumor markers in head and neck neoplasms-A consecutive study on CA-50, CA 19-9, and CEA. J Cancer Res Clin Oncol 1998; 114: 394.

[20] Walther EK, Dahlmann N and Gorgulla HT. Tumor markers in the diagnosis and follow-up of head and neck cancer: role of CEA, CA19-9, SCC, TK, and dTTPase. Head Neck 1993; 15: 230.

[21] Rosin MP, Dunn Bp and Stich HF. Use of intermediate endpoints in quantitating the response of precancerous lesions to chemopreventive agents. Can J Physiol Pharmacol 1987; 65: 483.

[22] Benner SE, Lippman SM, Wargovich MJ, Lee JJ, Velasco M, Martin JW, Toth BB, Hong WK. Micronuclei, a biomarker for chemoprevention trials: results of a randomized study in oral premalignancy. Int J Cancer 1994; 59: 457.

[23] Stich HF, Rosin MP and Hornby AP. Remission of oral leukoplakias and micronuclei in tobacco/betel quid chewers treated with beta-carotene and betacarotene and vitamin A. Int J Cancer 1988; 42: 195.

[24] Osella P, Carlson A, Wyandt H, Milunsky A. Cytogenetic studies of eight squamous cell carcinomas of the head and neck. Cancer Gent Cytogenet 1992; 59: 73.

[25] Carey TE, Van Dyke DL and Worsham MJ. Nonrandom chromosome aberrations and clonal populations in head and neck cancer. Anticancer Res 1993; 13: 2561.

[26] Pinkel D, Straume T and Gray JW. Cytogenetic analysis using quantitative, high sensitivity fluorescence hybridization. Proc Natl Acad Sci U S A 1986; 83: 2934.

[27] Akervall JA, Michalides RJ, Mineta H, Balm A, Borg A, Dictor MR, Jin Y, Loftus B, Mertens F, Wennerberg JP. Amplification of Cyclin D1 in squamous cell carcinoma of the head and neck and the prognostic value of chromosomal abnormalities and cyclin D1 overexpression. Cancer 1997; 79: 380.

[28] Bradford CR, Kimmel KA, VanDyke DL. Chromosome 11p deletions and breakpoints in squamous cell carcinoma: association with altered reactivity with the UM-E7 antibody. Genes Chromosomes Cancer 1991; 3: 272.

[29] Rosen MP, Cheng X, Poh C, Lam WL, Huang Y, Lovas J, Berean K, Epstein JB, Priddy R, Le ND, Zhang L. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. Clin Cancer Res 2000; 6: 357.

[30] Elledge R. Current concepts in research related to oncogenes implicated in salivary gland tumourigenesis: a review of the literature. Oral Dis 2009; 15: 249-54.

[31] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.

[32] The Catalogue of Somatic Mutations in Cancer (COSMIC) database. Available at: http://www.sanger.ac.uk/cosmic Accessed August 18, 2009.

[33] Sudbo J, Kildal W, Risberg B. DNA content as a prognostic marker in patients with oral leukoplakia. New Eng J Med 2001; 344: 1270.

[34] Sudbo J, Lippman SM, Lee JJ. The influence of resection and aneuploidy on mortality in oral leukoplakia. New Eng J Med 2004; 350: 1405.

[35] Mao L, El-Naggar AK, Papadimitrakopoulou V, Shin DM, Shin HC, Fan Y, Zhou X, Clayman G, Lee JJ, Lee JS, Hittelman WN, Lippman SM,

Hong WK. Phenotype and genotype of advanced premalignant head and neck lesions after chemopreventive therapy. J Natl Cancer Inst 1998; 90: 1545.

[36] Mao, L, Lee D and Tockman M. Microsatellite alterations as clonal markers in the detection of human cancer. Proc Natl Acad Sci U S A 1994; 91: 9871.

[37] Nawroz H, Koch W, Anker P, Stroun M, Sidransky D. Microsatellite alterations in serum DNA of head and neck cancer patients. Nature Med 1996; 2: 1035.

[38] Mao L, Lee JS, Fan YH, Ro JY, Batsakis JG, Lippman S, Hittelman W, Hong WK. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. Nat Med 1996; 2: 682-5.

[39] Ha PK, Pilkington TA, Westra WH, Sciubba J, Sidransky D, Califano JA. Progression of microsatellite instability from premalignant lesions to tumors of the head and neck. Int J Cancer 2002; 102: 615.

[40] Temam S, Casiraghi O, Lahaye JB, Bosq J, Zhou X, Julieron M, Mamelle G, Lee JJ, Mao L, Luboinski B, Benard J, Janot F. Tetranucleotide microsatellite instability in surgical margins for prediction of local recurrence of head and neck squamous cell carcinoma. Clin Ca Res 2004; 10: 4022.

[41] Somers KD, Merrick MA, Lopez ME. Frequent p53 mutations increases with progression of head and neck cancer. Cancer Res 1993; 53: 4477.

