Chapter 7
The PI3K Signaling Pathway in Head and Neck Squamous Cell Carcinoma

Jason D. Howard and Christine H. Chung

Abstract The PI3K/PTEN/AKT/mTOR signaling axis has been intensively studied in many cancer systems. Current evidence suggests deregulation of this pathway plays a unique role in the initiation, development, and recurrence of head and neck squamous cell carcinoma (HNSCC). A heterogeneous disease by nature, HNSCC encompasses a disparate collection of anatomical sites with complex tumor biology. Yet, PI3K/PTEN/AKT/mTOR signaling has an intimate role in nearly every facet of this disease. In this chapter, we will provide a brief introduction to the mechanisms involved in PI3K/PTEN/AKT/mTOR signaling and how specific alterations in these signaling nodes enable HNSCC development. We will also discuss differences in PI3K/PTEN/AKT/mTOR signaling with respect to HPV status. A number of inhibitors targeting multiple nodes in this pathway have been developed as agents have broad application across many cancer types. We will briefly review how these therapeutic agents are being evaluated and what predictive biomarkers have been established in HNSCC for these drugs. Finally, PI3K/PTEN/AKT/mTOR signaling represents an important source of resistance to radiation, chemotherapy, and other targeted agents. We will also speculate on how PI3K/PTEN/AKT/mTOR inhibitors may increase the efficacy of these established therapies. Although PI3K/PTEN/AKT/mTOR investigations are relatively new to HNSCC research, early evidence suggests further evaluation of this essential signal transduction pathway is warranted.

Keywords PI3K · PTEN · AKT · mTOR · HPV · HNSCC · Biomarkers

C. H. Chung (✉)
Department of Oncology, Sidney Kimmel
Cancer Center, Johns Hopkins Medical Institute, 1650 Orleans Street,
CRB-1 rm. 344, Baltimore, MD 21231, USA
e-mail: cchung11@jhmi.edu

J. D. Howard
Department of Oncology, Sidney Kimmel Cancer Center, Johns Hopkins
Medical Institute, Baltimore, MD 21231, USA

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7.1 Introduction

The PI3K/PTEN/AKT/mTOR pathway is a critical signaling axis which consolidates and regulates the myriad extracellular signals required for complex, multicellular organisms. The end result of appropriate PI3K/PTEN/AKT/mTOR signaling is homeostasis: the careful balance of proliferation, metabolism, autophagy, cap-dependent translation, migration, apoptosis, and many other cellular requirements. Given the magnitude of functionalities associated with this pathway, deregulation at any of its signaling nodes can have dire biological consequences. Thus, many of the proteins in this pathway have been established as bona fide oncogenes or tumor suppressors. Recent evidence suggests that at least 47 % of head and neck squamous cell carcinomas (HNSCCs) have at least one molecular alteration in this pathway [1]. In this chapter, we will summarize key features of this pathway, and how these molecular alterations are associated with HNSCC development and progression. In addition, we will provide some perspective regarding the translational potential of known therapeutic targets involved in this signaling network and development of biomarkers for assessing clinical outcomes. Ideally, these targeted agents would ultimately exploit an oncogenic dependence unique to HNSCC, or subvert acquired resistance mechanisms mediated by PI3K/AKT/mTOR signaling to enhance the efficacy of previously established therapies.

7.2 PI3K/PTEN/AKT/mTOR Signal Transduction

7.2.1 Phosphoinositide 3-kinase (PI3K)

The intracellular transduction of extracellular stimuli often requires receptor-mediated signaling. Thus, membrane-bound receptors translate extracellular ligand binding into intracellular signaling cascades to various downstream cellular compartments (Fig. 7.1). Adaptor proteins and second messengers play an important role in correctly mediating and regulating these signals. One group of second messengers is the class I phosphoinositide 3-kinase (PI3K) family (p110α, p110β, p110γ, and p110*δ*), a common signaling mechanism utilized by a wide array of receptor tyrosine kinases (RTKs) and G-protein-coupled receptors (GPCRs). A functional PI3K-signaling unit contains one regulatory (typically p85) and one catalytic (p110) protein, creating a heterodimeric kinase with enzymatic activity for lipid and protein substrates [2], [3]. However, only the lipid kinase activity is required for oncogenic signaling [4]. When a receptor is activated, PI3K translocates to the cell membrane where it associates with the receptor through p85 and various adaptor proteins (i.e., IRS1) [5], [6]. This binding relinquishes p85-negative regulation of p110, initiating catalytic activity. PI3K can also be positively affected by Ras, a critical GTPase which may facilitate PI3K membrane localization [7]–[9].

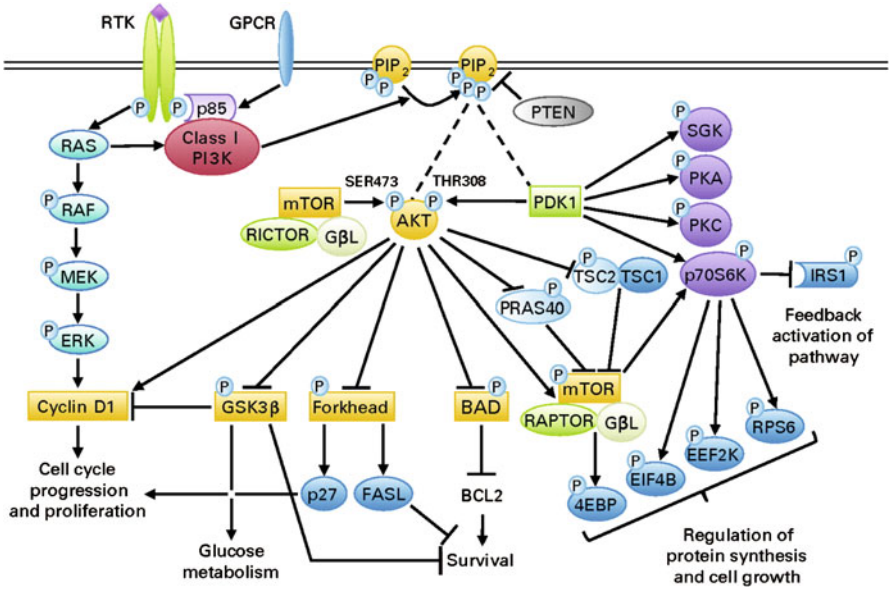

Fig. 7.1 Schematic diagram of the PI3K/PTEN/AKT/mTOR pathway. (Reprinted with permission, ©2012 American Society of Clinical Oncology. All rights reserved [181])

Once active, PI3K catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP_2) to phosphatidylinositol 3,4,5-trisphosphate (PIP_3). PIP_3 then serves to localize AKT and its activating kinase, 3-phosphoinositide-dependent kinase 1 (PDK1), to the cell membrane (Fig. 7.1). Following PDK1-mediated AKT activation at threonine 308 (T308) [10], AKT exerts considerable downstream effects on transcription, protein synthesis, metabolism, proliferation, and apoptosis. Aside from the protein phosphatases which carefully balance the activity of these kinases, the pathway is also negatively regulated by phosphatase and tensin homologue (PTEN), which catalyzes the dephosphorylation of PIP_3 to PIP_2 [11], [12].

