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INVITED MEDICAL REVIEW

The PI3K/Akt/mTOR axis in head and neck cancer: functions, aberrations, cross-talk, and therapies

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Head and neck squamous cell carcinoma (HNSCC) is one of the most morbid, mortal, and genetically diverse malignancies. Although HNSCC is heterogeneous in nature, alterations in major components of the PI3K/Akt/mTOR pathway are consistently observed throughout the majority of HNSCC cases. These alterations include genetic aberrations, such as mutations or DNA copy number variations, and dysregulation of mRNA or protein expression. In normal physiology, the PI3K/Akt/mTOR axis regulates cell survival, growth, and metabolism. However, alterations in this pathway lead to the malignant phenotype which characterizes HNSCC, among many other cancers. For this reason, both pharmaceutical companies and academic institutions are actively developing and investigating inhibitors of PI3K, Akt, and mTOR in preclinical and clinical studies of HNSCC. Many of these inhibitors have shown promise, while the effects of others are tempered by the mechanisms through which HNSCC can evade therapy. As such, current research aimed at elucidating the interactions between PI3K/Akt/mTOR and other important signaling pathways which may drive resistance in HNSCC, such as p53, NF-κB, and MAPK, has become a prominent focus toward better understanding how to most effectively treat HNSCC.

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Keywords: head and neck cancer; PI3K; Akt; mTOR; NF-κB; p53

Introduction

Head and neck cancer is the sixth most common cancer worldwide and has a 5-year morbidity rate of ~50% (Siegel *et al*, 2013). The histological diagnosis of head and neck squamous cell carcinoma (HNSCC) accounts for over 95% of all clinical cases of head and neck cancer and is limited anatomically to one of five primary sites: oral cavity, oropharynx, nasopharynx, hypopharynx, or larynx (Leemans *et al*, 2011). In terms of genetic alterations, HNSCC is one of the most heterogeneous of all cancers (Vogelstein *et al*, 2013). However, The Cancer Genome Atlas (TCGA; cancergenome.nih.gov) and other studies recently revealed that the majority of HNSCC possess alterations in the PI3K/Akt/mTOR pathway (Grandis *et al*, 2012; Hayes *et al*, 2013), a critical regulatory axis for cell growth, survival, motility, and metabolism in both normal physiology and cancer.

Phosphoinositide 3-kinase (PI3K), is composed of two subunits – an 85-kDa regulatory subunit and a 110-kDa catalytic subunit. It is activated in response to somatic mutation, stimulated receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor 1 (IGF-1R), or G-protein-coupled receptors (GPCRs). PI3K catalytic subunit alpha isoform *(PIK3CA)*, the gene that programs for the p110α isoform of PI3K, is one of the most frequently mutated and amplified oncogenes in human cancers (Yuan and Cantley, 2008), including HNSCC. In normal physiology, PI3K is regulated by the tumor suppressor, Phosphatase and tensin homolog (PTEN). However, inactivating mutation or loss of *PTEN* is a frequent alteration in cancer and leads to hyperactivity of the PI3K pathway (Courtney *et al*, 2010). Protein kinase B (Akt) and mammalian target of rapamycin (mTOR) are effector proteins downstream of PI3K and also play important roles in carcinogenesis. Together, the members of the PI3K/Akt/mTOR axis interact with and contribute to the regulation of several other signaling molecules in HNSCC, including tumor suppressor, tumor protein p53 [p53 (*TP53*)], nuclear factor-kappa B (NF-κB), and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) (Figure 1). For these reasons, there has been a surge in the development and use of pharmacologic inhibitors targeting PI3K, Akt, and mTOR for cancers such as HNSCC (Table 1). Initial preclinical and clinical studies indicate

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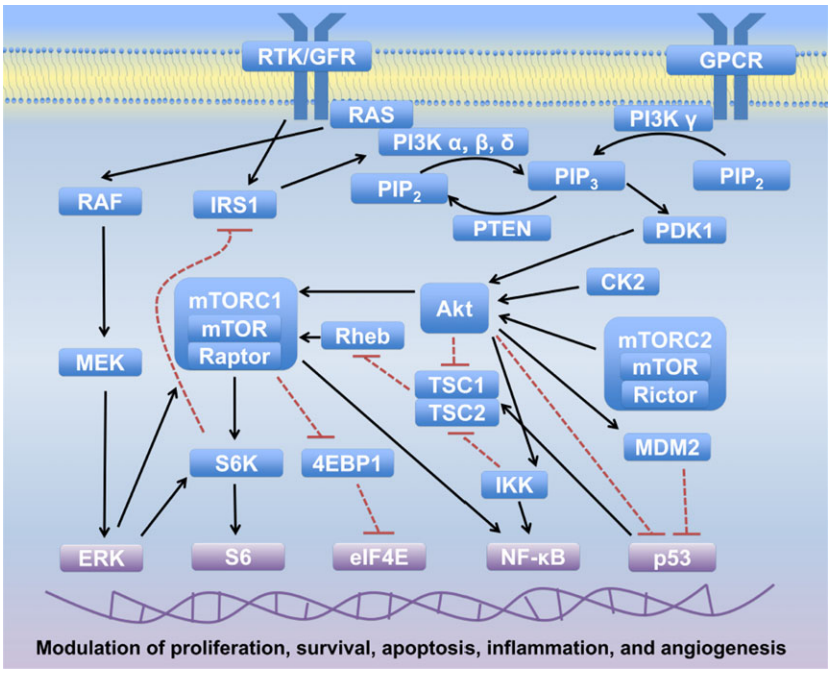