[42] Alsner J, Sorensen SB and Overgaard J. TP53 mutation is related to poor prognosis after radiotherapy, but not surgery, in squamous cell carcinoma of the head and neck. Radiother Oncol 2001; 59: 179.

[43] Carlos de Vicente, J, Gutierrez LMJ, Zapatera AH, Fresno Forcelledo MF, Hernández-Vallejo G, López Arranz JS. Prognostic significance of p53 expression in oral squamous cell carcinoma without neck noted metastases. Head Neck 2004; 26: 22.

[44] Yamazaki Y, Chiba I, Hirai A. Specific p53 mutation predict poor prognosis in oral squamous cell carcinoma. Oral Oncology 2002; 39: 163.

[45] Casado S, Forteza J, Dominguez S, Abad MT, Perez I, Intxaurbe I, del Campo JM, Lopez R. Predictive value of P53, BCL-2, and BAX in advanced head and neck carcinoma. Am J Clin Oncol 2002; 25: 288.

[46] Ioachim E, Peschos D, Goussia A, Mittari E, Charalabopoulos K, Michael m, Salmas M, Vougiouklakis T, Assimakopoulos D, Agnantis NJ. Expression patterns of cyclins D1, E in laryngeal epithelial lesions: correlation with other cell cycle regulators (p53, pRb, Ki-67 and PCNA) and clinicopathological features. J Exp Clin Res 2004; 23: 277-83.

[47] Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, Ridge JA, Goodwin J, Kenady D, Saunders J, Westra W, Sidransky D, Koch WM. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N Engl J Med 2007; 357: 2552-61.

[48] Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, Fakhry C, Xie TX, Zhang J, Wang J, Zhang N, El-Naggar AK, Jasser SA, Weinstein JN, Treviño L, Drummond JA, Muzny DM, Wu Y, Wood LD, Hruban RH, Westra WH, Koch WM, Califano JA, Gibbs RA, Sidransky D, Vogelstein B, Velculescu VE, Papadopoulos N, Wheeler DA, Kinzler KW, Myers JN. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science 2011; 333: 1154.

[49] Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, Kryukov GV, Lawrence MS, Sougnez C, McKenna A, Shefler E, Ramos AH, Stojanov P, Carter SL, Voet D, Cortés ML, Auclair D, Berger MF, Saksena G, Guiducci C, Onofrio RC, Parkin M, Romkes M, Weissfeld JL, Seethala RR, Wang L, Rangel-Escareño C, Fernandez-Lopez JC, Hidalgo-Miranda A, Melendez-Zajgla J, Winckler W, Ardlie K, Gabriel SB, Meyerson M, Lander ES, Getz G, Golub TR, Garraway LA, Grandis JR. The mutational landscape of head and neck squamous cell carcinoma. Science 2011; 333: 1157.

[50] Tan M, Myers JN, Agrawal N. Oral cavity and oropharyngeal squamous cell carcinoma genomics. Otolaryngol Clin North Am 2013; 46: 545-566.

[51] Reed AL, Califano J, Cairns P, Westra WH, Jones RM, Koch W, Ahrendt S, Eby Y, Sewell D, Nawroz H, Bartek J, Sidransky D. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. Cancer Res 1996; 56: 3630.

[52] Ohta S, Uemura H, Matsui Y, Ishiguro H, Fujinami K, Kondo K, Miyamoto H, Yazawa T, Danenberg K, Danenberg PV, Tohnai I, Kubota Y. Alterations of p16 and p14ARF genes and their 9p21 locus in oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 107: 81.

[53] Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson E, Burger PC, Baylin SB, Sidransky D. 5' CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. Nat Med 1995; 1: 686.

[54] Chen PL, Scully P, Shew JY, Wang JY, Lee WH. Phosphorylation of the retinoblastoma gene product is modulated during the cell cycle and cellular differentiation. Cell 1989; 58: 1193.

[55] Lukas J, Parry D, Aagaard L, Mann DJ, Bartkova J, Strauss M, Peters G, Bartek J. Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. Nature 1995; 375: 503.

[56] Mihara K, Cao XR, Yen A, Chandler S, Driscoll B, Murphree AL, T'Ang A, Fung YK. Cell cycle-dependent regulation of phosphorylation of the human retinoblastoma gene product. Science 1989; 246: 1300.

[57] Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, Corio R, Lee D, Greenberg B, Koch W, Sidransky D. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res 1996; 56: 2488.

[58] Sobin LH, Wittekind CH. TNM Classification of Malignant Tumors. 6th edition. New York, NY: Wiley-Liss; 2002.

[59] Mohr C, Molls M, Streffer C, Pelzer T. Prospective flow cytometric analysis of head and neck carcinomas. Prognostic relevance of DNA content and S-fraction. J Craniomaxillofac Surg 1992; 20: 8-13.

