Biomarkers in Head and Neck Cancer

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Abstract

Biomarker research provides the opportunity to risk stratify patients based on identified prognostic and predictive markers. The need for such biomarkers is evident to improve response and survival outcomes in head and neck cancer through more rational patient selection for intensive curative regimens as well as palliative treatments. Advances in our understanding of genomics, epigenetics, and immunology of head and neck cancer are accelerating the discovery of new biomarkers. With the increasing availability of molecularly targeted therapeutics, it is very important to identify and validate biomarkers in the appropriate clinical setting to translate the advances into improved clinical outcome. This chapter focuses on human papillomavirus (HPV) status as a validated prognostic biomarker and discusses emerging prognostic and/or predictive biomarkers with potential for testing through prospective clinical trials. The availability of validated diagnostic assays and required multi-institutional trials for selected patients presents logistical challenges in biomarker research for head and neck cancer.

Keywords

Molecular biomarkers • Head and neck cancer • Therapeutic targets • Predictive and prognostic markers

7.1 Introduction

Head and neck cancer is a heterogeneous disease which includes cancers arising from the paranasal sinuses, nasal cavity, oral cavity, pharynx, larynx, salivary glands, and thyroid. Head and neck squamous cell carcinoma (HNSCC) refers to a major subset of head and neck cancer that arise in the mucosal epithelium of the oral cavity, pharynx, and larynx. The management of patients with HNSCC has changed dramatically over the past 30 years from a surgically dominated specialty to a multidisciplinary decision-making approach. Nearly all patients presenting with locally advanced cancers now receive chemotherapy combined with radiotherapy as a part of their treatment, often as a strategy to preserve organ function or as an adjuvant therapy following surgery. Advances have also occurred in radiation technology for treatment planning and dose delivery to improve local control and reduce the volume of normal tissue treated and risk of late effects. The introduction of novel therapeutics including molecularly targeted therapy and immune therapy offers an exciting opportunity to improve upon the outcomes achievable with standard cytotoxic chemotherapy and radiotherapy.

The National Cancer Institute defines biomarker as "a biological molecule found in the blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition." [1]. Biomarkers can be prognostic or predictive; prognostic biomarkers provide long-term outcome of a disease process independent of treatment, whereas predictive

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biomarkers offer information on outcome associated with a specific treatment. A biomarker can be both prognostic and predictive. Successful implementation of a biomarker requires an extensive validation process to ensure robust clinical performance, a streamlined assay process to ensure a short turnaround time, and reasonable cost to ensure feasibility [2].

The current standard for assessing risk in HNSCC largely depends on clinical tumor staging which encompasses histopathology and imaging; the approach has limited ability to stratify patients for specific risk of metastasis, local–regional recurrence, or development of a second primary. The human papillomavirus (HPV) has been established as a prognostic biomarker in oropharyngeal squamous cell carcinoma (OPSCC) [3], but no validated predictive biomarkers have been identified in HNSCC yet. Further identification of prognostic and predictive markers is a logical and rational next step to achieve improvement in outcome without increasing acute and chronic toxicity associated with treatment. Recent advances in genomics have provided detailed data on genetic alterations in HNSCC from large genome-wide sequencing studies [4–7]. Most of the mutations were found in tumor suppressor genes (TSGs), which are harder to target, rather than oncogenes. Some of these mutations, such as *TP53* and *CDKN2A*, were consistent with previously known alterations in a multistep model of tobacco-related HNSCC carcinogenesis, but novel mutations such as *NOTCH1* were also identified [8]. These genetic alterations have great potential to serve as reliable predictive biomarkers against targeted therapy as each of them may represent a distinct biological process in individual cancer.

This chapter will focus on an established biomarker in HNSCC, HPV, and p16 and emerging biomarkers including predictive biomarkers to existing treatments, genomic alterations, gene-expression profile, and immunotherapy-related biomarkers.

7.2 Established Biomarker: HPV and p16

HPV is associated with a subset of HNSCC that is biologically very distinct from non-HPV-related HNSCC [9]. Among greater than 100 subtypes of HPV, HPV 16 is the subtype most frequently associated with HNSCC; it is also associated with cervical and vulvar cancers in women, anal cancer in men and women, and penile cancer [10]. Over the past decade, the incidence of oropharynx cancers has been rising, especially in younger individuals in the US and Europe who have little or no history of exposure to two major risk factors, tobacco and alcohol [11].

HPV, which is a circular double-stranded DNA virus, causes inactivation of p53 and retinoblastoma (RB) mediated by two viral oncoproteins, E6 and E7, respectively [12, 13]. HPV integrates its DNA into the host cell genome, encodes for E6 and E7 genes, and dysregulates the cell cycle. The E6 oncoprotein promotes ubiquitination and degradation of p53, promoting cell survival. The E7 oncoprotein binds and inactivates the retinoblastoma tumor suppressor protein leading to upregulation of p16, low expression of cyclin D1, cell-cycle disruption, proliferation, and malignant transformation (Fig. 7.1).

Patients with HPV-related OPSCC are more likely to be nonsmokers and nondrinkers. As HPV is a sexually transmitted virus, the major risk factors appear to be a high number of lifetime sexual partners, younger age at first intercourse, a history of genital warts, and possibly marijuana use [18]. Patients with HPV-positive head and neck cancer commonly present with large cystic neck nodes and a small primary (low T stage) in the tonsil or base of the tongue [19]. Histologically, these cancers are usually nonkeratinizing, poorly differentiated squamous carcinomas with basaloid features [20].

HPV status in a tumor tissue can be determined by detection of the presence of HPV DNA or mRNA or by detection of p16 which is overexpressed by the downstream effect of viral oncoprotein E7. The gold standard for detection of HPV in a tumor is detection of high-risk HPV E6/E7 oncogene expression through reverse transcriptase–polymerase chain reaction (PCR), which is currently not available in most clinical laboratory settings [21]. Commonly used detection methods include HPV DNA in situ hybridization (ISH), HPV RNA ISH, and p16 immunohistochemistry (IHC) [22]. It has been shown in multiple studies that p16 IHC or ISH/FISH are very sensitive and specific at least in OPSCC (Table 7.1).