Figure 1 Interactive signaling of PI3K/Akt/mTOR and other pathways. Relationships between well-characterized molecular signaling routes in HNSCC (EGFR/PI3K/PTEN/Akt/mTOR, RAS/RAF/MEK/ERK, IKK/NF-κB, and MDM2/p53) are shown by black arrows (activating interactions) and dashed red lines (negative regulation). PI3K/Akt/mTOR activation or inhibition ultimately regulates the activity of many kinases and transcription factors (purple) important in the nuclear control of cell survival and the malignant phenotype

promise for PI3K and mTOR inhibitors to benefit patients with HNSCC.

EGFR/PI3K/Akt/mTOR signaling in normal physiology

The EGFR/PI3K/Akt/mTOR signaling cascade is critical to cell growth, development, survival, and differentiation. Epidermal growth factor receptor (EGFR), also known as ErbB-1 or HER1, contains an extracellular ligand-binding domain, a hydrophobic transmembrane portion, and a cytoplasmic domain containing tyrosine kinase activity and multiple phosphorylation sites (Kalyankrishna and Grandis, 2006; Normanno *et al*, 2006; Freudlsperger *et al*, 2011). At least 30 ligands of the four ErbB family members have been characterized (Rogers *et al* 2005). Ligand binding triggers receptor activation through dimerization and auto- or cross-phosphorylation of the tyrosine kinase domains (Lu *et al*, 2012), which initiates EGFR signal transduction through multiple intracellular pathways, including the PI3K/Akt/mTOR pathway.

There are three classes of PI3Ks (I, II, and III), classified by structure and substrate preference. Class I PI3Ks, the subject of this review, are divided into subclasses IA and IB (Vanhaesebroeck *et al*, 2012). While initially postulated that IA enzymes are activated by RTKs, and IB enzymes are stimulated through GPCRs, recent work shows crossover in these stimuli (Katso *et al*, 2001). PI3K IA catalytic subunits include p110α (*PIK3CA*), p110*β*, and p110*δ* – all of which contain an N-terminal p85 regulatory subunit-binding domain and a Ras-binding domain (Domin and Waterfield, 1997; Fruman *et al*, 1998). p110α and p110*β* are ubiquitously expressed, while p110*δ* is mainly found in leukocytes, as it is a key player in lymphoid differentiation and development (Vanhaesebroeck *et al*, 2010). PI3K class IB includes the catalytic p110*γ* subunit, which is similar to the p110α-*δ* subunits, but does not contain a p85-binding site (Katso *et al*, 2001). Activated EGFR recruits and activates class IA PI3K at the membrane through direct binding of the Src homology 2 domains of p85 with the phosphotyrosine residues of the activated receptor (Engelman, 2009). Upon activation, the catalytic p110 subunit phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP_2), converting it into phosphatidylinositol-3,4,5-trisphosphate (PIP_3). Tumor suppressor PTEN antagonizes PI3K function by dephosphorylating PIP_3 to regenerate PIP_2. PIP_3 functions as a second messenger with binding sites for signaling proteins with pleckstrin homology (PH) domains including 3-phosphoinositide-dependent protein kinase-1 (PDK1) and Akt (protein kinase B) (Liu *et al*, 2009).

Akt is a serine/threonine protein kinase with three isoforms (Akt1, Akt2, and Akt3) (Bellacosa *et al*, 1991), which require phosphorylation for activation (Scheid and Woodgett, 2001). Akt's N-terminal region contains a PH domain that interacts with PI3K products and initiates its recruitment to the plasma membrane, conformational change, and subsequent phosphorylation. Upon binding with PIP_3, Akt is phosphorylated at three different sites: threonine 308 stabilizing the activation loop of the catalytic kinase domain by Phosphoinositide dependent kinase (PDK1) (Vivanco and Sawyers, 2002), serine 473 in the hydrophobic COOH-terminal domain by mTORC2 (mTOR complex 2) (Sarbassov *et al*, 2005), and serine 129 between the PH and catalytic domains by casein kinase 2 (CK2) (Di Maira *et al*, 2005). Activated Akt has multiple downstream targets involved in cell survival, growth, proliferation, angiogenesis, metabolism, and migration (Manning and Cantley, 2007).