[60] Corvò R, Giaretti W, Sanguineti G, Geido E, Orecchia R, Barra S, Margarino G, Bacigalupo A, Vitale V. Potential doubling time in head and neck tumors treated by primary radiotherapy: preliminary evidence for a prognostic significance in local control. Int J Radiat Oncol Biol Phys 1993; 27: 1165-1172.

[61] Cooke LD, Cooke TG, Forster G, Jones AS, Stell PM. Prospective evaluation of cell kinetics in head and neck squamous carcinoma: the relationship to tumour factors and survival. Br J Cancer 1994; 69: 717-720.

[62] Roland NJ, Caslin AW, Bowie GL, Jones AS. Has the cellular proliferation marker Ki67 any clinical relevance in squamous cell carcinoma of the head and neck? Clin Otolaryngol 1994; 19: 13-18.

[63] Pich A, Chiusa L, Navone R. Prognostic relevance of cell proliferation in head and neck tumors. Ann Oncol 2004; 15: 1319-1329.

[64] Sittel C, Ruiz S, Volling P, Kvasnicka HM, Jungehülsing M, Eckel HE. Prognostic significance of Ki-67 (MIB 1), PCNA and p53 in cancer of the oropharynx and oral cavity. Oral Oncol 1999; 35: 583-589.

[65] Piffkò J, Bànkfalvi A, Ofner D, Rasch D, Joos U, Schmid KW. Standardized AgNOR analysis of the invasive tumour front in oral squamous cell carcinomas. J Pathol 1997; 182: 450-456.

[66] Bryne M, Koppang HS, Lilleng R, Kjaerheim A. Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. J Pathol 1992; 166: 375-381.

[67] Fracchiolla NS, Pignataro L, Capaccio P, Trecca D, Boletini A, Ottaviani A, Polli E, Maiolo AT, Neri A. Multiple genetic lesions in laryngeal squamous cell carcinomas. Cancer 1995; 75: 1292.

[68] Bartkova J, Lukas J, Müller H, Strauss M, Gusterson B, Bartek J. Abnormal patterns of D-type cyclin expression and G1 regulation in human head and neck cancer. Cancer Res 1995; 55: 949.

[69] Akervall JA, Michalides RJ, Mineta H, Balm A, Borg A, Dictor MR, Jin Y, Loftus B, Mertens F, Wennerberg JP. Amplification of cyclin D1 in squamous cell carcinoma of the head and neck and the prognostic value of chromosomal abnormalities and cyclin D1 overexpression. Cancer 1997; 79: 380.

[70] Bova RJ, Quinn DI, Naankervis JS, Cole IE, Sheridan BF, Jensen MJ, Morgan GJ, Hughes CJ, Sutherland RL. Cyclin D1 and p16INK4A expression predict reduced survival in carcinoma of the anterior tongue. Clin Cancer Res 1999; 5: 2810.

[71] Masuda M, Hirakawa N, Nakashima T, Kuratomi Y, Komiyama S. Cyclin D1 overexpression in primary hypopharyngeal carcinomas. Cancer 1996; 78: 390-5.

[72] Mineta H, Miura K, Takebayashi S, Ueda Y, Misawa K, Harada H, Wennerberg J, Dictor M. Cyclin D1 overexpression correlates with poor prognosis in patients with tongue squamous cell carcinoma. Oral Oncol 2000; 36: 194-8.

[73] Jares P, Fernández PL, Campo E, Nadal A, Bosch F, Aiza G, Nayach I, Traserra J, Cardesa A. PRAD-1/cyclin D1 gene amplification correlates with messenger RNA overexpression and tumor progression in human laryngeal carcinomas. Cancer Res 1994; 54: 4813-7.

[74] Perisanidis C, Perisanidis B, Wrba F, Brandstetter A, El Gazzar S, Papadogeorgakis N, Seemann R, Ewers R, Kyzas PA, Filipits M. Evaluation of immunohistochemical expression of p53, p21, p27, cyclin D1, and Ki67 in oral and oropharyngeal squamous cell carcinoma. J Oral Pathol Med 2012; 41: 40-6.

[75] Smith BD, Smith GL, Carter D, DiGiovanna MP, Kasowitz KM, Sasaki CT, Haffty BG. Molecular marker expression in oral and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2001; 127: 780-5.

[76] Michalides RJ, van Veelen NM, Kristel PM, Hart AA, Loftus BM, Hilgers FJ, Balm AJ. Overexpression of cyclin d1 indicates a poor prognosis in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 1997; 123: 497-502.

[77] Rasamny JJ, Allak A, Krook KA, Jo VY, Policarpio-Nicolas ML, Sumner HM, Moskaluk CA, Frierson HF Jr, Jameson MJ. Cyclin D1 and FADD as Biomarkers in Head and Neck Squamous Cell Carcinoma. Otolaryngology. Head Neck Surg 2012; 146: 923-31.