Investigations of oncogenic PI3K have been focused largely on p110α (*PIK3CA*). Exome sequencing projects have determined this isoform represents the vast majority of cancer-related PI3K mutations [13]. It is currently estimated that p110α is mutated in 6–20 % of HNSCCs [14]–[17]. Unlike tumor suppressors, these mutations are not spread throughout the gene. Accordingly, 80 % of these cancer-associated modifications occur within three "hot spot" locations: E542, E545, and H1047. The first two mutations decouple p110 from p85, releasing the inhibitory effect of the regulatory subunit [18], [19]. The third mutation introduces a conformational change in the activation loop [18], possibly mimicking Ras-mediated activation [20]. Other cancer-specific mutations do exist within the gene; however, they have lower oncogenic activity and provide less of a selective advantage for tumorigenesis [21], [22].

7.2.2 PTEN

PTEN is a critical tumor suppressor, originally discovered because complete or partial deletion of chromosome 10 is a common event in a number of cancers, including brain, bladder, and prostate [23]. At least 80 % of Cowden's disease patients harbor heritable, germline mutations in PTEN which confer a rare familial cancer syndrome [24]–[26]. Although mostly known for catalyzing the reaction of PIP_3 back to PIP_2, this gene encodes a protein which possesses both peptide and phospholipid phosphatase activity (Fig. 7.1; [11], [12]). Loss of PTEN function causes an accumulation of PIP_3 at the cell membrane. This enriched pool of PIP_3 recruits AKT/mTOR pathway members (AKT isoforms, PDK1, etc.) to the cell membrane and inappropriately initiates the activation of this central signaling axis.

Knockout experiments have determined that PTEN, while essential for viable development, also has tumor suppressive functions in endometrial, liver, prostate, gastrointestinal, thyroid, and thymus tissues [27]. Haploinsufficiency is often sufficient to mediate a loss of PTEN function [28], [29]. Due to its singular importance, PTEN function is regulated, and consequently deregulated, by a myriad of mechanisms: mutation, deletion, epigenetic silencing, transcriptional, post-transcriptional, and microRNA (miRNA) regulation, post-translational modification, and various protein–protein interactions. The effect of PTEN on PI3K/AKT/mTOR pathway activity is well established; however, multiple tumor suppressors exist within this pathway. As PTEN is the most frequently deregulated tumor suppressor associated with this pathway, additional functions independent of PI3K/AKT/mTOR likely imbue PTEN with added functional importance. For example, a loss of PTEN causes PIP_2 depletion, an important membrane-associated regulator of cell polarity. This morphological modulation initiates a loss of epithelial characteristics, similar to epithelial-to-mesenchymal transition (EMT) [30], a hallmark of particularly aggressive cancers. Following EMT, neoplastic cells have increased cell motility and are often more resistant to standard therapy (reviewed in [31]). PTEN also localizes to the nucleus and is involved in maintaining chromosomal stability. Consequently, a loss of nuclear PTEN enhances chromosomal instability and leads to spontaneous DNA double-strand breaks [32]. Furthermore, PTEN only exhibits PIP_3 phosphatase activity in the cytoplasm, thus PTEN may affect genomic stability and cell cycle progression in the nucleus by lipid phosphatase-independent mechanisms [33]. Due to its numerous PI3K-dependent and -independent functions, PTEN is widely considered a critical tumor suppressor with loss of function often resulting in cancer.

7.2.3 AKT

AKT, also known as protein kinase B (PKB), is a critical node for mammalian signal transduction and the major effector of PI3K signaling. This vital serine/threonine protein kinase was originally discovered as the human homolog of *v-akt*, an oncogene transduced by the murine retrovirus AKT8 [34]–[37]. The AKT family is represented

by three isoforms: AKT1, AKT2, and AKT3. AKT1 is ubiquitously expressed at high levels [36]–[38], while the remaining isoforms are expressed in a more tissue-specific manner. Insulin-sensitive cells, such as liver, skeletal muscle, and adipose tissue demonstrate high levels of AKT2 expression [39], [40]. Meanwhile, AKT3 is highly expressed in the brain and testes, with lower levels of expression observed in muscle and intestinal organs [41]. While cancer-related AKT research largely focuses on AKT1, large-scale cancer sequencing projects have uncovered single nucleotide polymorphisms (SNPs) and somatic mutations associated with AKT2 and AKT3 [42], [43]. Mouse knockout models of the various AKT isoforms demonstrate specific mutant phenotypes, but are all viable [44]–[48]. Thus, the lack of embryonic lethality suggests that while each AKT isoform has characteristic signaling functions, they share a degree of functional compensation.

AKT kinases are comprised of an N-terminal pleckstrin homology (PH) domain, a flexible linker, and a C-terminal catalytic domain. While PIP_3 interacts with AKT via the PH domain [49], AKT is phosphorylated by PDK1 on the C-terminal activation loop (T308) and at serine 473 (S473) by mTORC2 (Fig. 7.1; [50]) to achieve full kinase activity [51]. While these mechanisms represent canonical AKT activation, a number of PIP_3-independent mechanisms also initiate AKT signaling. Activated CDC42 kinase 1 (Ack1 or TNK2) [52], [53], Src [54], protein-tyrosine kinase 6 (PTK6) [55], and serine/threonine-protein kinase 1 (TBK1) [56]–[58] all possess the ability to modulate AKT activity by noncanonical means. Once activated, AKT phosphorylates downstream targets altering cell survival, growth, proliferation, metabolism, and crosstalk with other signaling pathways. The most important downstream target of AKT is mammalian target of rapamycin (mTOR), a master regulator of cell growth, metabolism, translation initiation, and ribosome biogenesis. AKT also affects cell survival by negatively regulating proapoptotic proteins such as FOXO and MDM2, a negative regulator of p53 [59], [60]. AKT can also enhance cell-cycle turnover by phosphorylating glycogen synthase kinase 3 (GSK-3), which stabilizes cyclin D/E, c-jun, and c-myc proteins [61]–[64].