A major downstream effector of Akt is mTOR, also a serine/threonine protein kinase, and the catalytic subunit of mTOR complex 1 (mTORC1). mTORC1 regulates protein translation through phosphorylation of protein synthe-

Table 1 Currently ongoing clinical trials of PI3K/Akt/mTOR inhibitors targeting HNSCC and solid tumors. All ongoing clinical trials of PI3K/Akt/mTOR inhibitors that include any patients with HNSCC are listed, unless noted otherwise. The three most common adverse events associated with each agent are presented in order of frequency reported, to date. Clinical trial IDs are indexed by http://clinicaltrials.gov (National Institutes of Health)

Drug (company)	*Combination*	*Status*	*Clinical trial ID*	*Condition*	*Adverse events*
PI3K Inhibitors					
BKM120 (Novartis, East Hanover, NJ, USA)	Cetuximab	Phase I/II	NCT01816984	Recurrent or metastatic HNSCC	Rash, hyperglycemia, diarrhea
	Single agent	Phase II	NCT01737450	Metastatic, recurrent, or progressive HNSCC	
PX-866 (Oncothyreon, Seattle, WA, USA)	Cetuximab	Phase I/II	NCT01252628	Incurable, progressive, recurrent or metastatic HNSCC	Diarrhea, nausea, vomiting
	Docetaxel	Phase I/II	NCT01204099	Locally advanced, recurrent or metastatic HNSCC	
BYL719 (Novartis)	Cetuximab	Phase Ib/II	NCT01602315	Recurrent or metastatic HNSCC	Nausea, hyperglycemia, diarrhea
	Single agent	Phase Ia	NCT01219699	Advanced solid malignancies, including HNSCC, with an alteration of *PIK3CA*	
mTOR Inhibitors					
Everolimus (Novartis)	Single agent	Phase II	NCT01051791	HNSCC	Rash, mucositis, fatigue
	Single agent	Phase II	NCT01133678	HNSCC	
	Carboplatin, cetuximab	Phase I/II	NCT01283334	Advanced HNSCC	
	Carboplatin, paclitaxel	Phase I/II	NCT01333085	Unresectable or inoperable locally advanced HNSCC	
	Cetuximab	Phase I	NCT01637194	Metastatic or recurrent HNSCC	
	Erlotinib	Phase II	NCT00942734	HNSCC	
Rapamycin (Pfizer, New York, NY, USA)	Single agent	Phase I/II	NCT01195922	Previously untreated HNSCC	Hyperglycemia, nausea, anemia
Temsirolimus (Pfizer)	Single agent	Phase II	NCT01172769	HNSCC	Rash, mucositis, mood disturbance
	Cetuximab	Phase II	NCT01256385	Recurrent or metastatic HNSCC	
	Carboplatin, paclitaxel	Phase I/II	NCT01016769	HNSCC	
PI3K/mT0R Dual Inhibitors					
BEZ235 (Novartis)	Everolimus	Phase Ib	NCT01508104	Metastatic or unresectable advanced solid tumors[a]	Fatigue, nausea, diarrhea
PF-04691502 (Pfizer)	Single agent	Phase I	NCT00927823	Advanced solid tumors including HNSCC	Fatigue, nausea, vomiting
PF-05212384 (Pfizer)	Docetaxel, cisplatin, dacomitinib	Phase I	NCT01920061	Advanced solid tumors[a]	Mucositis, nausea, fatigue
	PD-901, irinotecan	Phase I	NCT01347866	Advanced solid tumors[a]	
SAR245409 (Sanofi, Bridgewater, NJ, USA)	Pimasertib	Phase I	NCT01390818	Advanced solid tumors[a]	Rash, diarrhea, fatigue
Akt Inhibitors					
MK2206 (Merck, Whitehouse Station, NJ, USA)	Single Agent	Phase II	NCT01349933	Recurrent or metastatic HNSCC	Rash, nausea, pruritis

HNSCC, Head and neck squamous cell carcinoma.
[a]Open to all solid tumors including non-small-cell lung carcinoma, but might not include Head and neck squamous cell carcinoma HNSCC.

sis components based on growth factor and nutrient availability (Hennessy *et al*, 2005). mTORC1 and mTORC2 are structurally and functionally unique complexes; mTORC1 contains raptor (rapamycin-sensitive adaptor protein of mTOR), while mTORC2 binds rictor (rapamycin-insensitive companion protein of mTOR). Activation of mTORC1 occurs through Akt-mediated inactivation of the tuberous sclerosis complex (TSC). The TSC consists of a heterodimer of tumor suppressor proteins hamartin (TSC1) and tuberin (TSC2) and antagonizes mTOR signaling by inhibiting the Ras-like small GTPase, Ras-homolog enriched in brain (Rheb), a direct regulator of mTOR signaling (Li *et al*, 2004; Pan *et al*, 2004). Well-characterized targets of mTORC1 include ribosomal protein S6 kinase (RPS6K or S6K) and eukaryote initiation factor 4E-binding protein 1 (EIF4EBP1 or 4EBP1), both key regulators of protein translation. S6K phosphorylation of the S6 protein increases translation of ribosomal proteins and translation regulators (Meyuhas, 2000; Inoki *et al*, 2005). 4EBP1, a translation inhibitor, binds and inhibits eukaryotic translation initiation factor 4E (eIF-4E), which initiates translation (Gingras *et al*, 1999). mTORC2 regulates actin cytoskeletal reorganization and serves as a hydrophobic motif kinase for Akt at serine 473 (Bhaskar and Hay, 2007).

