

A Focus on Current Molecular Pathways in Head and Neck

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Abstract

Early stages of head and neck cancer require concomitant administration of chemotherapy and radiotherapy. Currently platinum, taxane and fluoruracil analogs are being administered. On the other hand, for advanced stages extensive surgery is required. During the last years, several molecular characteristics have been identified in the tissue of head and neck squamous cell carcinoma, and novel treatment options are being pursued. In this review we will elucidate these molecular pathways that could be used as a possible treatment.

Key words: head and neck cancer, chemotherapy, radiotherapy, molecular pathways.

Introduction

Treatment of head and neck squamous cell carcinoma (HNSCC) in early stages requires a non-invasive approach with concomitant chemotherapy and radiotherapy. There are still new data to come from ongoing studies whether taxane analogs have better efficiency compared to platinum analogs [1]. Advanced stages require extensive surgery. Unfortunately, HNSCC of head and neck cancer has substantial heterogeneity, at the clinical and molecular level therefore it cannot be considered as a single disease entity. Currently two large studies investigate the mutational landscape of HNSCC [2, 3] as well as the pending release of data from the Cancer Genome Atlas Project (TCGA). This new information will shed light to novel therapies.

EGFR: New Data and Best Use of Inhibitors

Epidermal growth factor receptor (EGFR) was the first molecular pathway to be investigated. EGFR

is a member of a family of receptor tyrosine kinases whose other members include ERBB2, ERBB3, and ERBB4. This pathway is responsible for the regulation of host of cellular activities including cell division, differentiation, and migration [4]. It has been demonstrated that EGFR overexpression is an early and very frequent molecular change in HNSCC, and the intensity of its expression is associated with reduced survival [5, 6]. EGFR is targeted either by small molecule adenosine-triphosphate competitive tyrosine kinase inhibitors (TKIs) specific to EGFR which bind to cytoplasmic region, such as gefitinib, erlotinib, or lapatinib, or by monoclonal antibodies (mAbs) directed at the extracellular domain of EGFR [7]. Cetuximab is the only U.S. FDA-approved targeted therapy available for HNSCC. Cetuximab is a chimeric IgG1-human monoclonal antibody against the extracellular domain of EGFR, which blocks the ligand binding to the receptor. Initial studies which

evaluated cetuximab in human cancer cell lines in vitro and human tumor xenografts in vivo showed its potent antitumor activity [8]. Cetuximab involves several mechanisms, such as; induction of apoptosis, including inhibition of cell cycle progression, inhibition of metastasis, inhibition of angiogenesis, and its ability to enhance the response to chemotherapy and radiation [9]. The FDA has previously approved the addition of cetuximab to radiation for the treatment of locally advanced HNSCC. Cetuximab has been approved as a single agent for the treatment of patients with recurrent or metastatic HNSCC for patients whom platinum-based therapy has failed. Cetuximab has been administered with radiotherapy and was compared with radiotherapy alone in a multinational randomized study. During a 54 month follow up, the median survival time was 49 months in patients treated with cetuximab plus radiotherapy compared with 29.3 months in those treated with radiotherapy alone ($p = 0.03$) [10]. A 5-year evaluation of the overall survival results was conducted. The addition of cetuximab to radiotherapy significantly improved the survival of patients with HNSCC ($p = 0.018$) [11]. To date cisplatin chemoradiotherapy is considered the best choice for locally advanced, unresectable HNSCC, since no direct comparison between cetuximab radiotherapy and cisplatin chemoradiotherapy has been performed. Cetuximab currently is also being studied as a substitute to cisplatin in locally advanced HPV-associated oropharyngeal cancers (OSCC). Moreover; there two ongoing phase III studies which compare cetuximab with intensity modulated radiation therapy (IMRT) with cisplatin chemoradiotherapy in HPV-associated locally advanced oral squamous cell carcinoma [12]. In another phase II study The Eastern Cooperative Oncology Group (ECOG) (E1308) evaluated whether the increased response to platinum-based induction chemotherapy could be used to select patients who can then safely receive a lower dose of intensity-modulated radiation therapy (IMRT).

There are data in in vitro studies with HNSCC cell lines that show a synergistic effect of cetuximab with cisplatin [13]. Moreover; clinical data in the recurrent/metastatic setting confirmed the activity of this combination [14, 15]. However; there is the lack of survival from the addition of cetuximab to cisplatin chemoradiation because possibly cetuximab and cisplatin share the same mechanism of action. Therefore, it has proposed that other agents such as docetaxel might be better partners to cetuximab/radiotherapy.