Recent evidence suggests that subcellular localization is an important determinant of AKT activity and downstream signaling. In fact, two important AKT substrates (FOXO proteins and p300) are sequestered solely in the nucleus [65], [66]. Despite lacking a nuclear localization signal, AKT likely translocates to the nucleus by interacting with members of the T-cell leukemia-1 (TCL1) family of oncoproteins. These proteins are capable of complexing with AKT to serve as coactivators, shuttling AKT to the nucleus [67], [68]. Increased nuclear phospho-AKT has been observed in acute myeloid leukemia [69], [70], lung [71], breast [72], thyroid [73], and prostate cancers [74]. Nuclear phospho-AKT detection has also been positively correlated with prostate cancer progression [74] and Gleason score [75]. Nuclear AKT activity may have specific oncogenic effects as promyelocytic leukemia protein (PML), which functions to dephosphorylate AKT within the nucleus and is a known tumor suppressor [76].

Due to the staggering number of pathways dependent on AKT signaling, deregulation of this enzyme by alterations in associative proteins or changes in subcellular

localization can have disastrous biological consequences. For example, mosaic expression of an AKT point mutant (AKT E17K) is responsible for almost 90 % of Proteus syndrome cases, the debilitating growth disorder suffered by Joseph Merrick, popularly known as "The Elephant Man" [77]. Proteus syndrome is characterized by segmental overgrowth and hyperplasia of a variety of tissues and organs, which also includes an increased risk of tumorigenesis [78], [79]. The rare nature of this crippling disease (< 1 case/1 million) lies in its dependence on mosaic expression, as constitutive somatic or germline expression of this mutant is lethal. Not surprisingly, this mutation has been detected in a variety of cancers including breast [80], urinary tract [81], and endometrial cancers [82]. Although the incidence of AKT E17K in patient tumor samples is low (1–4 %), it nonetheless represents an important component of the total PI3K/PTEN/AKT/mTOR deregulation that occurs during tumorigenesis.

7.2.4 mTOR

As mentioned above, mTOR is the single most important effector of AKT signaling. Serving as the catalytic subunit of two macromolecular complexes (mTORC1 and mTORC2), mTOR is a master regulator of cell growth. Although mTOR is shared between these two complexes, the associative proteins unique to each tune the activity of this enzyme for distinct substrates and sources of regulation [83]–[89]. mTORC1 consists of mTOR, Deptor, Raptor, mLST8, and PRAS40 [90], [91]. This complex is rapamycin sensitive [92]–[94], and S6K1 and 4E-BP1 are its most important downstream targets (Fig. 7.1; [95]–[97]). Phosphorylation of S6K1 promotes mRNA translation by facilitating initiation and elongation complex formation at the mRNA transcript. Activation of 4E-BP1 allows eIF4E to recruit eIF4G and initiate 5′ mRNA translation. Aside from protein synthesis, mTORC1 also regulates ribosome biogenesis and autophagy [98]–[100]. Recent studies have shown that mTORC1 activation is sufficient to inhibit autophagy, which is reversible following mTORC1 inhibition [101].

mTORC2 also contains Deptor and mLST8; however, additional associative proteins include Rictor, mSIN1, and Protor [89], [102], [103]. Differential phosphorylation of AKT (T308 vs. S473) had long been understood, with PDK1 mediating T308 activation. However, it was recently discovered that mTORC2 is the complex responsible for "PDK2" activity, phosphorylating AKT at S473 [50]. Consequently, this functionality places mTORC2 in a positive feedback loop within the pathway, allowing AKT to achieve full activation. This function was initially difficult to elucidate as mTORC2 is rapamycin insensitive during acute treatment [92]–[94]. Along with AKT, mTORC2 can also activate serum- and glucocorticoid-regulated kinase (SGK) and protein kinase C (PKC) [50], [104]–[106].

Because mTOR has a central role in controlling cell growth, appropriate regulation of mTOR itself is paramount to maintaining homeostasis. Thus, it is not surprising

that a number of familial cancer syndromes involve germline mutations of mTOR-negative regulators (Cowden disease, tuberous sclerosis) [26], [107]. Transgenic mice have also provided experimental evidence for the importance of appropriate mTOR regulation. Mice heterozygous for beclin or autophagy-related 4C (ATG4C), both critical regulators of autophagy, are prone to tumor formation due to defects in autophagosome formation [108]–[110]. As a negative regulator of autophagy, sustained mTORC1 activation has the ability to mimic these genetic modifications and enhance tumor development. Sustained mTORC2 activity is also capable of driving tumorigenesis through constitutive activation of AKT and SGK. Furthermore, expression of Rictor is required for tumor cell line and prostate tumor growth in PTEN-deficient mice [111], [112]. Consequently, tumor-associated defects in PI3K, PTEN, or AKT all have the potential to initiate pathological mTOR signaling. However, multiple routes of deregulation may provide important biomarkers and potential targets of therapeutic intervention to alleviate the oncogenic effects of mTOR signaling in HNSCC.

7.3 PI3K/PTEN/AKT/mTOR Deregulation in HNSCC

7.3.1 Genetic Alterations of PIK3CA in HNSCC

PI3K functions with critical importance to potentiate and regulate receptor-mediated extracellular stimuli. This vital second messenger has been intensively studied in cancer progression, including in HNSCC. PI3K, and more specifically *PIK3CA*, is a bona fide oncogene in HNSCC. As mentioned above, *PIK3CA* contains activating point mutations (commonly E542, E545, or H1047) in 6–20 % of HNSCC tumor samples (Table 7.1; [14], [15]). In fact, two sequencing projects independently identified *PIK3CA* as a significantly mutated oncogene in HNSCC tumor samples [16], [17]. Stransky et al. determined 8 % (6/74) of their tumor samples had *PIK3CA*-activating mutations: 1-R115 L (rare), 3-E542–545, 2-H1047 [16]. Agrawal et al. reported 6 % of their tumors harbored *PIK3CA* mutations: all three H1047 mutants [17]. In addition to these activating point mutations, copy number gains within the *PIK3CA* locus (3q26) are extremely common [113].

Current evidence suggests *PIK3CA* copy number gain is an early event in HNSCC development. Oral premalignant lesions commonly demonstrate an increase in *PIK3CA* copy number (39 %) [1]. An equivalent incidence of *PIK3CA* copy number gain is also noted in HNSCC tumors (32–37 %) [1], [114]. Along with alterations in ERK/MAPK, fibroblast growth factor (FGF), and p53, deregulated PTEN and PI3K/AKT pathway members delineate high-grade premalignant lesions from low-grade dysplasias [115]. Increased *PIK3CA* copy number is also associated with early HNSCC recurrence, but this difference is only statistically significant in patients without lymph node metastases ($p = 0.026$) [114].