EGFR/PI3K/Akt/mTOR pathway aberrations in HNSCC

Recent developments in deep sequencing of tumor tissues have paved a powerful path for discovery of the most frequent genetic alterations in HNSCC. Unbiased high-throughput approaches have revealed that *PIK3CA* is one of the most commonly mutated and amplified genes in the

protein-coding human HNSCC genome (Agrawal *et al*, 2011; Stransky *et al*, 2011), which validates earlier *PIK3CA*-targeted studies (Okami *et al*, 1998; Kozaki *et al*, 2006; Qiu *et al*, 2006; Murugan *et al*, 2008; Cohen *et al*, 2011; Morris *et al*, 2011). *PIK3CA* missense mutations are concentrated in three 'hotspots' on chromosome 3q26 (H1047R, E545K, and E542K) known to affect constitutive PI3K activity (Samuels *et al*, 2004). Recent mutation analysis of 279 patients with HNSCC by TCGA has provided evidence that *PIK3CA* mutations are among the most frequent mutations in HNSCC (18% in HPV-negative cancers and 37% in HPV-positive cancers) (Grandis *et al*, 2012; Hayes *et al*, 2013). Altogether, genetic changes (mutation or DNA copy number variation) in major components of the PI3K pathway are represented in 66% of the TCGA HNSCC cases studied (Figure 2). When including changes in mRNA expression, this statistic jumps to over 90% (http://www.cbioportal.org), with alterations in one component of the pathway often being mutually exclusive with changes in a different component. *PIK3CA* DNA copy number is amplified in 20% of TCGA cases and mRNA expression is upregulated in 52% the TCGA tumors (Iglesias-Bartolome *et al*, 2013), with reduced PTEN expression in ~30% of another HNSCC cohort (Squarize *et al*, 2013). As a whole, the cumulative changes in PI3K/Akt/mTOR and molecules that communicate with this axis are represented at a significant rate in HNSCC. Consequently, it serves as an attractive target for molecular-oriented therapy.

A recent analysis showed that druggable events (copy gain with increased expression or activating mutation) associated with the *PIK3CA* or *AKT1/2/3* loci could be targeted in 12/35 (34%) HNSCC tumors sequenced (Pickering *et al*, 2013). In a functional test of the druggability of *PIK3CA* mutations in HNSCC patient tumor grafts, mutated rather than wild-type *PIK3CA* conferred a greater sensitivity to the PI3K/mTOR inhibitor BEZ-235 (Lui *et al*, 2013). The same result held true for the PI3K inhibitor PX-866 in HNSCC patient tumor grafts (Keysar *et al*, 2013). Thus, the predilection of PI3K inhibitors activity for HNSCC with *PIK3CA* activating mutations provides one rationale for using *PIK3CA* mutational status as a predictor of treatment selection. When considering the importance of human papilloma virus (HPV) oncoproteins E6 and E7 in the etiology of a subset of HNSCC, and the association of HPV with PI3K pathway mutations, this rationale is strengthened. In a directed analysis of 15 genes in 64 HNSCC tumors, *PIK3CA* mutation was the most abundant mutation, found at a much higher frequency in the oropharynx than in any other primary site (McBride *et al*, 2013). This observation is consistent with a putative association between higher rates of HPV infection in oropharynx than in other head and neck cancer anatomic sites (Gillison *et al*, 2000), and higher rates of *PIK3CA* mutation in HPV+ HNSCC than in HPV- HNSCC (Lui *et al*, 2013; Nichols *et al*, 2013).

PI3K/Akt/mTOR aberrations promote the malignant phenotype

Functionally, aberrations in the PI3K/Akt/mTOR pathway are associated with many of the malignant characteristics of HNSCC, including immune suppression and inflammation, angiogenesis, survival, invasion, and metastasis. Inflammation, in specific, regulates many of these processes simultaneously (Grivennikov *et al*, 2010). In contrast to ubiquitously expressed PI3K p110-α and -β isoforms, p110-δ is mainly present in leukocytes and