The presence of HPV or p16 has been consistently shown to be a strong prognostic factor of favorable outcome with significant improvement in both overall survival and progression-free survival in locally advanced OPSCC in multiple phase 2–3 clinical trials (Table 7.2) [3, 23–26]. The presence of p16 in OPSCC appears to remain as an important prognostic factor for patients who had surgery followed by adjuvant concurrent chemoradiotherapy or patients who develop recurrent and/or metastatic disease. A recent study showed that patients with HPV-positive OPSCC had better locoregional control and longer survival after postoperative platinum-based concurrent chemoradiotherapy regardless of p53 expression (by IHC) and the presence of extracapsular extension [27]. Another study reported that patients with p16-positive OPSCC had better overall survival (HR 0.48, 95 %CI 0.31–0.74), independent of initial tumor stage, progression type (distant versus locoregional), salvage surgery, and smoking status compared to p16-negative patients after progression of disease in a combined analysis of two large prospective clinical trials [28]. Even in non-OPSCC tumors, p16 expression was shown to be associated with better progression-free survival (HR 0.63, 95 %CI 0.42–0.95) and overall survival (HR 0.56, 95 %CI 0.35–0.89) in analyses of three phase 2–3 clinical trials [29]. These trials, though, did

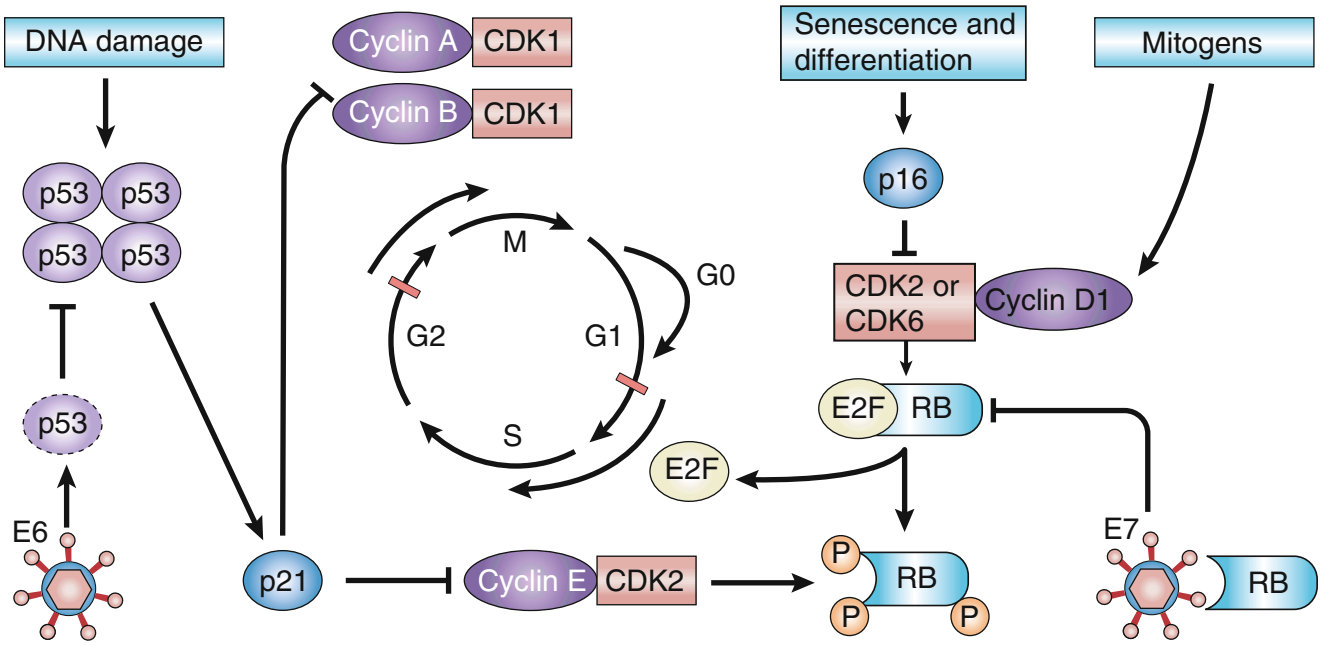

Fig. 7.1 Cell-cycle deregulation by human papillomavirus. Schematic diagram of molecular pathogenesis of HPV-related HNSCC. HPV can cause cell-cycle dysregulation and result in genomic instability and therefore promote malignant transformation. (1) Ubiquitination by viral E6 leads to p53 degradation [13, 14]. (2) Ubiquitination by viral E7 leads to pRb degradation [15, 16]. (3) Increased expression of $p16^{INK4A}$ as a consequence of increased S-phase gene expressions from the absence of pRb function [17]. *Abbreviation*: *CDK* cyclin-dependent kinase [Reprinted from Leemans CR, Braakhuis BJM, Brakenhoff RH. The molecular biology of head and neck cancer. Nature Rev Cancer 2011;11(1):12. With permission from Nature Publishing Group]

Table 7.1 Comparison of HPV detection methods in OPSCC[a]

Study	Number of samples	HPV-DNA PCR		ISH/FISH		$p16^{INK4A}$ IHC		$p16^{INK4A}$ IHC interpretation		
		Sens.	Spec.	Sens.	Spec.	Sens.	Spec.	Intensity	%	Pattern
Smeets et al. [115]	19	100 %	92 %	83 %	100 %	100 %	70 %	≥1+	>10	N or C
Shi et al. [116]	111	NA	NA	84 %	92 %	89 %	81 %	Strong	N/A	N and C
Schache et al. [117]	95	97 %	87 %	88 %	88 %	94 %	82 %	Strong	>70	N and C
Schlecht et al. [118]	21	NA	NA	38 %	100 %	90 %	100 %	≥2+	≥75	N and C
Rotnaglova et al. [119]	109	100 %	89 %	NA	NA	94 %	96 %	≥1+	>50	N or C
Jordan et al. [21]	235	99 %	63 %	88 %	95 %	97 %	84 %	≥2+	>70	N and C
						92 %	90 %	H score[b] ≥ 60		

From Kang H, Kiess AP and Chung CH. Emerging biomarkers in head and neck cancer in the era of genomics. Nature Rev Clin Oncol 2015;12(1):14. Reprint permission waived (authored by Kang H and Chung CH)

C cytoplasmic, *FISH* fluorescence in situ hybridization, *HPV* human papillomavirus, *IHC* immunohistochemistry, *N* nuclear, *OSCC* oropharyngeal squamous cell carcinoma, *Sens.* sensitivity, *Spec.* specificity

[a]Sensitivities and specificities are based on gold standard of E6 mRNA qRT-PCR

[b]H score is derived from cross product of the intensity score (0–3) and from the percentage of tumor staining at the highest intensity (0–100 %)

not include oral cavity squamous cell carcinoma (OCSCC), 6 % of which can be positive for HPV16. In OCSCC, p16 expression by IHC has a poor positive predictive value [30] for HPV infection and thus should not be used as a surrogate marker for the presence of HR-HPV. Also, the prognostic role of either p16 or HPV DNA/RNA has not been established in OCSCC [31].