[78] Hong AM, Dobbins TA, Lee CS, Jones D, Fei J, Clark JR, Armstrong BK, Harnett GB, Milross CG, Tran N, Peculis LD, Ng C, Milne AG, Loo C, Hughes LJ, Forstner DF, O'Brien CJ, Rose BR. Use of cyclin D1 in conjunction with human papillomavirus status to predict outcome in oropharyngeal cancer. Int J Cancer 2011; 128: 1532-45.

[79] Yu Z, Weinberger PM, Haffty BG, Sasaki C, Zerillo C, Joe J, Kowalski D, Dziura J, Camp RL, Rimm DL, Psyrri A. Cyclin D1 is a valuable prognostic marker in oropharyngeal squamous cell carcinoma. Clin Cancer Res 2005; 11: 1160-6.

[80] Capaccio P, Pruneri G, Carboni N, Pagliari AV, Quatela M, Cesana BM, Pignataro L. Cyclin D1 expression is predictive of occult metastases in head and neck cancer patients with clinically negative cervical lymph nodes. Head Neck 2000; 22: 234-40.

[81] Baldin V, Lukas J, Marcote MJ, Pagano M, Draetta G. Cyclin D1 is a nuclear protein required for cell cycle progression in G1. Genes Dev 1993; 7: 812-21.

[82] Li Y, Nichols MA, Shay JW, Xiong Y. Transcriptional repression of the D-type cyclin-dependent kinase inhibitor p16 by the retinoblastoma susceptibility gene product pRb. Cancer Res 1994; 54: 6078-82.

[83] Andl T, Kahn T, Pfuhl A, Nicola T, Erber R, Conradt C, Klein W, Helbig M, Dietz A, Weidauer H, Bosch FX. Etiological involvement of oncogenic human papillomavirus in tonsillar squamous cell carcinomas lacking retinoblastoma cell cycle control. Cancer Res 1998; 58: 5-13.

[84] Li W, Thompson CH, Cossart YE, O'Brien CJ, McNeil EB, Scolyer RA, Rose BR. The expression of key cell cycle markers and presence of human papillomavirus in squamous cell carcinoma of the tonsil. Head Neck 2004; 26: 1-9.

[85] Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science 2002; 298: 1912-34.

[86] Lazo PA, Vega FM, Sevilla A. Vaccinia-related kinase-1. Mol Cell Biol 2004; 24: 10366-80.

[87] Santos CR, Rodríguez-Pinilla M, Vega FM, Rodríguez-Peralto JL, Blanco S, Sevilla A, Valbuena A, Hernández T, van Wijnen AJ, Li F, de Alava E, Sánchez-Céspedes M, Lazo PA. VRK1 signaling pathway in the context of the proliferation phenotype in head and neck squamous cell carcinoma. Mol Cancer Res 2006; 4: 177-185.

[88] Lopez-Borges S, Lazo PA. The human vaccinia-related kinase 1 (VRK1) phosphorylates threonine-18 within the mdm-2 binding site of the p53 tumour suppressor protein. Oncogene 2000; 19: 3656-64.

[89] Sevilla A, Santos CR, Barcia R, Vega FM, Lazo PA. c-Jun phosphorylation by the human vaccinia-related kinase 1 (VRK1) and its cooperation with the Nterminal kinase of c-Jun (JNK). Oncogene 2004; 23: 8950-8.

[90] Sevilla A, Santos CR, Vega FM, Lazo PA. Human vaccinia-related kinase 1 (VRK1) activates the ATF2 transcriptional activity by novel phosphorylation on Thr-73 and Ser-62 and cooperates with JNK. J Biol Chem 2004; 279: 27458-65.

[91] Wahl GM, Carr AM. The evolution of diverse biological responses to DNA damage: insights from yeast and p53. Nat Cell Biol 2001; 3: E277-86.

[92] van Dam H, Wilhelm D, Herr I, Steffen A, Herrlich P, Angel P. ATF-2 is preferentially activated by stress-activated protein kinases to mediate c-jun induction in response to genotoxic agents. EMBO J 1995; 14: 1798-811.

[93] Sugden PH, Clerk A. Cell stresses regulate the phosphorylation state of c-Jun and ATF2 in ventricular myocytes. Biochem Soc Trans 1997; 25: 221S.

[94] Barcia R, Lopez-Borges S, Vega FM, Lazo PA. Kinetic properties of p53 phosphorylation by the human vaccinia-related kinase 1. Arch Biochem Biophys 2002; 399: 1-5.

[95] Vega FM, Sevilla A, Lazo PA. p53 stabilization and accumulation induced by human vaccinia-related kinase 1. Mol Cell Biol 2004; 24: 10366-80.

[96] Maurizi M, Almadori G, Ferradina G, Distefano M, Romanini ME, Cadoni G, Benedetti-Panici P, Paludetti G, Scambia G, Mancuso S. Prognostic significance of epidermal growth factor receptor in laryngeal squamous cell carcinoma. Br J Cancer 1996; 74: 1253-1257.

[97] Rubin Grandis J, Tweardy DJ. Elevated levels of transforming growth factor a and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res 1993; 53: 3579-3584.