Furthermore, administration of cetuximab has

presented improved outcome of chemotherapy in the recurrent/metastatic setting. In another study it was reported that improved response rates were observed with the addition of cetuximab to cisplatin in ECOG 5397 [16]. In the study, conducted by Vermorken and colleagues, 442 eligible patients with previously untreated recurrent or metastatic HNSCC were randomly assigned it was observed that the addition of cetuximab to platinum-based chemotherapy with fluorouracil resulted in improvement in median OS from 7.4 to 10.1 months ($p = 0.04$). Moreover; median PFS was significantly prolonged from 3.3 to 5.6 months ($p < 0.001$), and the response rate increased from 20% to 36% ($p < 0.001$).

Furthermore, administration panitumumab which is a fully human monoclonal antibody against EGFR has been administered. Until now, there have been two phase II randomized studies, CONCERT 1 and 2, have been conducted in the unresectable locally advanced setting. Regarding CONCERT 1[17], it is a randomized phase II study of chemoradiotherapy with and without panitumumab. Regarding CONCERT 2 it is a randomized comparison of panitumumab radiotherapy to cisplatin chemoradiotherapy. However; both studies did not demonstrate superiority of the panitumumab arm, in specific it was observed that the addition of panitumumab to cisplatin chemoradiotherapy was associated with increased toxicity. Moreover; in the SPECTRUM study, which is a randomized phase III trial of chemotherapy with cisplatin/5-fluorouracil with or without panitumumab OS was not met. Until now EGFR TKIs have been tested in HNSCC but none have received FDA approval. In a recent phase II trial patients with recurrent or metastatic HNSCC received gefitinib, however; an overall response rate was only 11% [18]. Another a phase II study evaluated the association between adaptive dose-escalation to skin toxicity in the recurrent/metastatic setting and disease response [19]. Dose escalation was not associated with increased activity. In a phase III randomized study, the ECOG-E1302, docetaxel was compared to docetaxel plus gefitinib in patients with recurrent or metastatic HNSCC with PS 2 or who had been previously treated. The study was terminated early, because no statistically significant improvement in response rate, PFS, or OS compared with the docetaxel arm was observed. In another phase I study chemoradiotherapy was administered with lapatinib, a dual inhibitor which also disrupts the HER2 pathway, for locally advanced HNSCC reported an overall response rate of 81% [20]. Lapatinib has been observed to have little activity in EGFR inhibitor-naïve or -refractory subsets.

Heterodimerization of EGFR with other HER family members are involved in resistance to EGFR inhibitors. Afatinib has a broad activity against multiple receptors in the HER family, making it theoretically more effective against tumor cells bearing several ErbB family members and heterodimerizations. In another, study a randomized phase II study compared weekly cetuximab (400 mg/m² loading dose and 250 mg/m² thereafter) with 50 mg of afatinib daily in 74 patients with recurrent or metastatic HNSCC in whom platinum-based therapy had failed [21]. Initial safety results analysis showed a side effect profile consistent with other EGFR inhibitors, with diarrhea and rash. It was observed that afatinib has activity in patients with recurrent/metastatic HNSCC in whom platinum-based therapy has failed and compares favorably to cetuximab. The major problem in EGFR-targeted therapy in HNSCC is patient selection, since the basic mechanism of resistance has not been identified. Regarding non-small cell lung cancer (NSCLC), patients with activating mutations in the EGFR tyrosine kinase domain demonstrate significantly higher response to EGFR TKIs (erlotinib, gefitinib and afatinib). However; mutations in EGFR tyrosine kinase domain are rare in HNSCC. There are several investigators reporting detection of the type III mutated variant (EGFRvIII) in a variable proportion of HNSCC ranging from 0% to 40%, [22] however; there are still data to be clarified.

EGFRvIII mutated variant is characterized by an in-frame deletion from exons 2 through 7 in the extracellular domain irreversible EGFR inhibitors such as afatinib in preclinical studies have been found to be effective against EGFRvIII.

It has been observed that overexpression of ERBB2-induced gefitinib resistance, and also the combination of pertuzumab (which targets HER2) with gefitinib in HNSCC cell lines resistant to gefitinib resulted in increased inhibition of cell growth. [23] It has been observed that mutations in *KRAS* are extremely rare in HNSCC, however; *HRAS* mutations have been reported [2].

Another molecular pathway the *MET* overexpression was found in 84% of HNSCC cases; The *MET* mutations and gene amplification have been reported in 13.5% and 13% of the cases, respectively. It was also found that a combination of a *MET* inhibitor with erlotinib resulted in greater-than-additive inhibition of cell growth by ErbB3/AKT signaling [24].

Future research will elucidate the factors that correlate with response of EGFR-targeted therapies.