Although HPV status or p16 expression have been well established as a strong prognostic marker for OPSCC, whether it can serve as a predictive biomarker for certain therapies is still not clear. HPV-negative tumors tend to have higher total and phosphorylated epidermal growth factor receptor (EGFR) protein expression than HPV-positive tumors [32]; thus, there is a possibility that EGFR-targeting

Table 7.2 Impact of HPV status on outcome of HNSCC

Study	Site	Detection method	Number of patients	PFS rate			OS rate		
				HPV^+	HPV^-	HR	HPV^+	HPV^-	HR
Fakhry et al. [23]	OP; L	DNA ISH	96	86 % at 2 years	53 % at 2 years	3.57 (1.33–9.09)	95 % at 2 year	62 % at 2 year	2.86 (1.25–6.67)
Ang et al. [3]	OP	DNA ISH	323	73.7 % at 3 years	43.4 % at 3 years	2.50 (1.75–3.45)	82.4 % at 3 year	57.1 % at 3 year	2.63 (1.82–3.85)
Rischin et al. [24]	OP	$p16^{INK4A}$ IHC	185	87 % at 2 years	72 % at 2 years	2.56 (1.35–5)	91 % at 2 year	74 % at 2 year	2.78 (1.35–5.88)
Posner et al. [25]	OP	DNA PCR for E6/E7	111	78 % at 5 years	28 % at 5 years	NA	82 % at 5 year	35 % at 5 year	5.00 (2.63–10.00)
Lassen et al. [26]	OP; OC; L; P	$p16^{INK4A}$ IHC	794	68 % at 5 years	57 % at 5 years	1.52 (1.14–2.04)	62 % at 5 year	47 % at 5 year	1.61 (1.28–2.04)

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HNSCC head and neck squamous cell carcinoma, *HPV* human papillomavirus, *HR* hazard ratio, *ISH* in situ hybridization, *IHC* immunohistochemistry, *L* larynx, *NA* not available, *OC* oral cavity, *OP* oropharynx, *OS* overall survival, *P* pharynx, *PFS* progression-free survival

therapy may work better for HPV-negative HNSCC. In a retrospective subset analysis of the SPECTRUM study, in which patients received panitumumab, a monoclonal antibody against EGFR, in combination with cisplatin and 5-FU, a survival benefit from the addition of panitumumab was limited to p16-negative patients [33]. Another study, comparing MEHD7945A, a dual-action antibody against EGFR and HER3, and cetuximab in a second-line systemic therapy of recurrent or metastatic HNSCC, showed that the response to either MEHD7945A or cetuximab is limited to HPV-negative patients [34]. However, a retrospective analysis of the EXTREME study demonstrated that the benefit of cetuximab is not limited to HPV-negative patients [35]. As these studies are all retrospective, unplanned analyses of prospective studies of limited numbers of patients, a prospective study will be required to address this question.

As the long-term survival of patients with HPV-positive HNSCC treated with current standard of care multimodality regimens is excellent, current clinical trials are focused on de-intensification of multimodality treatment [36]. HPV status may become a biomarker for less intensive curative intent treatment if randomized controlled de-intensification trials demonstrate comparable outcome. Also, HPV status may become a predictive biomarker for HPV-targeted therapies in the future, such as therapeutic HPV vaccines [37].

7.3 Emerging Biomarkers

Recent advances in tumor biology and multiplex genomic analysis have enabled us to access expansive information on genetic and epigenetic alterations of HNSCC. Comprehensive genome-wide sequencing data from several studies available to date have shown that there are more alterations in tumor suppressor genes (TSGs) rather than in oncogenes (Fig. 7.2) [4–6, 38, 39]. TSG mutations are more difficult to target than oncogene mutations, as it is harder to restore loss of function than to suppress gain of function. In addition, while oncogene mutations tend to occur in certain hotspots, TSG mutations tend to occur scattered throughout the gene [40].

Genomic analyses clearly demonstrate distinct biologic difference between HPV-positive and HPV-negative tumors. HPV-positive tumors tend to have fewer mutations per tumor and frequently have helical domain mutations of the oncogene, *PIK3CA*. This is not very surprising given that all HPV-positive tumors have already altered p53 and Rb pathways from actions of viral oncoproteins, E6 and E7. Almost all HPV-negative tumors show loss-of-function *TP53* mutations and *CDKN2A* inactivation which leads to $p16^{INK4A}$ functional loss (Table 7.3) [38]. These biological differences support clinical observations and may provide further insight on development of specific treatments for each type of HNSCC.

7.3.1 Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein and a member of the human epidermal receptor (HER) family receptor tyrosine kinases. EGFR is composed of an extracellular ligand-binding domain, a transmembrane region, and an intracellular domain that includes the tyrosine kinase enzyme. When a ligand binds to the receptor, it undergoes a conformational change and dimerization with another EGFR or other HER family members such as HER2, HER3, or HER4. Dimerization results in activation of intracellular tyrosine kinase, protein phosphorylation and stimulation of various cell signaling pathways that mediate cell-cycle progression, angiogenesis, inhibition of apoptosis, tumor invasion, and metastasis [41].

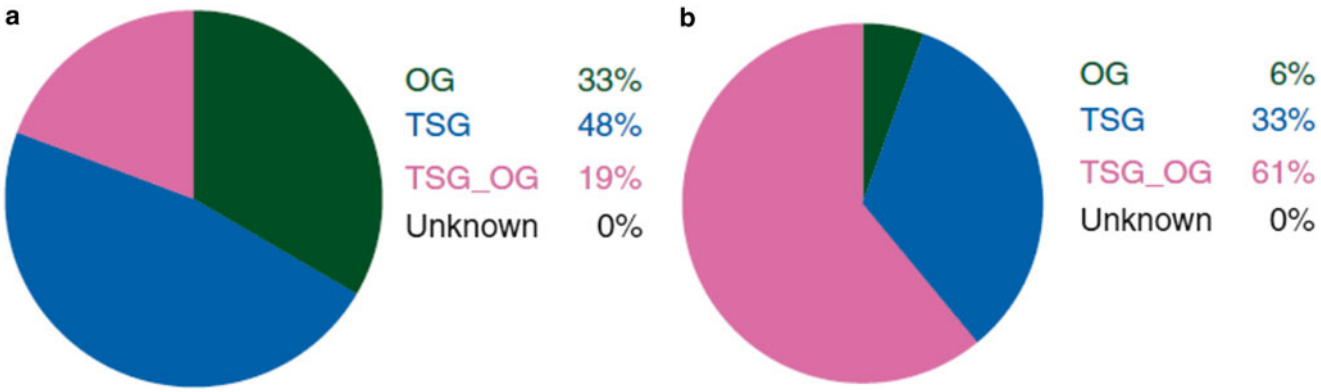

Fig. 7.2 Tumors with alterations in oncogenes (OG), tumor suppressor genes (TSG), or both (TSG and OG) based on selected 236 cancer-related gene sequencing, (**a**) HPV-positive HNSCC, (**b**) HPV-negative HNSCC [Reprinted from Chung CH, Guthrie VB, Masica DL et al. Genomic alterations in head and neck squamous cell carcinoma determined by cancer gene-targeted sequencing. Ann Oncol 2015 2015 Jun;26(6):1216–23. With permission from Oxford University Press]