[98] Dassonville O, Formento JL, Francoual M, Ramaioli A, Santini J, Schneider M, Demard F, Milano G. Expression of epidermal growth factor receptor and survival in upper aerodigestive tract cancer. J Clin Oncol 1993; 11: 1873-1878.

[99] Grandis JR and Twardy DJ. Elevated levels of transforming growth factoralpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res 1993; 53: 3579.

[100] Atkins D, Reiffen KA, Tegtmeier CL, Winther H, Bonato MS, Störkel S. Immunohistochemical detection of EGFR in paraffin-embedded tumor tissues: variation in staining intensity due to choice of fixative and storage time of tissue sections. J Histochem Cytochem 2004; 52: 893-901.

[101] Vermorken JB, Mesia R, Remenar E. Predictors of efficacy in the EXTREME study: cetuximab plus platinum-based therapy firstline in patients with recurrent and/or metastatic (R/M) squamous cell carcinoma of the head and neck (SCCHN) [abstract]. Ann Oncol 2012; 39: 2489-507.

[102] Psyrri A, Yu Z, Weinberger PM, Sasaki C, Haffty B, Camp R, Rimm D, Burtness BA. Quantitative determination of nuclear and cytoplasmic epidermal growth factor receptor expression in oropharyngeal squamous cell cancer by using automated quantitative analysis. Clin Cancer Res 2005; 11: 5856-5862.

[103] Ang KK, Berkey BA, Tu X, Zhang HZ, Katz R, Hammond EH, Fu KK, Milas L. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res 2002; 62: 7350-7356.

[104] Kim S, Grandis JR, Rinaldo A, Takes RP, Ferlito A. Emerging perspectives in epidermal growth factor receptor targeting in head and neck cancer. Head Neck 2008; 30: 667-74.

[105] Grandis JR, Melhem MF, Gooding WE, Day R, Holst VA, Wagener MM, Drenning SD, Tweardy DJ. Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. J Natl Cancer Inst 1998; 90: 824-32.

[106] Burtness B, Goldwasser MA, Flood W, Mattar B, Forastiere AA; Eastern Cooperative Oncology Group. Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: an Eastern Cooperative Oncology Group study. J Clin Oncol 2005; 24: 8646-8654.

[107] Sok JC, Coppelli FM, Thomas SM, Lango MN, Xi S, Hunt JL, Freilino ML, Graner MW, Wikstrand CJ, Bigner DD, Gooding WE, Furnari FB, Grandis JR. Mutant epidermal growth factor receptor (EGFRvIII) contributes to head and neck cancer growth and resistance to EGFR targeting. Clin Cancer Res 2006; 12: 5064-5073.

[108] Loeffler-Ragg J, Witsch-Baumgartner M, Tzankov A, Hilbe W, Schwentner I, Sprinzl GM, Utermann G, Zwierzina H. Low incidence of mutations in EGFR kinase domain in Caucasian patients with head and neck squamous cell carcinoma. Eur J Cancer 2006; 42: 109-111.

[109] Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, Ince WL, Jänne PA, Januario T, Johnson DH, Klein P, Miller VA, Ostland MA, Ramies DA, Sebisanovic D, Stinson JA, Zhang YR, Seshagiri S, Hillan KJ. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol 2005; 23: 5900-5909.

[110] Lee JW, Soung YH, Kim SY, Nam HK, Park WS, Nam SW, Kim MS, Sun DI, Lee YS, Jang JJ, Lee JY, Yoo NJ, Lee SH. Somatic mutations of EGFR gene in squamous cell carcinoma of the head and neck. Clin Cancer Res 2005; 11: 2879-2882.

[111] Field JK. Genomic instability in squamous cell carcinoma of the head and neck. Anticancer Res 1996; 16: 2421.

[112] Williams HK. Molecular pathogenesis of oral squamous carcinoma. Mol Pathol 2000; 53: 165-172.

[113] Rathcke IO, Gottschlich S, Görögh T, Lippert BM, Werner JA. Incidence of point mutations in Ki-ras codon 12 and 13 in squamous epithelial carcinomas of the head-neck region. Laryngorhinootologie 1996; 75: 465-470.

[114] Yarbrough WG, Shores C, Witsell DL, Weissler MC, Fidler ME, Gilmer TM. Ras mutations and expression in head and neck squamous cell carcinomas. Laryngoscope 1994; 104: 1337-1347.

[115] Bissada E, Abou-Chacra Z, Weng X, Nguyen-Tan PF, Tabet JC, Thibaudeau E, Lambert L, Audet ML, Fortin B, Soulières D. Prevalence of K-RAS codon 12 mutations in locally advanced head and neck squamous cell carcinoma. Int J Otolaryngol 2003; 2013: 848021.

[116] Hoa M, Davis SL, Ames SJ, Spanjaard RA. Amplification of wild-type K-RAS promotes growth of head and neck squamous cell carcinoma. Cancer Res 2002; 62: 7154-7156.