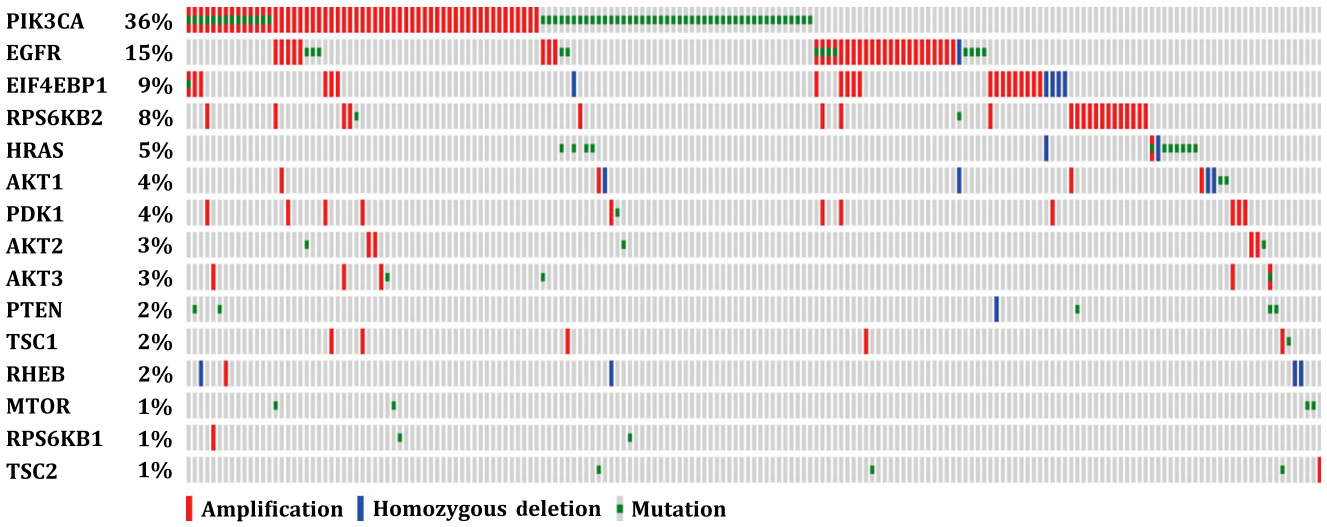

Figure 2 The frequency of genetic alterations of signaling molecules involved in PI3K pathway. The genetic data were obtained from cBioPortal for Cancer Genomics (http://www.cbioportal.org), where the data from The Cancer Genome Atlas (TCGA) project were deposited. The data were collected from 279 HNSCC tumor samples through high-throughput sequencing and other array technologies. Samples with genomic amplification (red), homozygous deletion (blue), and/or mutation (green) are presented in the OncoPrint as individual bars or square dots. Only those samples with genetic alterations in the PI3K/Akt/mTOR pathway are presented (183/279 tumors = 66% of cases). Percentages reflect the frequency with which samples express any of the three alterations reported in a given gene. Copy number variations are putative. PI3K catalytic subunit alpha isoform (PIK3CA); epidermal growth factor receptor (EGFR); eIF-4E binding protein 1 (EIF4EBP1); ribosomal protein S6 kinase (RPS6KB); Harvey rat sacrcoma virus oncogene (HRAS); protein kinase B (AKT); 3-phosphoinositide dependent protein kinase 1 (PDK1); phosphatase and tensin homolog (PTEN); tuberous sclerosis (TSC); Ras-homolog enriched in brain (RHEB); mammalian target of rapamycin (mTOR)

p110-γ in myeloid cells (Koyasu, 2003; Vanhaesebroeck *et al*, 2005). As such, both p110-δ and p110-γ are crucial for promoting inflammation in the cancer microenvironment. Although most of the PI3K pathway-directed anti-cancer therapies have aimed at p110-α suppression, isoform-specific inhibition of p110- δ or -γ also attenuates dysregulated inflammatory and immunosuppressive responses and may have an improved safety profile (Rommel *et al*, 2007; Fruman and Rommel, 2011; Schmid *et al*, 2011).

Dysregulated inflammatory signaling mediates angiogenesis, a critical process enabling tumor growth and spread. Angiogenesis is promoted by the secretion of cytokines such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), and interleukin-8 (IL-8) by tumor cells and associated myeloid lineage infiltrating cells. PI3K is activated downstream of the receptors for these signaling molecules and acts as a master regulator of angiogenesis and associated cytokine release (Yuan and Cantley, 2008). mTORC1 phosphorylation of eIF-4E facilitates tumor progression by increasing b-FGF and VEGF and increases risk for second primary in histologically cancer-free surgical margins (Nathan *et al*, 1999). While increased VEGF is associated with poor prognosis in HNSCC (Luangdilok *et al*, 2011), we have shown that pharmacologic inhibition of PI3K reduces phosphorylation of Akt in HNSCC cell lines, thereby blocking the secretion of VEGF and IL-8, even under epidermal growth factor (Bancroft *et al*, 2002) or hepatocyte growth factor stimulation (Dong *et al*, 2001a). In preclinical mouse studies, constitutive Akt activity causes abnormal vascular patterning (Hamada *et al*, 2005), and conditional class IA PI3K knockout compromises vascular integrity, limiting tumor size (Graupera *et al*, 2008; Yuan *et al*, 2008).