Table 7.1 Investigations of PI3K/PTEN/AKT/mTOR genetic alterations in premalignant lesions and squamous cell carcinoma of head and neck

Pathway member	Investigator	Samples	Methods	Result	Reference
PIK3CA	Redon et al.	45 HNSCC tumors (no nodal involvement)	CGH, FISH	60 % 3q26 gain (PIK3CA locus)	182
	Woenckhaus et al.	15 PMLs 15 HNSCC tumors	FISH	3q26 CN gain low to mod. PML: 1/6 high-grade PML: 7/9 HNSCC:11/11 3q26 amp low to mod. PML:0/6 High-grade PML: 1/7 HNSCC: 6/11	113
	Pedrero et al.	38 PMLs 117 HNSCC tumors	qRT-PCR	Amplified 39 % PMLs Amplified 37% HNSCC tumors	1
	Sticht et al.	280 HNSCC TMA	FISH	39 % CN gain	183
	Qiu et al.	30 HNSCC tumors 8 HNSCC cell lines	gPCR/sequencing	Total mutated samples: 11 %	14
	Fenic et al.	33 HNSCC tumors	qRT-PCR gPCR/sequencing	36.4 % CN gain 9 % amplified 48.5 % mRNA overexpression No mutations detected	184
	Murugan et al.	37 HNSCC tumors 17 HNSCC cell lines	gPCR/sequencing	Mutant cell lines: 29.4 % Mutant tumors: 10.5 %	15
	Pattje et al.	140 HNSCC tumors	IHC	High p110α: 41 %	163
	Stransky et al.	74 HNSCC tumors	WES	Mutant tumors: 8 %	16
	Agrawal et al.	120 HNSCC tumors	WES, gPCR/sequencing	Mutant tumors: 6 %	17
	Kondo et al.	16 HNSCC cell lines	gPCR/sequencing	Mutant cell lines: 12.5 %	185
	Morris et al.	31 HNSCC tumors	CGH gPCR/sequencing	45.2 % CN gain Mutant tumors: 6 %	186
	Suda et al.	115 HNSCC tumors	qRT-PCR, CGH gPCR/sequencing	32.2 % CN gain Mutant tumors: 2.6 %	114
PTEN	Shao et al.	19 HNSCC tumors	PCR, gPCR/sequencing	LOH: 40 %, mutant tumors 16 %	116
	Lee et al.	41 HNSCC tumors	IHC	PTEN negative: 29 %	117
	Pedrero et al.	117 HNSCC tumors	MPA, PCR	LOH: 14 %, no detectable homozygous deletions	1

Table 7.1 (continued)

Pathway member	Investigator	Samples	Methods	Result	Reference
	Nathan et al.	22 HNSCC tumors 23 tumor-free margins	FISH	Tumor PTEN abnormalities: 68 % Margin PTEN abnormalities: 35 %	187
	Murugan et al.	37 HNSCC tumors 17 HNSCC cell lines	gPCR/sequencing	No mutations detected	15
	Pattje et al.	140 HNSCC tumors	IHC	PTEN low expression: 69 %	163
	Stransky et al.	74 HNSCC tumors	WES	Mutant tumors: 7 %	16
	Kondo et al.	16 HNSCC cell lines	gPCR/sequencing	Mutant cell lines: 12.5 %	185
	Snietura et al.	147 HNSCC tumors	IHC	PTEN low expression: 59.9 %	164
AKT	Gupta et al.	38 HNSCC tumors	IHC	2–3+ pAKT staining: 65.8 %	160
	Pedrero et al.	117 HNSCC tumors	qRT-PCR, WB	pAKT detected: 17 % AKT2 amplification: 30 %	1
	Molinolo et al.	305 HNSCC TMA	IHC	pAKT473 0: 1 %, 1–2: 31 %, 3–4: 66 % pAKT308 0: 4 %, 1–2: 57 %, 3–4: 42 %	188
mTOR	Clark et al.	72 HNSCC tumors	IHC	p-mTOR detected: 59 %	189
	Morris et al.	31 HNSCC tumors	CGH	Rictor CN gain: 38.7 %	186
Downstream Targets	Nathan et al.	27 Tumor-free margins	IHC	eIF4E overexpressed: 70 % All eIF4E overexpressing tumors Exhibited high p-p70S6K	162
	Molinolo et al.	305 HNSCC TMA	IHC	pS6 0: 2 %, 1–2: 13 %, 3–4: 79 %	188
	Clark et al.	72 HNSCC tumors	IHC	p-4EBP1: 36 %	189

CGH comparitive genomic hybridization, *FISH* fluorescent in situ hybridization, *PML* premalignant lesion, *CN* copy number, *qRT-PCR* quantitative real-time polymerase chain reaction, *TMA* tissue microarray, *gPCR* genomic PCR, *IHC* immunohistochemistry, *WES* whole-exome sequencing, *LOH* loss of heterozygosity, *MPA* microsatellite pattern analysis, *pAKT* phospho-AKT, *WB* Western blot, *p-mTOR* phospho-mTOR, *p-p70S6K* phospho-p70S6K, *p-S6* phospho-S6, *p-4EBP1* phospho-4EBP1

7.3.2 PTEN Loss

A loss of PI3K-negative regulation has been observed in a number of independent HNSCC studies, as alterations in PTEN status are common (Table 7.1). Early efforts to catalog PTEN deregulation in HNSCC began with a screen of 19 tumors, which determined PTEN was mutated in three samples [116]. A loss of heterozygosity (LOH) also occurred within the PTEN locus (10q23) in 6 of the 15 evaluable samples. Within the mutant PTEN patients, two had stage IV disease while the third had recurrent, metastatic and stage III disease. In a larger study, targeted analysis of PI3K/AKT/mTOR HNSCC genetic alterations detected PTEN LOH in 14 % of the samples [1]. Three of the eight patients with PTEN LOH also demonstrated abnormal PTEN levels in the adjacent mucosa, suggesting both *PIK3CA* and PTEN deregulation are early events in HNSCC development. An additional investigation in squamous cell carcinoma of the tongue determined PTEN loss was evident in 29 % of the tumor samples [117]. Deregulated PTEN also correlated with decreased overall survival ($p = 0.03$) and event-free survival ($p = 0.01$). While these studies were targeted in nature, PTEN loss was also evident in one of the two HNSCC genome sequencing projects referenced above. Stransky et al. detected PTEN mutations in 7 % of their tumor samples [16], while PTEN abnormalities were not detected by Agrawal et al. [17].