Table 7.3 Frequently mutated genes in HPV-positive and HPV-negative tumors in selected studies

HPV-positive HNSCC				HPV-negative HNSCC			
Gene	TCGA (*N*=36) [38]	Chicago (*N*=51) [6]	Foundation medicine (*N*=84) [39]	Gene	TCGA (*N*=243) [38]	Chicago (*N*=69) [6]	Foundation medicine (*N*=168) [39]
PIK3CA	56 %	35 %	30 %	*TP53*	84 %	80 %	87 %
SOX2	28 %	NA	11 %	*CDKN2A/B*	57 %	32 %	54 %
MLL2 (KMT2D)	17 %	20 %	13 %	*FGF19*	32 %	NA	23 %
RB1	6 %	24 %	7 %	*FGF3*	31 %	NA	22 %
BCL6	25 %	18 %	1 %	*FGF4*	31 %	NA	22 %
EP300	14 %	12 %	10 %	*PIK3CA*	34 %	29 %	16 %
NOTCH1	11 %	18 %	6 %	*CCND1*	32 %	13 %	24 %
PTEN	3 %	8 %	15 %	*NOTCH1*	21 %	26 %	16 %
FGFR3	11 %	24 %	1 %	*LRP1B*	22 %	30 %	6 %
ASXL1	19 %	10 %	5 %	*SOX2*	21 %	NA	8 %

Modified from Chung CH, Guthrie VB, Masica DL et al. Genomic alterations in head and neck squamous cell carcinoma determined by cancer gene-targeted sequencing. Ann Oncol 2015 2015 Jun;26(6):1216–23. With permission from Oxford University Press
TCGA The Cancer Genome Atlas

EGFR has been a major therapeutic target in the treatment of HNSCC, as the majority of HNSCC (~90 %) overexpresses EGFR relative to normal tissue [42]. High EGFR expression has been associated with worse outcome in patients who were treated with resection or radiotherapy [43, 44]. However, EGFR expression detected by IHC has not been widely adopted as a biomarker because there is no standardized anti-EGFR antibody, staining protocol, or quality control measure for the assay. Furthermore, EGFR expression assessed by IHC has not been shown to be predictive of response to EGFR-targeting therapy, such as cetuximab, a chimeric monoclonal IgG1 antibody directed against EGFR [45].

EGFR amplification has been investigated as a prognostic factor in HNSCC. *EGFR* is amplified in 10–58 % of HNSCC and is measured by fluorescence in situ hybridization (FISH) and quantitative PCR and was associated with worse progression-free and overall survival in two independent studies [46, 47]. However, there is no evidence correlating gene amplification with response outcome to EGFR-targeting therapies in HNSCC. The Cancer Genome Atlas (TCGA) showed that only 15 % of HPV-negative HNSCC and 6 % of HPV-positive HNSCC have mutations or amplifications of *EGFR* [38], which suggests that the previous studies may have overestimated *EGFR* mutations or copy number variations. More investigations are needed to clarify the role of *EGFR* alterations as a predictive biomarker.

The resistance mechanisms against EGFR-targeting therapy provide insight into potential prognostic and predictive biomarkers and therapeutic targets. These include increased nuclear localization of EGFR, transactivation and dimerization with other HER family receptors, activation of other receptor tyrosine kinases such as MET or IGF-1R, or activation of downstream signaling molecules (Fig. 7.3) [48].