Although the PI3K/Akt/mTOR pathway enables vascular formation and growth factor delivery to tumors, it also confers a selective growth advantage and survival in conditions of nutrient deprivation. Normal cells live within 100–200 μm of a capillary bed, the diffusion limit of oxygen (Baish *et al*, 2011). Malignant cells with aberrations in *PIK3CA*, *PTEN*, or *MTOR,* however, are able to continue proliferating in hypoxic conditions outside of this range because of a modified cellular response to hypoxic stress, nutrient sensing, insulin signaling, and glucose uptake (Abraham, 2004; Kalaany and Sabatini, 2009; Kang *et al*, 2013; Vogelstein *et al*, 2013). Akt also promotes cell survival by attenuating pro-apoptotic factors, BAD, BAX, procaspase-9, and FOXO (Hennessy *et al*, 2005; Engelman *et al*, 2006).

Tumor outgrowths as a result of aberrant PI3K/Akt/mTOR signaling can subsequently lead to invasion (Samuels *et al*, 2005). Mechanistically, PI3K oncogenic disruption through *PTEN* loss disturbs membrane polarity and leads to epithelial-to-mesenchymal transition (Martin-Belmonte and Mostov, 2008), a key event causing invasion. 3q26 (*PIK3CA* locus) copy number gain and amplification is much more frequent in invasive carcinoma and high-grade dysplasia than in low-grade dysplasia (Woenckhaus *et al*, 2002). Late-stage HNSCC lesions also express higher levels of PI3K p110α mRNA and protein (Woenckhaus *et al*, 2002), lower levels of PTEN (Squarize *et al*, 2002, 2013), and more simultaneous mutations in multiple PI3K pathway genes (Lui *et al*, 2013). In addition, increased phospho-Akt and phospho-S6 expressions have been correlated with HNSCC progression (Amornphimoltham *et al*, 2004; Molinolo *et al*, 2007). This suggests that disruption of any number of components in the PI3K pathway might act as an oncogenic switch leading to invasion. Once invasion has occurred, PI3K-PTEN interplay regulates chemotaxis and intravasation of cells into endothelial networks (Kolsch *et al*, 2008). Consequently, the capacity of the PI3K/Akt/mTOR pathway to concurrently regulate the processes of angiogenesis, cell motility, and invasion also gives it solid footing for contributions to metastasis.

Accordingly, lymph node metastases are directly associated with *PIK3CA* gain and mutation (Fenic *et al*, 2007). Because metastasis is the leading cause of death for patients with solid tumors, PI3K activation portends a worse prognosis. Indeed, two independent studies of HNSCC show that 3q26 gain (*PIK3CA* locus) can predict poor clinical outcome for patients with early disease tumors that have not yet metastasized (Redon *et al*, 2001; Suda *et al*, 2012). Reduced PTEN expression is a separate prognostic indicator of poor clinical outcome (Lee *et al*, 2001). Additionally, mTOR blockade is able to diminish lymphangiogenesis and cervical lymph node spread, thereby increasing survival (Patel *et al*, 2011). Because PI3K, Akt, and mTOR activation and PTEN inactivation are all independently associated with adverse outcome in HNSCC (Nathan *et al*, 2004; Massarelli *et al*, 2005; Molinolo *et al*, 2009), they serve as logical targets in clinical trials.

PI3K/Akt/mTOR interactions with other signaling molecules

TP53 is one of the best-studied and most frequently deleted or mutated tumor suppressor genes. Inactivation of p53 can occur through physical association with murine double minute 2 homolog (MDM2), which results in centrosome hyperamplification, chromosomal instability, and aberrant mitosis (Carroll *et al*, 1999). PI3K phosphorylation of Akt activates MDM2 by translocating it from the cytoplasm to the nucleus, thereby diminishing cellular levels of p53 (Mayo and Donner, 2001). Concurrent inactivating mutations in *TP53* and activating abnormalities in *PIK3CA* are uncommon in cancer, implying that they are often mutually exclusive and independent promoters of malignancy. In HNSCC, *PI3KCA* activation confers resistance to p53-induced apoptosis, while p53-mediated apoptosis involves transcriptional inhibition of *PIK3CA* (Singh *et al*, 2002). We have shown that treatment with the PI3K/mTOR inhibitor, PF-04691502 (PF-502), induces apoptosis and expression of wild-type p53 in two separate *in vivo* models of HNSCC (Herzog *et al*, 2013). We have also provided evidence that Akt inhibition reverses the direct suppression of p53 by transcriptional and post-translational mechanisms (Friedman *et al*, 2013). In glioma and endothelial cells, PI3K inhibition has also been reported to increase p53 transactivation and transcription of its target genes (Su *et al*, 2003). Furthermore, p53 is able to induce PTEN, favoring cell death in malignancy (Mayo and Donner, 2002). Other studies have evinced that

mTOR signaling contributes to p53 inactivation through IGF-1R-mediated upregulation of MDM2 (Du *et al*, 2013), and the use of the mTOR inhibitor, rapamycin, is able to prevent oral cancer progression in a K-*ras*/*p53* double-knockout mouse model (Raimondi *et al*, 2009). Thus, therapies targeting PI3K/mTOR may be doubly effective because of their capacity to both limit PI3K/Akt/mTOR-mediated growth and survival and intensify p53-mediated apoptosis.