Although LOH and PTEN mutation have been described in many cancer systems, protein loss by miRNA deregulation is a relatively new field of study. These short, noncoding RNAs are capable of regulating a wide variety of proteins, and thus represent oncogenes and tumor suppressors in their own right. A recent study of HNSCC tumor samples and cell lines determined miR-21 is overexpressed with respect to normal tissue [118]. miR-21 overexpression downregulated HNSCC PTEN protein levels *in vitro*, activated phospho-AKT, and increased the proliferation of immortalized keratinocytes (HaCaT) [118]. Consequently, miR-21 has been described as a proto-oncogene in HNSCC. However, miR-21 is not the only miRNA capable of targeting PTEN protein expression. miR-9 is a frequently methylated gene in HNSCC tumor samples, with miR-9 expression levels closely correlating with methylation status [119]. When miR-9 is reexpressed with the use of a demethylating agent, a significant increase in PTEN and a concomitant decrease in cell growth is observed [119]. While the connection between miR-9 and PTEN is indirect, this study does provide additional evidence for miRNA-mediated PTEN modulation in HNSCC cells.

Recent evidence also suggests a powerful association between transforming growth factor-β (TGF-β) signaling and PTEN loss occurs in HNSCC development. The TGF-β superfamily of ligands and receptors represent a signaling pathway unified by a shared group of second messengers: SMADs. While SMAD4 knockdown is sufficient to develop HNSCC in mouse models [120], conditional knockdown of TGF-β receptor 1 (TGF-βRI) in the oral cavity of mice will only lead to early HNSCC development when combined with a topical carcinogen (DMBA) [121]. Consequently, this suggests that progressive disease requires an additional genetic

aberration provided by chemical treatment. Sixteen weeks after DMBA treatment, 45 % of the TGF-βRI conditional knockdown mice develop HNSCC. After 1 year, only 10 % of these mice develop HNSCC without DMBA treatment [122]. TGF-βRI knockdown tumors are characterized by an increase in AKT activity with a paradoxical upregulation of PTEN. However, when PTEN is conditionally knocked-down in combination with TGF-βRI loss, the mice develop benign papillomas within 4 weeks. After 10 weeks, 100 % of the mice develop HNSCC [122]. These tumors demonstrate an overexpression of EGFR and an activation of AKT, NF-κB, and STAT3 signaling, characteristic hallmarks of the human disease. Treatment of these animals with rapamycin effectively prevents tumorigenesis, thus carcinogenesis in this model is an mTOR-dependent event [123]. An overexpression of chemokines and recruitment of tumor-promoting myeloid-derived suppressor cells (CD11b +) is also observed in these carcinomas. Consequently, multiple routes of PI3K/PTEN/AKT/mTOR deregulation contribute to HNSCC initiation, development, and progression.

7.4 Differences in PI3K-Dependent Signaling Based on HPV Status

7.4.1 PI3K Signaling in HPV-negative HNSCC

The most common genetic abnormality associated with human papillomavirus (HPV)-negative HNSCC is a functional loss of p53 [16], [17], yet somatic ablation of this tumor suppressor in transgenic mice favors spontaneous tumor formation in the skin, rather than tumorigenesis in the oral mucosa [124]. However, when p53 loss is combined with constitutively active AKT (myrAKT), tumor formation within the oral cavity, palate, ventral side of the tongue, and lips is markedly increased [125]. These tumors also exhibit increased EGFR expression and potently activated NF-κB and STAT3 pathways, recapitulating the hallmarks of HPV-negative HNSCC. As the PI3K/PTEN/AKT/mTOR pathway can be activated through a multitude of mechanisms, these data suggest that any manner of AKT activation, when combined with p53 loss, may synergize to initiate HNSCC development and progression. This is consistent with the observation that both *PIK3CA* and PTEN deregulation are early events in HNSCC and targeting this pathway may have a role in chemoprevention for smokers.

The majority of patients with HPV-negative tumors have an extensive history of cigarette smoking, and these tumors are associated with an increased number of mutations compared to their HPV-positive cohorts [16], [17]. Tobacco use is a well-defined causal link for the development of HNSCC [126], and cigarette pack-years is a predictive variable for survival even among HPV-positive patients [127]. Studies have shown that nicotine and an additional tobacco carcinogen [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, NNK] are both capable of activating AKT by receptor-mediated signaling in normal human airway epithelial cells [128]. This mechanism

has since been observed in human head and neck epithelium as well, where activated AKT is four-times more likely in HNSCC-adjacent mucosa from smokers compared to nonsmokers [129]. Experiments with HNSCC cell lines have also determined NNK activation of AKT is PI3K dependent [129]. Furthermore, cigarette smoke condensate (CSC) also upregulates the multidrug-transporter ABCG2 in lung cancer and HNSCC-cell lines [130]. After CSC treatment, these cells are more resistant to doxorubicin and have upregulated drug efflux mechanisms; the latter effect can be abrogated by PI3K or nicotinic acetylcholine receptor inhibition [130]. As tobacco use is a strong predictor of HNSCC recurrence, and HNSCC patients have a 10–14 % risk of developing a second malignancy within 5 years of primary surgical treatment [127], [131], further studies of premalignant PI3K/AKT/mTOR activation may yield novel chemopreventive options to mitigate this public health challenge.

Initial studies in recurrence prevention have focused on the therapeutic value of 13-*cis*-retinoic acid (13-cRA). Early data suggested that second primary tumor (SPT) development or HNSCC recurrence may be prevented by 13-cRA treatment [132], [133]. However, in a follow-up phase III clinical trial, no significant difference in SPT or recurrence could be observed between placebo and low-dose 13-cRA in early-stage HNSCC patients [134]. A recent retrospective study characterized 137 SNPs as predictive biomarkers for recurrence in the aforementioned placebo cohort. While 22 SNPs were significantly associated with recurrence, 15 SNPs were detected in the majority of patients who recurred [135]. Ten of these fifteen SNPs were located in TSC1, a negative regulator of mTOR. When these SNPs were assayed in the 13-cRA treatment group, two of the TSC-1-associated SNPs yielded a 43 % decrease in SPT/recurrence with treatment. Variants of *PIK3CD* and *PTEN* were also associated with a decrease in SPT/recurrence risk with 13-cRA treatment [135]. Consequently, prospective analysis of early stage HNSCC for PI3K/PTEN/AKT/mTOR pathway genetic variants could increase the efficacy of 13-cRA chemoprevention.

7.4.2 PI3K Signaling in HPV-positive HNSCC

The incidence of HPV-negative HNSCC is decreasing worldwide following successful tobacco cessation campaigns; however, the overall incidence of HNSCC remains constant due to an increase in HPV-positive HNSCC [136], [137]. From 1988 to 2004, the incidence of HPV-positive HNSCC increased by 225 % in the USA, while the incidence of HPV-negative disease decreased by 50 % over the same time period [137]. HPV-positive HNSCC is primarily associated with tumors of the oropharynx, as HPV infection most commonly occurs in the palatine and lingual tonsils (reviewed in [138]). Mounting evidence suggests PI3K/PTEN/AKT/mTOR signaling has an important role in HPV infection and HPV-induced carcinogenesis.