[117] Nakayama H, Ikebe T, Beppu M, Shirasuna K. High expression levels of nuclear factor kB, IkB kinase alpha and Akt kinase in squamous cell carcinoma of the oral cavity. Cancer 2011; 95: 3037.

[118] Reiter R, Gais P, Jutting U, Steuer-Vogt MK, Pickhard A, Bink K, Rauser S, Lassmann S, Höfler H, Werner M, Walch A. Aurora kinase A messenger RNA overexpression is correlated with tumor progression and shortened survival in head and neck squamous cell carcinoma. Clin Cancer Res 2006; 12: 5136-41.

[119] Kwon HC, Roh MS, Oh SY, Kim SH, Kim MC, Kim JS, Kim HJ. Prognostic value of expression of ERCC1, thymidylate synthase, and glutathione S-transferase P1 for 5-fluoruracil/oxaliplatin chemotherapy in advanced gastric cancer. Ann Oncol 2007; 18: 504-509.

[120] Johnson SW, Perez RP, Godwin AK, Yeung AT, Handel LM, Ozols RF, Hamilton TC. Role of platinum-DNA adduct formation and removal in cisplatin resistance in human ovarian cancer cell lines. Biochem Pharmacol 1994; 47: 689-697.

[121] Johnson SW, Swiggard PA, Handel LM, Brennan JM, Godwin AK, Ozols RF, Hamilton TC. Relationship between platinum-DNA adduct formation and removal and cisplatin cytotoxicity in cisplatin-sensitive and -resistant human ovarian cancer cells. Cancer Res 1994; 54: 5911-5916.

[122] Reed E. Platinum-DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. Cancer Treat Rev 1998; 24: 331-344.

[123] Reardon JT, Vaisman A, Chaney SG, Sancar A. Efficient nucleotide excision repair of cisplatin, oxaliplatin, and bis-aceto-ammine-dichloro-cyclohexylamineplatinum (IV) (JM216) platinum intrastrand DNA diadducts. Cancer Res 1999; 59: 3968-3971.

[124] Zhen W, Link CJ Jr, O'Connor PM, Reed E, Parker R, Howell SB, Bohr VA. Increased gene-specific repair of cisplatin interstrand cross-links in cisplatin-resistant human ovarian cancer cell lines. Mol Cell Biol 1992; 12: 3689-3698.

[125] Bramson J, Panasci L. Effect of ERCC-1 overexpression on sensitivity of Chinese hamster ovary cells to DNA damaging agents. Cancer Res 1993; 53: 3227-3240.

[126] Jiang H, Yang LY. Cell cycle checkpoint abrogator UCN-01 inhibits DNA repair: association with attenuation of the interaction of XPA and ERCC1 nucleotide excision repair protein. Cancer Res 1999; 59: 4529-4534.

[127] Selvakumaran M, Pisarcik DA, Bao R, Yeung AT, Hamilton TC. Enhanced cisplatin cytotoxicity by disturbing the nucleotide excision repair pathway in ovarian cancer cell lines. Cancer Res 2003; 63: 1311-1316.

[128] Chang IY, Kim MH, Kim HB, Lee DY, Kim SH, Kim HY, You HJ. Small interfering RNA induced suppression of ERCC1 enhances sensitivity of human cancer cells to cisplatin. Biochem Biophys Res Comm 2005; 327: 225-233.

[129] Handra-Luca A, Hernandez J, Mountzios G. Excision repair cross complementation group1 immunohistochemical expression predicts objective response and cancer-specific survival in patients treated with cisplatin-based induction chemotherapy for locally advanced head and neck squamous cell carcinoma. Clin Cancer Res 2007; 13: 3855-3859.

[130] Jun HJ, Ahn MJ, Kim HS, Yi SY, Han J, Lee SK, Ahn YC, Jeong HS, Son YI, Baek JH, Park K. ERCC1 expression as a predictive marker of squamous cell carcinoma of the head and neck treated with cisplatin-based concurrent chemoradiation. Br J Cancer 2008; 99: 167-172.

[131] Metzger R, Leichman CG, Danenberg KD, Danenberg PV, Lenz HJ, Hayashi K, Groshen S, Salonga D, Cohen H, Laine L, Crookes P, Silberman H, Baranda J, Konda B, Leichman L. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. J Clin Oncol 1998; 16: 309-316.

[132] Shirota Y, Stoehlmacher J, Brabender J, Xiong YP, Uetake H, Danenberg KD, Groshen S, Tsao-Wei DD, Danenberg PV, Lenz HJ. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. J Clin Oncol 2001; 19: 4298-4304.

[133] Moore-Joshi MB, Shirota Y, Danenberg KD, Conlon DH, Salonga DS, Herndon JE 2nd, Danenberg PV, Harpole DH Jr. High gene expression of TS1, GSTP1, and ERCC1 are risk factors for survival in patients treated with trimodality therapy for esophageal cancer. Clin Cancer Res 2005; 11: 2215-2221.