In contrast to loss of p53 as a mechanism for evading apoptosis, overactivity of the transcription factor, NF-κB, is another important anti-apoptotic and prosurvival event in HNSCC (Van Waes, 2007). We have compiled a large body of evidence showing that NF-κB regulates the gene expression and secretion of multiple cytokines and chemokines that favor tumor progression (Chen *et al*, 1998, 1999; Duffey *et al*, 1999; Loukinova *et al*, 2000; Dong *et al*, 2001b; Allen *et al*, 2007). Activation and nuclear translocation of NF-κB occurs primarily through phosphorylation of inhibitor-κB kinases (IKK) (Van Waes *et al*, 2007; Nottingham *et al*, 2013), which can be phosphorylated downstream of active PI3K/Akt (Ozes *et al*, 1999; Romashkova and Makarov, 1999; Yang *et al*, 2001; Hutti *et al*, 2012) in a cell-type-specific manner (Gustin *et al*, 2004). Accordingly, we have shown that the PI3K inhibitor, LY-294002, is able to reduce both constitutive and inducible NF-κB activity in HNSCC (Bancroft *et al*, 2002). Furthermore, rapamycin has also been shown to inhibit IKK activity, as the stimulation of IKK through Akt requires the interaction of the mTOR-associated protein, raptor, with IKK (Dan *et al*, 2008). While the mechanisms of interaction between the PI3K/Akt/mTOR and NF-κB pathways have been elucidated in many cancers, it remains to be studied further in HNSCC.

The PI3K/Akt/mTOR signaling route is also activated in concert with the Ras/Raf (MAPK kinase kinase)/MAPK pathway downstream of EGFR. Although the contributions of these signaling pathways to malignancy are often independent of EGFR overactivity, recent evidence indicates that MAPK activity is differentially affected by the EGFR-dependent PI3K/Akt/mTOR axis. Many studies have shown that through various feedback loops or resistance mechanisms, inhibition of one pathway downstream of EGFR or IGF-1R causes compensatory signaling through other parallel pathways (Carracedo *et al*, 2008; Pernas *et al*, 2009; Ercan *et al*, 2012; Limesand *et al*, 2013). The pleiotropy and resistance to therapy of downstream EGFR signaling has led to preclinical and clinical trials of combinations of EGFR, PI3K, mTOR, MAPK kinase kinase, effector of Ras (RAF), and MAPK kinase (MEK)/ERK inhibitors. Although the interactions between the PI3K/Akt/mTOR axis and p53, NF-κB, and MAPK signaling pathways are perhaps the most studied, this is by no means an exhaustive list of its interactions. The EGFR/PI3K/Akt/mTOR axis has also been shown to be modified in HNSCC in response to the modulation of transforming growth factor beta (TGF-β) (Bian *et al*, 2012), signal transducer and activator of transcription 3 (STAT3) (Lee *et al*, 2008; Sen *et al*, 2012), and Wnt signaling (Kavitha *et al*, 2013), among others.

PI3K/Akt/mTOR inhibitors in clinical studies

The summary of currently ongoing clinical trials using PI3K pathway inhibitors for head and neck cancer is presented in Table 1. BKM120, a pan-isoform PI3K inhibitor which is furthest along in development, is currently in a phase III trial for breast cancer (NCT01633060). Several studies have been initiated to select for patients with HNSCC, including a phase II trial using BKM120 as a single agent and a phase I/II study combining BKM120 with cetuximab. The orally available, irreversible pan-isoform PI3K inhibitor PX-866 has also moved into phase II trials for HNSCC. Although showing limited effect as a single agent in solid tumors including HNSCC (Hong *et al*, 2012), phase I/II trials combining PX-866 with cetuximab and docetaxel have been initiated in patients with HNSCC and may provide further insight into the efficacy of this drug.

Several isoform-specific PI3K inhibitors are currently in clinical trials, offering the potential benefit of improved target selectivity with fewer off-target side effects. Of these, only BYL719, a PI3Kα-specific inhibitor, has been applied to patients with HNSCC. The phase I trial on this drug was the first to selectively enroll patients with advanced solid tumors with prescreened mutations or amplifications of *PIK3CA*. In the dose escalation portion, eight patients including those with oral cancers showed prolonged disease stabilization lasting $\geq$4 months (Juric *et al*, 2012), while the dose expansion study that followed reported a partial response in 7/39 patients, one of which had HNSCC (Gonzalez-Angulo, 2013). The safety profile for BYL719 was similar to pan-PI3K inhibitors studied, with the most common adverse effects including hyperglycemia, nausea, and GI toxicities. Based on the encouraging results from this initial trial, BYL719 is currently being evaluated in combination with cetuximab in a phase Ib/II trial in patients with HNSCC.