Recent studies have demonstrated that EGFR and PI3K signaling are required for viral entry into the cell. Pretreatment of HaCaT or cervical cancer cells (HeLa) *in vitro* with an EGFR inhibitor (gefitinib) is sufficient to inhibit HPV-16 endocytosis [139]. Additionally, two different PI3K inhibitors (PI-103, wortmannin) are also

capable of preventing viral entry [139]. As the same mechanism was observed in cells from different anatomical sites, these data suggest EGFR and PI3K activity share a common regulatory requirement for high-risk HPV infection.

However, following viral transformation, the PI3K pathway continues to play an important role in HPV-related tumorigenesis. Gene expression profile analysis of HNSCC patient samples has determined that HPV-positive tumors experience an upregulation in genes associated with the 3q26–29 chromosomal region [140]. This locus contains *PIK3CA*, and confirmatory analysis with RT-PCR confirmed that *PIK3CA* is upregulated in HPV-positive tumors compared to HPV-negative samples [141]. Immunohistochemical (IHC) analysis of HNSCC tissue samples has also demonstrated a strong correlation between p16 upregulation (surrogate marker for HPV-infection) and activated eIF4E ($p = 0.03$) [142]. Although an association between phospho-AKT and p16 expression trended towards significance ($p = 0.06$), its lack of concordance could be caused by additional signaling factors that regulate AKT activity compared to eIF4E function. Consequently, HPV infection is associated with mTOR-dependent activation of mRNA translation, including the upregulation of transformation-related and prosurvival pathway members [143], [144].

In a larger follow-up study, neither phospho-AKT (pAKT S473) nor phospho-S6 (mTOR target) was associated with HPV-positive HNSCCs [145]. In addition, the HPV-positive tumors were not associated with an activation of EGFR, as observed in the HPV-negative samples. Thus, the hyperactivation of selective mTOR targets noted in the prior study may be due to a different mechanism. However, detection of phosphorylated proteins such as AKT and S6 in clinical specimens is challenging due to rapid dephosphorylation and technical variations between studies [146]. To determine if this pathway represents a viable clinical target, HPV-positive HNSCC and cervical cancer xenografts were treated with rapamycin and RAD001 (everolimus). Both xenograft models demonstrated a durable, cytostatic response following mTOR inhibition [145]. Consequently, mTOR inhibition may represent an important therapeutic option in HNSCC patients, particularly those with HPV-positive disease.

7.5 PI3K/AKT/mTOR Inhibition as a Novel Therapeutic Option in HNSCC

Due to the overwhelming preclinical evidence that PI3K/AKT/mTOR signaling represents an integral component of HNSCC signal transduction, a number of clinical trials are currently underway to evaluate the efficacy of small molecules which inhibit key nodes of this pathway (Table 7.2). Currently, rapamycin and its associated analogs (rapalogs) are the most investigated PI3K/AKT/mTOR-targeted agents in HNSCC clinical trials. Rapamycin is a secondary metabolite produced by *Streptomyces hygroscopicus*, isolated from a soil sample collected on Easter Island (Rapa Nui) [147]. Owing to the evolutionarily conserved nature of mTOR, rapamycin exhibits a broad range of antiproliferative activity, and this compound was

Table 7.2 Active clinical trials evaluating PI3K/AKT/mTOR targeted agents for which locally advanced, and recurrent and/or metastatic HNSCC patients are eligible. (All trial data available at www.clinicaltrials.gov)

Targeted agent	Additional targeted agent	Additional therapy	Inclusion criteria	Phase	Clinical trial identifier
mTOR inhibitor					
Everolimus			Advanced HNSCC	II	NCT01111058
			Advanced/recurrent HNSCC	II	NCT01051791
		IMRT, cisplatin	Advanced HNSCC	I	NCT00858663
		Docetaxel, cisplatin	Advanced HNSCC	I	NCT00935961
		Carboplatin, paclitaxel	Advanced HNSCC	I/II	NCT01333085
	Cetuximab	Carboplatin, paclitaxel	Recurrent HNSCC	I/II	NCT01283334
	Cetuximab	Cisplatin, paclitaxel	Advanced HNSCC	II	NCT01133678
	Erlotinib		Recurrent HNSCC	II	NCT00942734
	Vatalinib		Advanced solid tumors	I	NCT00655655
Temsirolimus			Recurrent HNSCC	II	NCT01172769
		Carboplatin, paclitaxel	Advanced/recurrent HNSCC	I/II	NCT01016769
	Cetuximab		Recurrent HNSCC	II	NCT01256385
	Cetuximab	Cisplatin	Advanced/recurrent HNSCC	I/II	NCT01015664
	Erlotinib		Platinum-refractory or ineligible HNSCC	II	NCT01009203
Sirolimus			Advanced HNSCC	I/II	NCT01195922
		Grapefruit juice	Advanced solid tumors	I	NCT00375245
Ridaforolimus	Cetuximab		Advanced HNSCC/NSCLC/CRC	I	NCT01212627
Metformin		Paclitaxel	Recurrent/met HNSCC	II	NCT01333852
PI3K inhibitor					
PX-866		Docetaxel	Advanced HNSCC/NSCLC	I/II	NCT01204099
	Cetuximab		Advanced HNSCC/CRC	I/II	NCT01252628
Dual PI3K/mTOR inhibitor					
NVP-BEZ235			Advanced/met solid tumors	I	NCT01343498
	NVP-BKM120	Paclitaxel	Advanced/met solid tumors	I	NCT01285466
	MEK162		Advanced solid tumors	I	NCT01337765
	Everolimus		Advanced solid tumors	I/II	NCT01508104

HNSCC head and neck squamous cell carcinoma, *IMRT* intensity modulated radiation therapy, *NSCLC* non-small cell lung cancer, *CRC* colorectal cancer

invaluable in elucidating the mechanisms of PI3K/AKT/mTOR signaling. This molecule is an allosteric inhibitor of mTOR, creating a complex with FKBP12 which binds and prevents mTOR activation via the FKBP12-rapamycin-binding (FRB) domain. As this functional domain is unique to mTOR, rapamycin-induced inhibition of mTORC1 is highly selective; however, as reviewed above, mTORC2 is largely uninhibited by this compound in the acute setting [148].