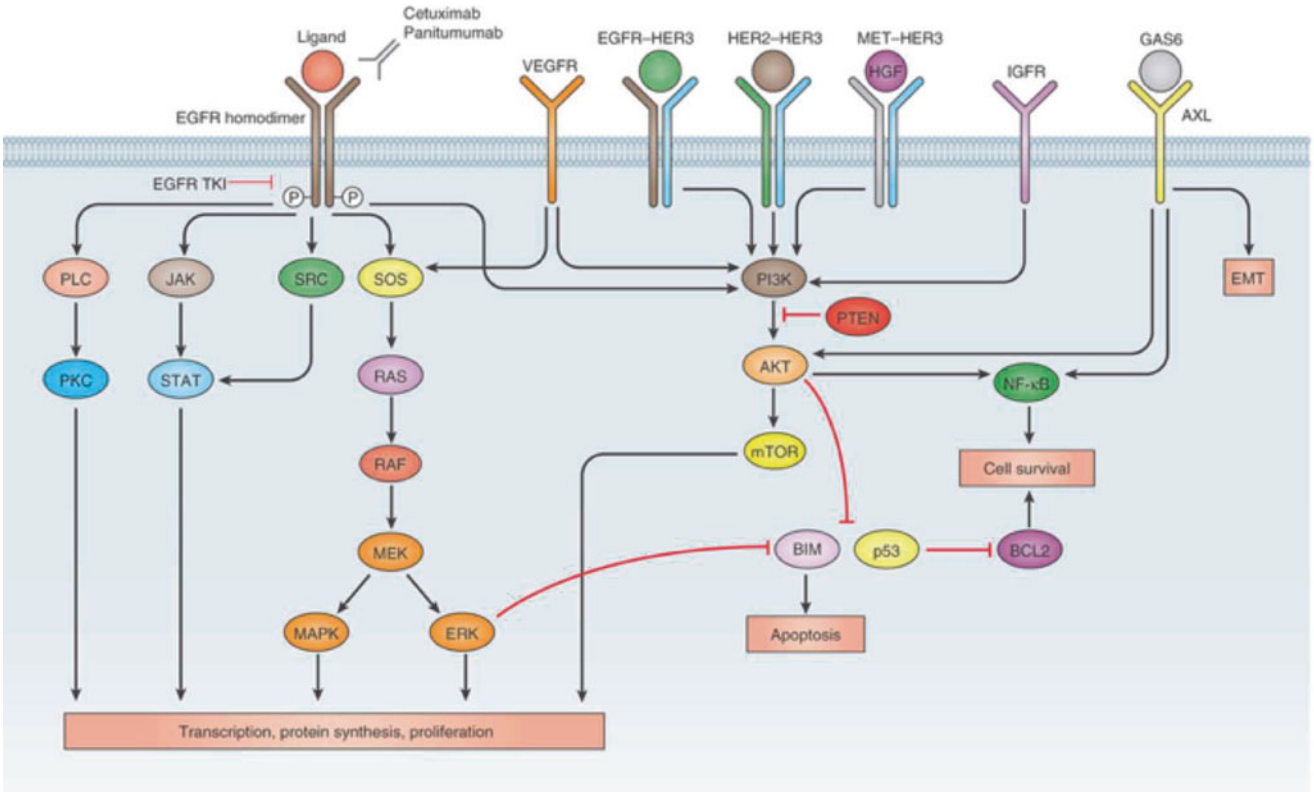

Fig. 7.3 EGFR and receptor tyrosine kinase signaling in head and neck cancer. Resistance to EGFR inhibitors can arise via signaling from redundant receptor tyrosine kinases, such as HER family members, MET, or IGF-1R, as well as the activation of downstream signaling intermediaries [Reprinted from Chong CR, Janne PA. The quest to overcome resistance to EGFR-targeted therapies in cancer. Nat Med 2013;19(11):1390. With permission from Nature Publishing Group]

7.3.2 *PIK3CA*

PIK3CA encodes p110α, a p110 catalytic subunit of phosphoinositol 3-kinase (PI3K), which is a family of lipid kinases that integrate signals from growth factors, cytokines, and other environmental cues, and relays them to intracellular signaling for such functions as cell growth, proliferation, and survival [49]. An example of a signaling cascade mediated by activated PI3K is shown in Fig. 7.4. PI3K activates AKT, which subsequently leads to the activation of the mammalian target of rapamycin (mTOR), triggering downstream effects on transcription, protein synthesis, metabolism, proliferation, and apoptosis (Fig. 7.4) [50].

PIK3CA is the most commonly mutated gene in HPV-positive HNSCC, and the mutation tends to be located in the helical domain (E542K and E545K), while *PIK3CA* mutations in HPV-negative HNSCC are more diverse throughout the gene [38, 51]. The distinctive mutation loci may result in functionally different mutant proteins that could serve as novel therapeutic targets and predictive biomarkers. In a comparative protein array study, HPV-positive and HPV-negative OPSCC differentially activate PI3K/AKT/mTOR pathway—*PIK3CA* mutations in HPV-positive OPSCC were associated with activation of mTOR but not AKT [32], suggesting that an mTOR inhibitor may have activity against HPV-positive *PIK3CA* mutant OPSCC. In a preclinical study, dual inhibition of mTOR/PI3K was shown to be effective in controlling a *PIK3CA* mutant patient tumor-derived xenograft mouse model [51].

Numerous clinical trials of drugs targeting the PI3K pathway are currently on-going. Early data from phase I/II trials have suggested limited efficacy as monotherapy in tumors with PI3K pathway activation partly because of lack of specificity and activation of alternate signaling pathways [52]. Clinical trials in nonselected RM-HNSCC population with an irreversible oral PI3K inhibitor, PX-866, in combination with either cetuximab or docetaxel did not show any improvement in the response rate or progressive-free survival [53, 54]. There was no correlation between the response and the PI3K mutation status, although only small number of patients harbored PI3K mutations (17 % and 8 %). Further development should account for the specific characteristics of *PIK3CA* mutations in HNSCC.

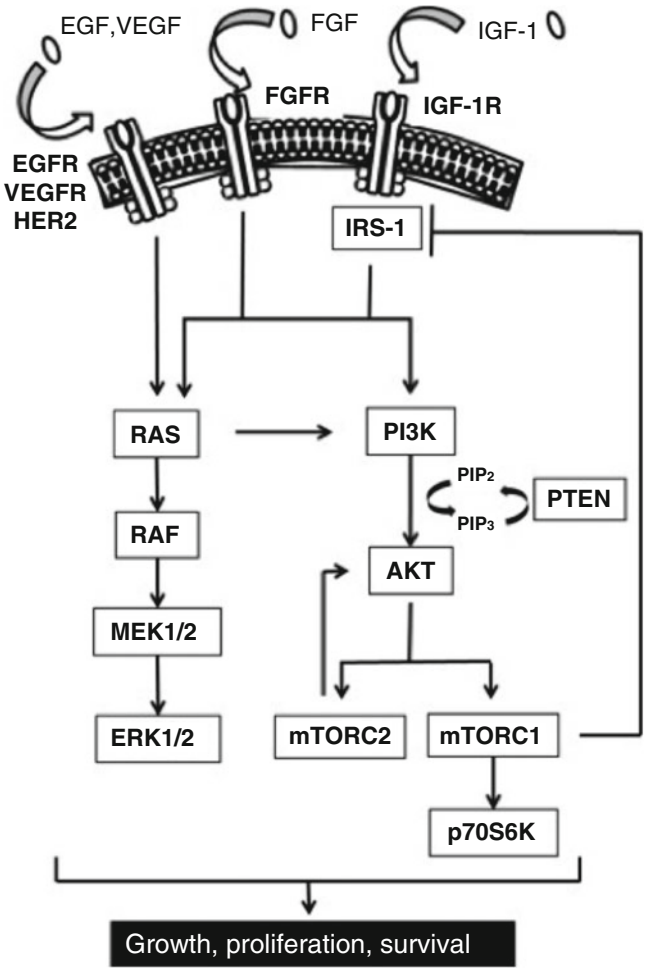

Fig. 7.4 The PI3K/AKT/mTOR pathway and associated signaling pathways. *AKT* protein kinase B, *EGFR* epidermal growth factor receptor, *ERK 1/2* extracellular signal-regulated kinase 1/2, *FGFR* fibroblast growth factor receptor, *HER2* human epidermal growth factor 2, *IGF* insulin-like growth factor, *IRS-1* insulin receptor substrate 1, *MEK 1/2* mitogen-activated protein kinase 1/2, *mTORC* mammalian target of rapamycin complex, *PI3K* phosphatidylinositol 3-kinase, *PIP2* phosphatidylinositol 4,5-bisphosphate, *PIP3* phosphatidylinositol (3,4,5)-trisphosphate, *PTEN* phosphatase and tensin homolog, *p70S6K* p70S6 kinase, *VEGFR* vascular endothelial growth factor [Reprinted from Simpson DR, Mell LK, Cohen EE. Targeting the PI3K/AKT/mTOR pathway in squamous cell carcinoma of the head and neck. Oral Oncol 2015;51(4):292. With permission from Elsevier]

7.3.3 Cyclin D1

Cyclin D1 is a protein expressed in a cell-cycle-dependent manner and plays an important role in regulating G1-S transition by forming a complex with cyclin-dependent kinases (CDKs), such as CDK4 and CDK6. This complex phosphorylates Rb and activates transcription factors, promoting proliferation through the expression of S-phase proteins [55]. Cyclin D1 also has non-catalytic functions independent of CDKs and can interact with various transcription factors [56] and regulate histone acetylation and methylation [57]. *CCND1*, which encodes for cyclin D1, was shown to be amplified in 28 % of HNSCC in TCGA, mostly in HPV-negative tumors (32 %) rather than in HPV-positive tumors (6 %) [38].