Phosphatidylinositide 3-Kinase Pathway Biology and Inhibitors

The PI3K-AKT-mTOR Pathway and Physiology

Phosphoinositide 3-kinases (PI3Ks) are a family of related enzymes that play a pivotal role to the cellular regulatory mechanisms. It has been observed that their function has been linked to the regulation of numerous biologic processes, including cell growth, migration, survival, proliferation, differentiation, and differentiation [25, 26]. According to structural and substrate specificity, PI3Ks are divided into 3 classes. Class 1 is currently the only clinically relevant subgroup and can be further divided into A and B subtypes: Class 1 A, Class 1 B enzymes, Class 2 and Class 3. PI3-kinases are linked with several cellular functions such as; cell growth, differentiation, motility, proliferation, survival, and intracellular trafficking. Several of these functions are related to the ability of class 1 PI3-kinases to activate protein kinase B (PKB, also commonly known as AKT). AKT, is a serine-threonine kinase, which has three different isoforms (AKT1, AKT2, AKT3). AKT activation is known to be initiated by translocation to the plasma membrane. AKT phosphorylation is carried out by 3-phosphoinositide-dependent protein kinase 1 (PDK1) and PDK2. It is currently thought that mTOR-ricor (rapamycin insensitive companion of mTOR) complex 2 (mTORC2) is the primary source of PDK2 activity under most circumstances.

It is known that there are several AKT-independent pathways, however; their role in cancer is not well defined. Upon AKT activation which is a phosphorylating source for many proteins, including glycogen synthase phosphate 3 and FOXOs, several diverse cellular functions are regulated. It is known that in most cancers, AKT activation by PI3K is either by tyrosine kinases (RTKs) or by somatic mutations [25, 26]. It has been found that PI3K-AKT signaling plays diverse role in normal and cancer physiology through multiple downstream pathways.

PTEN loss

It is known that PTEN functionally antagonizes PI3K activity through its intrinsic lipid phosphatase activity that reduces the cellular pool of PtdIns [3,4,5] P3 by converting PtdIns [3,4,5] P3 back to phosphatidylinositol-4,5-bisphosphate (PtdIns [4,5] P2). It has been observed that loss of PTEN results in unrestrained signaling by the PI3K pathway, which leads to cancer. It has been observed that PTEN loss is common in head and neck cancer [25, 27]. It is known that tumors with PTEN loss appear to signal primarily

using the p110beta (PIK3CB), this information is vital for the selection of appropriate inhibitors [28]. The loss of PTEN tumor suppressor is common, although mutations may not be the primary mechanism of PTEN loss in HNSCC [29].

Genetic Alterations in the PI3K Pathway in Head and Neck Cancer

PIK3CA mutations

It has been observed that there are genetic aberrations of the PI3K pathway in head and neck cancer. These mutations of the *PIK3CA* gene have been reported in 6% to 20% of head and neck tumors [2, 29]. Moreover; it has been observed that there are well-established oncogenic (canonical) *PIK3CA* mutations in HPV-positive head and neck cancers [30].

There are also other genetic mutations such as; *PIK3R1* mutations, AKT mutations and amplification, TSC1/2 or PDK1 aberrations, INPP4/PHLPP tumor suppressor loss, or loss of NF2 [25, 26]. However; it remains unclear whether these genetic mutations occur in HNSCC. Regarding epidermal growth factor which is amplified in 10% to 15% of HNSCC, [29] signaling may not solely rely on PI3K [25, 26].

Translational Implications

It has been observed in vitro that PI3K mutations may induce increased resistance to EGFR inhibition [31]. *PIK3CA* may be biomarker for EGFR resistance.

PI3k Inhibitors in Clinical Testing and Trials in Hnscc

There are three classes of PI3K inhibitors that can be differentiated: Combined inhibitors of PI3K/mTOR, Pan-Class I PI3K inhibitors, Alpha-specific (p110alpha specific) inhibitors.

It remains unclear which type of PI3K inhibitors could be the most promising, however; the vast genetic aberration in the PI3K pathway and early clinical data indicate that PI3K could be a promising target for HNSCC [27].

New Signaling Pathways and Targets in the Hnscc Horizon

NOTCH

There is also the NOTCH family which consists of four receptors (*NOTCH1-4*), which interact with the Delta-like (Dll1, Dll3 and Dll4) and Jagged (Jag1 and Jag2) families of ligands. These are normally bound to the cell membrane. It is known that ligand binding is followed by two cleavages of the NOTCH receptor by ADAM metalloprotease and

gamma-secretase complex, leading to the release of the NOTCH intracellular domain (NICD). It has been observed that NICD is associated with the CSL/MAM complex which is able to bind DNA and promote transcription [32]. NOTCH signaling pathway is known to be a pro-tumorigenic pathway. When activated then mutations and translocations are observed in the genes for NOTCH receptors or their regulators. *NOTCH1* heterodimerization (HD) domain mutations can result in ligand independent proteolytic activation which then drive proliferation in T-ALL [33]. Furthermore, these activating mutations result in *HES 1* upregulation, which in turn induce suppression of transcription of *PTEN*. It has been previously observed that *PTEN* loss is a gateway to resistance to *NOTCH1* inhibition in T-ALL [34].