A panel of rapamycin analogs have been synthesized, however intellectual property concerns were not the driving factors in rapalog development. The current array of rapalogs were designed to improve the pharmacokinetics of the parent compound. Temsirolimus (Torisel; Wyeth) is a water-soluble ester of rapamycin (sirolimus) for oral or IV administration (reviewed in [149]). Everolimus (Afinitor; Novartis) is a hydroxyethyl ether derivative, also demonstrating increased solubility relative to the parent compound (reviewed in [150]). Current HNSCC clinical trials are investigating these compounds as single agents or in combination with previously established radiation and chemotherapy regimens. Metformin, while not a direct inhibitor of mTOR, is also being investigated as a chemotherapeutic in this patient setting. Metformin is currently used to control type II diabetes as this compound indirectly inhibits mTORC1 by increasing intracellular AMP levels. Current evidence suggests metformin-induced mTOR inhibition can be mediated by AMPK-dependent and -independent mechanisms [151]–[153]. Additionally, this drug has demonstrated chemopreventive activity for a number of different cancers in diabetic patients (reviewed in [154]). Thus, additional studies are warranted to determine whether this well-characterized compound will have similar chemopreventive or chemotherapeutic effects in non-diabetic patients.

Additional compounds acting upstream of mTOR are also being evaluated in HNSCC. MK-2206 is an allosteric AKT inhibitor developed by Merck. Synergistic anticancer properties have been observed *in vitro* when this compound is used in combination with erlotinib [non-small cell lung cancer, (NSCLC)] or lapatinib (breast cancer) [155]. PX-866 is a synthetic derivative of wortmannin with antineoplastic activity and reduced liver toxicity with respect to the parent compound [156]. Aside from increased safety, PX-866 also demonstrates superior water solubility, bioavailability, and AKT inhibition. However, due to the positive feedback and compensation that can occur via mTORC2, single target inhibition has demonstrated acquired resistance in preclinical and clinical trial investigations. Consequently, additional studies are investigating dual-target inhibitors. NVP-BEZ235 is an orally available, dual PI3K/mTOR inhibitor which reversibly inhibits class I PI3K through ATP competition. This compound is unique because it simultaneously inhibits mTOR catalytic activity while preserving off-target protein kinase function [157], although initial studies suggest the potency of NVP-BEZ235 is not equivalent for each target. In breast cancer cells, NVP-BEZ235 exerts anti-mTOR activity at lower doses (< 100 nM) while dual PI3K/mTOR blockade occurs at higher concentrations (> 500 nM) [158].

7.6 PI3K Pathway Biomarkers

7.6.1 Activation of PI3K Pathway Prognostic Biomarkers

Studies evaluating deregulated PI3K/AKT/mTOR pathway members as prognostic biomarkers in HNSCC are currently ongoing. However, validated data are sparse due to small sample sizes, technical limitations, and the intrinsic biological heterogeneity of HNSCC. To date, the most established prognostic biomarkers for HNSCC outcome are EGFR overexpression and HPV status [127], [159], and the contribution of PI3K/PTEN/AKT/mTOR signaling needs to be evaluated in the context of these well-characterized biomarkers for clinical translation. Initially, investigators have looked at hyperphosphorylation of PI3K/AKT/mTOR pathway members to establish pathway activation. One potential prognostic biomarker is phospho-AKT. Activated AKT is associated with poor local control in HNSCC tumor samples [160]. eIF4E, a downstream target of mTOR, is also upregulated in many HNSCC tumor samples [161]. This protein is also associated with disease recurrence when upregulated eIF4E is detected in tumor-free margins [162].

However, phospho-protein detection in clinical samples can present a challenge as outcomes can differ depending on the fixation protocol and handling time *ex vivo*. Thus, biomarkers utilizing total protein fractions may be preferable from a technical standpoint. One controversy in the literature involves the prognostic value of PTEN in predicting outcome following HNSCC surgery and radiotherapy. In a recent IHC analysis of 140 HNSCC tissue microarray samples, PTEN-positive tumors were associated with worse locoregional control (LRC) than PTEN-negative tumors following surgery and radiation therapy (HR: 2.4) [163]. Additionally, phospho-AKT was also associated with poor LRC (HR: 2.2) and the authors suggest PTEN-positive tumors exhibit increased EGFR activity and subsequent PI3K/AKT/mTOR activation provides a protective effect from ionizing radiation. However, a similar study of 147 HNSCC patients also treated with surgery and radiotherapy observed the opposite association between PTEN and LRC. In this study, the 5-year LRC-free rate for PTEN-low tumors was 52.3 %, while 80.9 % of PTEN-high patients were recurrence free over the same time period ($p = 0.0007$) [164]. Furthermore, PTEN-status did not correlate with 5-year risk of metastasis in this study ($p = 0.49$) [164]. The observed discrepancy between these two studies highlights the difficulty in utilizing a tumor suppressor as a predictive biomarker. In the first study, the authors utilized a 7.5 % tumor cell cutoff to characterize a tumor as PTEN positive or negative by IHC [163]. In the latter study, the intensity of staining was scored by a pathologist, and tumors were described as PTEN high or PTEN low. As PTEN haploinsufficiency can be tumorigenic [28], [29], and techniques for accurate, quantitative assessment of this target from clinical samples are lacking, further studies are required to establish this protein as a bona fide prognostic marker in HNSCC.

On the other hand, detection of *PIK3CA* mutations is more straightforward with current technology. As reviewed above, mutations in exon 9 and 20 are the predominant *PIK3CA* lesions associated with most cancers, and current evidence suggests

these species may contribute differently to clinical outcome. Although important, the lower incidence of these mutations in HNSCC will require large sample sizes to achieve the statistical power needed to delineate any prognostic significance between *PIK3CA* alterations. For example, studies in breast cancer have shown that *PIK3CA* exon 9 mutations (E542/E545) are independently associated with shorter disease-free survival ($p = 0.0003$) and overall survival ($p = 0.001$) [165]. Conversely, exon 20 mutations (H1047) are associated with better overall survival. In this case, the relevance of these biomarkers is intrinsically linked with breast cancer-specific treatments; however, early evidence suggests these mutations need to be evaluated independently rather than in the context of a simplified binomial analysis.

7.6.2 PI3K Pathway Members as Predictive Biomarkers for Radiation Therapy and PI3K/mTOR Inhibitor Response

The association of increased phospho-AKT and poor LRC suggests that AKT activation may be a predictive marker of radiation resistance in HNSCC. While *in vitro* studies have indicated PI3K inhibition increases the radiosensitivity of HNSCC cell lines [160], a recent case report may provide preliminary clinical evidence for an expansion of PI3K/AKT/mTOR combination therapies in HNSCC patients. In this report, the authors describe a patient treated with radiotherapy for squamous cell carcinoma of the larynx (T2N0M0). This patient had also received a liver transplant and was being treated with sirolimus to prevent transplant rejection. After seven fractions of radiation, the patient experienced a complete response, an early response time compared to historical norms. However, treatment-associated toxicities required cessation of radiotherapy [166]. Future investigations of mTOR inhibition as a radiosensitizer in HNSCC treatment may establish an optimal treatment regimen and determine the maximum tolerated dose for this disease.