Overexpression of cyclin D1 or amplification of *CCND1* has been associated with poor outcome and resistance to EGFR-targeted therapy in HNSCC [58, 59]. This interaction may be further perturbed by inactivation of $p16^{INK4a}$, an inhibitor of CDK4 and CDK6. Inactivation of $p16^{INK4a}$ by deletion of *CDKN2A* (which is documented in 57 % of HPV-negative HNSCC) has been associated with poor prognosis [58, 60]. Increased cyclin D1 expression and loss of $p16^{INK4a}$ expression is associated with particularly poor clinical outcome in HNSCC [61], and there seems to be an inverse correlation between expressions of cyclin D1 and $p16^{INK4a}$ [62, 63]. As direct targeting of cyclin D1 is very difficult at this time, indirect targeting through inhibitors for CDK4/CDK6 is in development and might play role in patients with *CCND1* amplification.

7.3.4 Fibroblast Growth Factor Receptor

The FGF and fibroblast growth factor receptor (FGFR) pathway regulate developmental pathways, angiogenesis, wound repair, proliferation, differentiation, and survival. FGFRs are a family of highly conserved transmembrane tyrosine kinase receptors (FGFR1–4), which are activated by 18 ligands (FGFs) [64, 65]. The activated FGFR phosphorylates FGFR substrate 2 (FRS2) on several sites, allowing recruitment of the adaptor proteins, which in turn activate RAS–RAF–MAPK pathways and PI3K–AKT–mTOR pathways [64].

In HPV-negative HNSCC, *FGFR1*, *FGFR2*, *FGFR3*, and *FGFR4* are amplified or mutated in 10 %, 2 %, 2 %, and 0.4 %, respectively. HPV-positive HNSCC did not demonstrate any alteration in *FGFR1* and *FGFR2*, but *FGFR3* mutation or fusion was seen in 11 %, and *FGFR4* mutation was seen in 3 % [38]. In a preclinical study, FGF2 and FGFR2 and FGFR3 were found to be frequently expressed in HNSCC cell lines, forming an autocrine signaling network [66]. In a predominantly HPV-negative cohort primarily treated with surgery followed by radiation, FGF2 overexpression was shown to be independently associated with worse outcome after adjusting clinical factors and HPV status [67]. Inhibition of FGFR1 was shown to suppress cell growth and reverses epithelial–mesenchymal transition (EMT) features in HNSCC preclinical models [68]. Further investigation will be needed to validate this target.

7.3.5 *KRAS* Variant

KRAS is a well-known oncogene, although its alteration is rarely reported in HNSCC (amplification or mutation in 3 % of samples in TCGA) [38, 69]. A single nucleotide polymorphism

(SNP) in its 3′ UTR, *rs61764370*, has been associated with increased risk of non-small cell lung cancer [70], ovarian cancer [71], and triple-negative breast cancer [72]. The variant *KRAS* has altered *let*-7 miRNA complementary site (LCS) and is thought to cause decreased degradation of *KRAS* mRNA. The presence of *KRAS* variant was shown to be associated with higher mortality from ovarian cancer and a greater chance of platinum resistance [73].

In HNSCC, prevalence of the *KRAS* variant was reported to be around 20–30 % [74, 75] and associated with reduced survival [74]. A retrospective analysis of several prospective studies showed that the *KRAS* variant was associated with worse progression-free survival when treated with platinum-containing chemotherapy (cisplatin ± cetuximab). However, in patients treated with non-platinum-containing chemotherapy (docetaxel + bortezomib), no difference was observed in PFS between the *KRAS* variant group and the *KRAS* wild-type group [75]. This observation suggests that the *KRAS* variant may serve as a predictive biomarker for platinum response, and further studies are warranted.

7.3.6 *TP53*

Discovered in 1979 and characterized as a tumor suppressor in 1983, p53 is a highly studied, critical element of cell-cycle regulation and is mutated in over half of all human malignancies [76]. The normal role of p53 is to respond to an enormous variety of stress signals by modulating cellular responses, including transient cell-cycle arrest, cellular senescence, and apoptosis (Fig. 7.5) [77].

TP53 is the most commonly mutated gene in all cancers [78], and the mutation is found in 84 % of HPV-negative HNSCC tumors [38]. Including the inactivation of p53 by HPV viral oncoprotein E6 in HPV-positive OPSCC, functional loss of p53 occurs in more than 90 % of HNSCC [13, 79]. The majority of *TP53* mutations in human cancers are missense mutations (80 %) [80], leading to the substitution of a single amino acid in the p53 protein that can be stably expressed in the tumor cell. These mutations can occur anywhere in the gene but are most commonly found in the DNA-binding domain of p53 [81]. Diverse mutations may function differently in the different context, reflecting diverse expression patterns of target proteins of p53 [82]. Besides mutations resulting in loss of wild-type p53 functions, certain missense mutations exhibit gain-of-function properties [83], which is described to be oncogenic in HNSCC cell lines through inhibition of tumor-suppressive AMP-activated protein kinase (AMPK) signaling [84].

Loss of p53 function has been investigated as a prognostic biomarker in HNSCC, but early studies were confounded by poor assays, small sample size, and a lack of distinction between functional and nonfunctional alterations [85]. In several studies, disruptive *TP53* mutations which cause truncated p53 have been associated with worse clinical outcome in HNSCC patients [86, 87]. A recent study reported an evolutionary action score of *TP53* (EAp53) that identified high-risk mutations associated with decreased survival and increased distant metastases in HNSCC patients [88]. Cells harboring the high-risk *TP53* mutations tended to have decreased expression of certain p53 target genes, such as p21, Notch1, and BTG2. The same authors reported that high-risk *TP53* mutations identified by EAp53 were associated with decreased sensitivity to cisplatin in both preclinical tumor models and in patients treated with platinum-based chemotherapy [89]. These findings highly suggest that the functional status of p53, rather than the presence or absence of *TP53* alteration, may act as a prognostic biomarker in HNSCC.