Previously, the Akt inhibitor perifosine showed promising results in preclinical studies, but when moved to phase II clinical trials in HNSCC, it failed to show any antitumor effect (Argiris *et al*, 2006; Hideshima *et al*, 2006). More recently, a newer Akt inhibitor MK-2206 has shown synergistic activity with paclitaxel in preclinical models and phase I trials of HNSCC (Ahmed *et al*, 2013). It is currently being evaluated in phase II trials as a single agent in recurrent or metastatic HNSCC and may prove to be more effective than previous agents.

Rapamycin, the first mTOR inhibitor, targets mTOR exclusively and has shown anti-tumor activity in preclinical models (Amornphimoltham *et al*, 2005). The first clinical trial of rapamycin as a single agent in HNSCC is ongoing in phase I/II. Several rapamycin analogues have been developed, including temsirolimus (CCI-779) and everolimus (RAD001). Temsirolimus, after showing initial safety and strong inhibition of mTOR targets S6 and 4E-BP1 in HNSCC patient tumors (Ekshyyan *et al*, 2010), has moved to phase II trials in HNSCC as a single agent, in combination with carboplatin and paclitaxel or with cetuximab. Meanwhile, everolimus is also being evaluated extensively in HNSCC, with two phase I trials that have recently completed. In the first trial, combining everolimus

with cisplatin and docetaxel as induction therapy for radiation, 2-year overall survival rate was 91% and progression-free survival rate at 2 years was 76.6% (Fury *et al*, 2013b). In the second trial, everolimus was given with cisplatin and concurrent radiation, showing a 2-year overall survival rate of 92% and 2-year progression-free survival rate of 85% (Fury *et al*, 2013a). The most impressive response, however, has come from the ongoing phase I-II trials of everolimus in combination with carboplatin and paclitaxel as induction therapy in patients with unresectable locally advanced HNSCC. Recently presented results showed a 2.6% complete response, 76.3% partial response, as well as 21% stable disease in the 38 evaluable patients (Raymond *et al*, 2013). Everolimus continues to be investigated alone and in combinations in patients with HNSCC in five additional phase I-II trials (Table 1).

An important drawback in selectively inhibiting mTOR is the negative feedback loop that exists, in which S6K phosphorylates and blocks insulin receptor substrate 1 (IRS1), leading to the activation of PI3K and subsequent tumor growth (O'Reilly *et al*, 2006; Huang and Manning, 2009). To overcome this drawback, there is growing interest in the development of dual PI3K-mTOR inhibitors that can target the pathway at two points simultaneously. Dual PI3K-mTOR inhibitors that have shown promising results in preclinical studies of HNSCC include the orally available PF-502 (Herzog *et al*, 2013) and intravenously administered PF-05212384 (PF-384). Preliminary results from a phase I trial of PF-502, which is currently open to HNSCC, but to date only includes other advanced solid tumors such as non-small-cell lung carcinoma (NSCLC) and colorectal carcinoma, have shown no objective tumor reduction, but stable disease ≥16 weeks in approximately 16% of patients (LoRusso *et al*, 2011). Similarly, approximately 25% of patients with solid tumors including NSCLC given weekly PF-384 as a single agent achieved stable disease ≥16 weeks, although no objective tumor reduction was observed (Tabernero *et al*, 2011). Additional phase I trials of PF-384 in combination with docetaxel, cisplatin, and dacomitinib (EGFR inhibitor), as well as irinotecan (topoisomerase 1 inhibitor) and PD-901 (MEK inhibitor), may prove to have greater anti-tumor effects than single agents alone in HNSCC.

Conclusion and future directions

Following these initial clinical trials of agents targeting the PI3K pathway, several key questions remain. Additional studies will necessitate decision making about the most biologically active dose for each drug, the ideal method of administration, and the acceptance of adverse events including on-target effects such as hyperglycemia. It remains to be determined which class of agents targeting PI3K, mTOR, or Akt will be most effective in HNSCC, and whether there are factors that would make one class superior to the other in specific cases. Several clinical trials have retroactively analyzed *PIK3CA* mutation status in patient samples, while few have limited enrollment to patients with known PI3K pathway mutations. There has been some evidence that preselected patients with mutations in PI3K have better response to treatment, revealing improved outcomes compared to non-selective trials. Prescreening for molecular alterations has proven beneficial in preclinical studies as well, as it has uncovered critical mechanistic information regarding sensitivity or resistance and effects on other pathways. PI3K pathway cross-talk with other signaling routes, such as p53, NF-κB, and MAPK, should be investigated more extensively when designing future clinical trials. As large-scale screening protocols for molecular alterations become more prevalent in drug development trials, the feasibility of translating genetic information about the PI3K/Akt/mTOR axis to the clinic increases. Ultimately, this will allow clinicians to tailor treatment to an individual molecular profile and improve patient care.

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