Aside from radiotherapy, PI3K pathway deregulation may also serve as a biomarker for response to PI3K/AKT/mTOR-targeted agents. A recent retrospective analysis determined the PI3K mutational status of various solid tumors from clinical trials investigating PI3K/AKT/mTOR inhibitors [167]. Of the 1,012 patients in this study, 105 were prospectively selected and 66 of these patients harbored *PIK3CA* mutations. Although these patients had tumors of varying anatomical location, those possessing an exon 20 mutation responded better to PI3K/AKT/mTOR therapies than other PI3K mutants (PR rate: 38 vs. 10 %, $p = 0.018$). Unfortunately, an increase in progression-free survival only trended towards statistical significance (5.7 vs. 2 months, $p = 0.06$). While this study was hampered by a heterogeneous tumor population and multiple treatment regimens, PI3K/AKT/mTOR-treatment efficacy in patients with exon 20 mutations is an intriguing finding that requires further study. Of the 66 prospectively selected patients from this study, four individuals had HNSCC and the best responder possessed an H1047R mutation. Although these data indicate PI3K mutations may sensitize HNSCC tumors to PI3K/AKT/mTOR inhibitor treatment, wild-type PI3K status may not preclude the use of these drugs in this

patient population. While HNSCC cell lines have demonstrated *in vitro* sensitivity to PI3K inhibition, the efficacy of this treatment option is enhanced when combined with vorinostat, a histone deacetylase (HDAC) inhibitor [168]. This combination treatment is capable of increasing reactive oxygen species (ROS) production in a manner previously observed with other efficacious cytotoxic chemotherapeutics [169], and is only toxic to HNSCC cell lines, not keratinocytes [168].

7.6.3 *Predictive Biomarkers for Receptor Tyrosine Kinase (RTK) Inhibitor Resistance*

While the identification of predictive biomarkers for RTK inhibitor response is of paramount concern, equally important is the investigation of biomarkers for resistance. For example, additional HER receptors, aside from EGFR, signal through PI3K in HNSCC. A positive feedback loop has been reported between HER2 and ADAM12 in HNSCC cell lines. ADAM12 is a multifunctional protein with an intracellular domain capable of second messenger signaling and an extracellular domain capable of cleaving extracellular matrix substrates and activating EGFR ligands [170]–[172]. HER2 and ADAM12 have the ability to upregulate each other in HNSCC cell lines, and this positive feedback loop is dependent on PI3K and JNK signaling [173]. Additionally, ADAM12 upregulation confers increased migratory and invasive phenotypes to these cells. This signaling mechanism may have clinical significance as HER2 activation and total HER3 expression are predictive of *de novo* resistance to gefitinib (EGFR-targeted TKI) [174]. While upregulated ligand converting enzymes can potentially serve as biomarkers of therapy resistance, their cleavage products can also subvert targeted therapeutic response. For example, an upregulation of heparin-binding EGF (HB-EGF) has been observed in HNSCC cell lines with acquired resistance to cetuximab [175]. Increased serum HB-EGF plasma levels are also detected in patients with recurrent disease compared to those who are newly diagnosed.

A strong relationship also exists between Met and PI3K signaling in HNSCC. Consequently, Met activation represents another potential source of PI3K-mediated RTK inhibitor resistance. To address this concern, potent Met inhibitors (SU11274 and PF-2341066) have been developed and pretreatment of HNSCC cell lines with these compounds *in vitro* does prevent ligand-induced AKT activation [176], [177]. However, the degree of concordance between AKT inhibition and pharmacologic Met inhibition depends on which AKT phosphorylation site is studied. One investigation demonstrated consistent AKT inhibition with Met inhibitors when utilizing the mTORC2 phosphorylation site (S473) as a readout of AKT activity [176]. Meanwhile a similar investigation observed modest AKT inhibition across a panel of HNSCC cell lines while employing the PDK-1 phosphorylation site (T308) as a marker [177]. Due to the differential regulation of these sites, it is quite possible both observations are valid and these parallel studies provide further insight into the PI3K/PTEN/AKT/mTOR signaling occurring downstream of Met. From a clinical

perspective, the dual regulation of AKT may explain why combined treatments of EGFR and Met TKIs have potent, additive effects on HNSCC growth inhibition [177], [178]. Met activation following the addition of EGFR ligands has also been observed, suggesting crosstalk between these two receptors may have an important functional role [178]. As discussed above, while multiple pathways interact with Met, current evidence suggests PI3K/AKT/mTOR signaling is specifically capable of mediating pathologic signal transduction downstream of this receptor.

An additional pathway providing inhibitor resistance during HNSCC treatment is the TGF-β pathway. Aside from ligand-mediated signaling, noncanonical TGF-β activation can occur through the downstream pathways shared with EGFR and Met (MAPK, PI3K/AKT, and Rho GTPase) [179]. However, recent evidence suggests that TGF-β-induced changes in the tumor microenvironment can inhibit ADCC while simultaneously activating tumor-associated AKT signaling [180]. In this paradigm, TGF-β1 reduces the efficacy of immune-associated responses to cetuximab treatment while concurrently providing a proliferative signal to the tumor. In support of this hypothesis, HNSCC xenografts selected *in vivo* for cetuximab-resistance display increased TGF-β expression and TGF-β-dependent AKT activation [180]. This resistance is reversible with a TGF-β inhibitor, providing strong preclinical evidence for this therapeutic option in cetuximab-refractory HNSCCs.

7.7 Conclusion

Overwhelming evidence suggests the PI3K/PTEN/AKT/mTOR pathway is commonly deregulated in HNSCC. Although excellent preclinical and clinical studies have begun evaluating the therapeutic potential of this pathway in HNSCC, additional work is required to verify nodes of oncogenic dependency and addiction in this signaling network. Once these targets are fully validated in the laboratory, we can translate these findings to the clinic and identify the appropriate population of need. While single agent therapies targeting this pathway may represent future clinical endeavors, these compounds may also serve to enhance the efficacy of standard therapy options in use today. Whether these targeted agents will prove efficacious in HNSCC treatment is not clear, but the role of PI3K/PTEN/AKT/mTOR pathway deregulation in HNSCC certainly warrants further investigation.

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