Traditionally TSGs such as *TP53* have been regarded as hard to target. Recently, the concept of synthetic lethality in which a combination of mutations in two or more separate genes leads to cell death [90] has gained attention as a way to target TSGs. Synthetic lethality can be exploited when a maladaptive genetic change, not lethal by itself, makes cancer cells vulnerable to specific targeted therapies [91]. A high-throughput RNA interference functional genomic screen of the human kinome in HNSCC cell lines has shown that inhibition of WEE1, a G2-M cell-cycle-regulating protein, can render synthetic lethality in *TP53*-mutated tumors [92]. A WEE1 inhibitor, MK-1775, has been shown to sensitize platinum-resistant HNSCC cells with *TP53* mutations to cisplatin treatment in vitro and in vivo [93]. A similar approach can be taken by inhibiting CHK1, another G2-M cell-cycle-regulating protein, and a Chk inhibitor, AZD7762, has been shown to sensitize HNSCC cells with loss of functional p53 to cisplatin (Fig. 7.6) [94]. *TP53* mutations can be a potential predictive marker for these synthetic lethal approaches, in the context of functional disruption of p53.

7.3.7 Excision Repair Cross Complementing Group 1

The excision repair cross complementing group 1 (ERCC1)/xeroderma pigmentosum-complementation group F (XPF) is a heterodimeric DNA structure-specific endonuclease complex. This enzyme plays a key role in several DNA-repair pathways, particularly in repairing ultraviolet-induced lesions and intra- or interstrand cross-linked DNA adducts created by alkylating agents, such as cisplatin [95]. As platinum-based chemotherapy is routinely used in the management of HNSCC, ERCC1 has been investigated as a potential predictive biomarker.

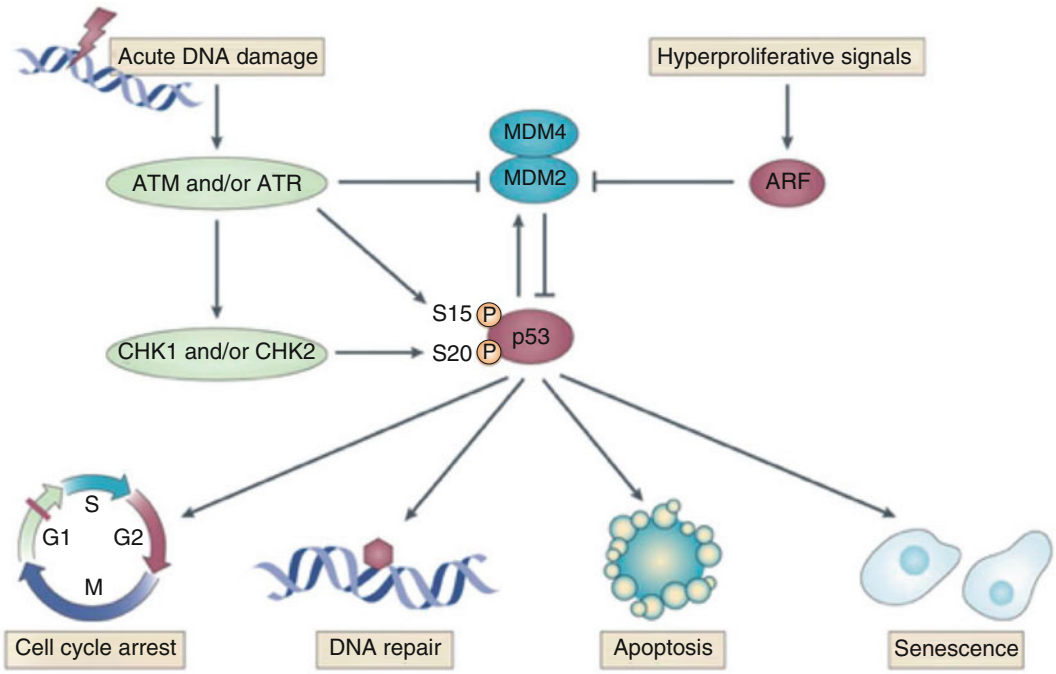

Fig. 7.5 p53 activation and response. In response to acute DNA damage, ataxia telangiectasia mutated (ATM) and/or ataxia telangiectasia and RAD3 related (ATR) is recruited and activates CHK1 and/or CHK2. ATM, ATR, CHK,1 or CHK2 can phosphorylate p53 and then stabilize it. MDM2 and MDM4 can bind to the transcriptional activation domain of p53 and can inhibit p53 transactivation function. Activation of p53 regulates crucial cellular processes by modulating cell-cycle arrest, DNA repair, apoptosis, and senescence [Reprinted from Bieging KT, Spano Mello S and Attardi LD. Unraveling mechanisms of p53-mediated tumor suppression. Nat Rev Cancer 2014;14(5):361. With permission from Nature Publishing Group]

Overexpression of ERCC1 determined by IHC has been associated with lower response rates to cisplatin-containing chemotherapy [96]. Results from various studies have not been consistent as there were inconsistencies in assay and interpretation, although there has been a strong signal that ERCC1 may be a useful predictive biomarker to platinum therapy [97]. A recent study with improved assays using specific antibodies and automatic quantitative analysis (AQUA) has shown that low ERCC1 expression was associated with improved outcome in patients treated with surgery followed by concurrent chemoradiation with cisplatin. There was no difference in survival between ERCC1 high and ERCC1 low group in patients treated only with surgery [98]. A retrospective analysis of patients treated with cisplatin-based concurrent chemoradiotherapy showed that higher ERCC1 expression determined by AQUA was associated with inferior PFS, irrespective of HPV status [99]. Similarly, low expression of XPF, a binding partner of ERCC1, has been associated with poor clinical outcome in HNSCC patients treated with platinum-based induction chemotherapy [100]. ERCC1 is a promising potential predictive biomarker for response to platinum chemotherapy, but these findings are needed to be validated in prospective studies.

7.3.8 Classification by Gene Expression Profiles

HNSCC can be classified based on gene-expression profiles using expression arrays. Four distinct subtypes have been identified as "basal," "mesenchymal," "atypical," and "classical" to reflect specific molecular characteristics [101]. This classification was validated in two independent cohorts [102]. These studies included only a small number of HPV-positive tumors which were classified in the "atypical" subtype. Recent analysis of an HPV-positive tumor-enriched cohort has led to the revision of the classification into five categories—"basal HPV," "classic HPV," "classic non-HPV," "mesenchymal HPV," and "mesenchymal non-HPV" [103]. Regardless of HPV status, the mesenchymal subtype was associated with the expression of immune response genes such as *CD8*, *ICOS*, *LAG3*, and *HLA-DRA* which could be used as predictive biomarkers for immune-based therapy in the future. In addition, a meta-analysis with a publicly available nine microarray gene-expression dataset in HNSCC showed a robust association of a 172-gene-expression signature with prognosis of patients regardless of HPV status [104]. Future studies will focus more on pathway-based analyses that integrate genomic data.