[134] Lord RV, Brabender J, Gandara D, Alberola V, Camps C, Domine M, Cardenal F, Sánchez JM, Gumerlock PH, Tarón M, Sánchez JJ, Danenberg KD, Danenberg PV, Rosell R. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in no small cell lung cancer. Clin Cancer Res 2002; 8: 2286-2291.

[135] Vaasa A, Feldman CH, Niedernhofer NJ. ERCC1 and XRCC1 as biomarkers for lung and head and neck cancer. Pharmacogenomics Pers Med 2011; 4: 47-63.

[136] Nix P, Greenman J, Stafford N, Cawkwell L. Expression of XRCC1 and ERCC1 proteins in radioresistant and radiosensitive laryngeal cancer. Cancer Therapy 2004; 47-53.

[137] Howell GM, Grandis JR. Molecular mediators of metastasis in head and neck squamous cell carcinoma. Head Neck 2005; 27: 710-7.

[138] Mandal M, Myers JN, Lippman SM, Johnson FM, Williams MD, Rayala S. Epithelial to mesenchymal transition in head and neck squamous carcinoma: association of Src activation with Ecadherin down-regulation, vimentin expression, and aggressive tumor features. Cancer 2008; 112: 2088-100.

[139] Kavallaris M, Kuo DY, Burkhart CA, Regl DL, Norris MD, Haber M, Horwitz SB. Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific beta-tubulin isotypes. J Clin Invest 1997; 100: 1282-1293.

[140] Ranganathan S, Dexter DW, Benetatos CA, Hudes GR. Cloning and sequencing of human betaIII-tubulin cDNA: induction of betaIII isotype in human prostate carcinoma cells by acute exposure to antimicrotubule agents. Biochim Biophys Acta 1998; 1395: 237-245.

[141] Cullen KJ, Schumaker L, Nikitakis N, Goloubeva O, Tan M, Sarlis NJ, Haddad RI, Posner MR. Beta-tubulin-II expression strongly predicts outcome in patients receiving induction chemotherapy for locally advanced squamous carcinoma of the head and neck: a companion analysis of the TAX 324 trial. J Clin Oncol 2009; 27: 6222-6228.

[142] Ioachim E, Assimakopoulos D, Goussia AC, Skevas A, Agnantis NJ. Glycoprotein CD44 expression in benign, premalignant and malignant epithelial lesions of the larynx: an immunohistochemical study including correlation with Rb, p53, Ki-67 and PCNA. Histol Histopathol 1999; 14: 1113-8.

[143] Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000; 92: 709-20.

[144] Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, Brandsma J, Sasaki C, Joe J, Camp RL, Rimm DL, Psyrri A. Molecular classification identifies a subset of human papillomavirus-associated oropharyngeal cancers with favorable prognosis. J Clin Oncol 2006; 24: 736-47.

[145] Chaturvedi A, Engels E, Anderson W. Incidence trends for human papillomavirus-related (HPV-R) and unrelated (HPV-U) head and neck squamous cell carcinomas (HNSCC) in the United States (US) [abstract]. J Clin Oncol 2007; 25 suppl: Abstract 6001.

[146] Ryerson AB, Peters ES, Coughlin SS, Chen VW, Gillison ML, Reichman ME, Wu X, Chaturvedi AK, Kawaoka K. Burden of potentially human papillomavirus-associated cancers of the oropharynx and oral cavity in the US, 1998-2003. Cancer 2008; 113 Suppl 10: 2901-2909.

[147] Ang KK, Harris J, Wheeler R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010; 363: 24-35.

[148] Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P, Rajkumar T, Sridhar H, Rose B, Pintos J, Fernández L, Idris A, Sánchez MJ, Nieto A, Talamini R, Tavani A, Bosch FX, Reidel U, Snijders PJ, Meijer CJ, Viscidi R, Muñoz N, Franceschi S; IARC Multicenter Oral Cancer Study Group. Human papillomavirus and oral cancer: the International Agency for Research on Cancer Multicenter Study. J Natl Cancer Inst 2003; 95: 1772-1783.

[149] Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML. Improved survival of patient with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 2008; 1000: 261-269.

[150] Syrjanen S. Human papillomavirus (HPV) in head and neck cancer. J Clin Virol 2005; 32: S59-S66.

[151] Pecoraro G, Morgan D, Defendi V. Differential effects of human papillomavirus type 6, 16, and 18 DNAs on immortalization and transformation of human cervical epithelial cells. Proc Natl Acad Sci U S A 1989; 86: 563-567.

[152] Park NH, Min BM, Li SL, Huang MZ, Cherick HM, Doniger J. Immortalization of normal human oral keratinocytes with type 16 human papillomavirus. Carcinogenesis 1991; 12: 1627-1631.