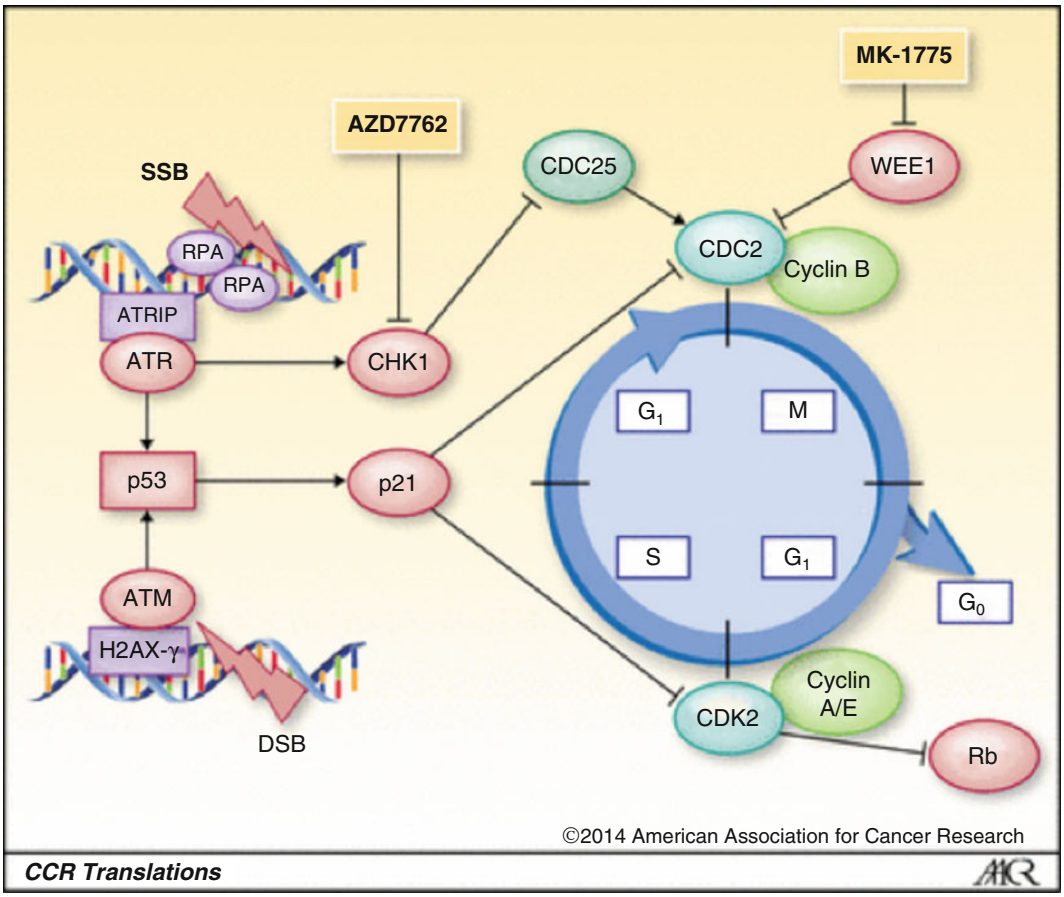

Fig. 7.6 Synthetic lethal approach for p53 dysfunctional tumors. Following DNA damage, ATR, and ATM initiate cell-cycle arrest, following their respective activation sites of single-strand (SSB) or double-strand breaks (DSB). ATR directly phosphorylates checkpoint kinase 1 (CHK1), whereas ATM activates p53 and CHK2, although there is extensive cross talk between these pathways. At the G2 checkpoint, G2-M arrest is triggered when CHK1 inhibits the activator of CDC2 and CDC25 or when WEE1 directly inactivates CDC2 [Reprinted from Bauman JE, Chung CH. CHK it out! Blocking WEE kinase routs TP53 mutant cancer. Clin Cancer Res 2014;20(16):4174. With permission from American Association for Cancer Research]

7.3.9 Immune-Related Biomarkers

Head and neck cancer is recognized as an immunosuppressive disease. Most patients demonstrate low absolute lymphocyte counts, impaired natural killer cell activity, and decreased antigen-presenting function [105–107]. Immune system evasion is mediated by several different mechanisms. The antigen-processing molecules, TAP 1/2, and the major histocompatibility complex (MHC) 1 are downregulated [108]. At the same time, co-inhibitory receptors, programmed death ligand 1 (PD-L1), and cytotoxic T-lymphocyte antigen 4 (CTLA-4), which induce immune tolerance to HNSCC, are frequently expressed on tumors [109, 110]. Immunosuppressive cytokines such as TGF-beta, VEGF, IL-6, and IL-10 are upregulated in the tumor microenvironment [111].

The recent success of immune checkpoint inhibitors in solid tumors along with the increased incidence of HPV-positive HNSCC has raised enthusiasm for novel immunotherapeutic approaches and identification of corresponding predictive biomarkers. Indeed, HPV-positive HNSCC arises from the deep crypts in lymphoid tissues of the lingual and palatine tonsils, and characteristic tumor-infiltrating lymphocytes (TILs) are found in the stroma and tumor nests [112]. Expression of PD-L1 is noted within deep tonsillar crypts as well as 70 % of HPV(+) HNSCC tumor cells. These PD-L1-expressing tumors were associated with an increased number of TILs [113]. High PD-L1 expression in the tumor or the tumor microenvironment, especially when it is expressed in tumor-infiltrating immune cells, seems to correlate with the likelihood of response in early clinical studies with PD1 pathway-targeting therapies [114]. Thus, the presence of TILs and expression of PD-L1 are promising candidates as predictive biomarkers for immune checkpoint inhibitors, but further evaluation is necessary.

7.4 Conclusion

Current research and patient care are influenced by the rapidly advancing knowledge of the molecular biology of head and neck cancer and of complex interconnecting pathways from cell surface receptors to transcriptional activation of genes that mediate uncontrolled cellular proliferation and survival. Molecular target identification and an array of new therapeutics present challenges to the standard methodologies for clinical trial designs, evaluation of efficacy, and toxicity. Risk stratification based on molecular prognostic and predictive markers is next on the horizon for advancing the field. This chapter has focused on markers with potential for testing in large-validation clinical trials. As yet, no predictive biomarker has been validated in the selection of therapy for individuals with head and neck cancer. HPV status, determined by p16 expression, HPV DNA, or RNA ISH, has been confirmed to be prognostic for better outcome. It is our responsibility to critically appraise and validate emerging biomarkers in prospective clinical trials to deliver optimal individualized care to patients with HNSCC.

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