[153] Andl T, Kahn T, Pfuhl A, Nicola T, Erber R, Conradt C, Klein W, Helbig M, Dietz A, Weidauer H, Bosch FX. Etiological involvement of oncogenic human papillomavirus in tonsillar squamous cell carcinomas lacking retinoblastoma cell cycle control. Cancer Res 1998; 58: 5-13.

[154] Adelstein DJ, Ridge JA, Gillison ML. Head and Neck Squamous Cell Cancer and the Human Papillomavirus: Summary of a National Cancer Institute State of the Science Meeting, November 9-10, 2008. 31st edition. Washington, D.C: Head & Neck; 2009. pp. 1393-422.

[155] Chai RL, Grandis JR. Advances in molecular diagnostics and therapeutics in head and neck cancer. Curr Treat Options Oncol 2006; 7: 3-11.

[156] Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, Møller B, Pukkala E, Schiller JT, Youngman L, Lehtinen M, Dillner J. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med 2001; 344: 1125-1131.

[157] Rischin D, Young R, Fisher R, Fox SB, Le QT, Peters LJ, Solomon B, Choi J, O'Sullivan B, Kenny LM, McArthur GA. Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. J Clin Oncol 2010; 28: 4142-4148.

[158] Lorch J, Posner M, Goloubeva O. Five-year survival (OS) and patterns of failure for human papillomavirus (HPV) positive and negative oropharynx cancer (OPC) in the TAX 324 clinical trial: results of sequential therapy [abstract]. Ann Oncol 2010; 21 Suppl 8: Abstract 10040.

[159] Center for Disease Control and Prevention. Human papillomavirus: HPV information for clinicians. Available at: http://www.cdc.gov/std/hpv/common-clinicians/ClinicianBro-fp.pdf Accessed August 2007; 18, 2009.

[160] Kirnbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT. A virus-like particle

enzyme linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. J Natl Cancer Inst 1994; 86: 494-499.

[161] Gasche JA and Goel A. Epigenetic mechanisms in oral carcinogenesis. Future Oncol 2012; 8: 1407-1425.

[162] Piyathilake CJ, Bell WC, Jones J, Henao OL, Heimburger DC, Niveleau A, Grizzle WE. Pattern of nonspecific (or global) DNA methylation in oral carcinogenesis. Head Neck 2005; 27: 1061-1067.

[163] Guerrero-Preston R, Baez A, Blanco A, Berdasco M, Fraga M, Esteller M. Global DNA methylation: a common early event in oral cancer cases with exposure to environmental carcinogens or viral agents. P R Health Sci J 2009; 28: 24-29.

[164] Baba S, Yamada Y, Hatano Y, Miyazaki Y, Mori H, Shibata T, Hara A. Global DNA hypomethylation suppresses squamous carcinogenesis in the tongue and esophagus. Cancer Sci 2009; 100: 1186-1191.

[165] Gasche JA, Hoffmann J, Boland CR, Goel A. Interleukin-6 promotes tumorigenesis by altering DNA methylation in oral cancer cells. Int J Cancer 2011; 129: 1053-1063.

[166] Supic G, Kozomara R, Jovic N, Zeljic K, Magic Z. Prognostic significance of tumor-related genes hypermethylation detected in cancer-free surgical margins of oral squamous cell carcinomas. Oral Oncol 2011; 47: 702-708.

[167] Mancuso M, Matassa DS, Conte M, Colella G, Rana G, Fucci L, Piscopo M. H3K4 histone methylation in oral squamous cell carcinoma. Acta Biochim Pol 2009; 56: 405-410.

[168] Chang CC, Lin BR, Chen ST, Hsieh TH, Li YJ, Kuo MY. HDAC2 promotes cell migration/invasion abilities through HIF-1α stabilization in human oral squamous cell carcinoma. J Oral Pathol Med 2011; 40: 567-575.

[169] Sakuma T, Uzawa K, Onda T, Shiiba M, Yokoe H, Shibahara T, Tanzawa H. Aberrant expression of histone deacetylase 6 in oral squamous cell carcinoma. Int J Oncol 2006; 29: 117-124.

[170] Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF, Yao TP. HDAC6 is a microtubule-associated deacetylase. Nature 2002; 417: 455-458

[171] Arif M, Vedamurthy BM, Choudhari R, Ostwal YB, Mantelingu K, Kodaganur GS, Kundu TK. Nitric oxide-mediated histone hyperacetylation in oral cancer: target for a water-soluble HAT inhibitor, CTK7A. Chem Biol 2010; 17: 903-913.

[172] Das BR. Increased ADP-ribosylation of histones in oral cancer. Cancer Lett 1993; 73: 29-34.

[173] Mascolo M, Ilardi G, Romano MF, Celetti A, Siano M, Romano S, Luise C, Merolla F, Rocco A, Vecchione ML, De Rosa G, Staibano S. Overexpression of chromatin assembly factor-1 p60, poly (ADP-ribose) polymerase 1 and nestin predicts metastasizing behaviour of oral cancer. Histopathology 2012; 61: 1089-105.