Moreover; several groups with the use of next-generation exome sequencing have observed that NOTCH mutations occur second in frequency only to *TP53* mutations in head and neck, lung squamous cell carcinomas (SCC) and skin [35].

Currently there is an interest in NOTCH signaling in HNSCC because identifying the pathways that become dysregulated after NOTCH inactivation is essential in order to identify effectiveness of a treatment approach.

ALK1

It is known that activin receptor-like kinase 1 (ALK1) is a type I receptor belonging to the transforming growth factor-beta (TGFβ) superfamily. This protein is selectively expressed on activated endothelial cells in response to injury or disease [36]. ALK1 has been hypothesized to play a key role in the development of functional vasculature.

ACE-041 protein binds to the ligands bone morphogenetic protein (BMP) 9 and BMP10 and inhibits their interaction with ALK1, and finally blocks ALK1-mediated signaling. ACE-041 binding disrupts the process of vascular development [37]. ACE-041 has been studied in a dose-escalation phase I study in order to determine its safety profile, optimal dosing strategy, tolerability, and antitumor activity in patients with advanced solid tumors [37]. The main toxicity observed was edema and fluid overload that were dose-dependent and responded to diuretic therapy. Based on the favorable outcome of this study currently there is an ongoing phase II clinical trial of ACE-041 in patients with refractory relapsed/metastatic (NCT01458392).

Hedgehog Pathway

It is known that the hedgehog pathway (HhP) is a validated anticancer target, and vismodegib (a small

molecule that inhibits the HhP) is used by patients with advanced basal cell carcinoma of the skin. Ligand activation by sonic hedgehog (SHH) leads to a cascade of signaling, which in turn modulates the expression of numerous cancer target genes. HhP signaling is also crucial for drug resistance [38, 39]. To date it has been observed that EGFR and HhP are implicated as key drivers of proliferation and survival of cancer cells.

Moreover, it has been observed that chronic gefitinib treatment generates a mesenchymal drug-resistant population in HNSCC cells independent of EGFR activation [40]. However; this dichotomy of EGFR-dependent population and -resistant states and the role of HhP signaling have not been yet clarified in HNSCC.

It has been observed that GLI1 is a key driver of tumor growth and metastasis in multiple cancers [41, 42]. Regarding head and neck squamous cell carcinoma nuclear GLI1 expression levels were determined in tumors from patients enrolled on RTOG 9003, a radiation fractionation trial [43]. In this study the results were also correlated with previously determined EGFR expression, and assessed in relation to time to metastasis (TTM). It was observed that GLI1 was associated with poorer outcomes, tumor/node/metastasis staging system stages, adjusted for age, and performance status. In this study, data suggested that GLI1 could serve as a marker in HNSCC, however, since several pathways other than HhP converge modulate GLI1, and thus the regulatory mechanisms, the significance of these findings are yet to be clarified.

Currently based on preclinical data using a vast variety of in vitro and patient-derived in vivo models of HNSCC and the HhP inhibitor IPI-926, [44]

cetuximab and IPI-926 (NCT01255800) are being administered with a dose escalation concept. In this study the authors investigate the transcriptome analysis in order to determine the molecular mechanisms underlying EGFR acquired resistance and how HhP modulation may modulate such EGFR dependence. Table 1, Figure 1.

Table 1. Current selected targeted agents from www.clinicaltrial.gov.

Drug Name (Tradename)	Target(s)	Phase of study in Head and Neck cancer
Cetuximab (Erbix) EGFR	EGFR	III
Gefitinib (Iressa) EGFR	EGFR	I/II/III
Sunitinib (Sutent) VEGF	VEGF	I/II
Bevacizumab (Avastin) VEGF	VEGF	I/II/III
Romidepsin Histone deacetylase	Histone deacetylase	I/II
Vorinostat (Zolinza) Histone deacetylase	Histone deacetylase	I/II
Dasatinib (Sprycel) Tyrosine kinases	Tyrosine kinases	II
Imatinib (Gleevec) Tyrosine kinases	Tyrosine kinases	II
Pazopanib VEGF, Tyrosine kinases	VEGF, Tyrosine kinases	II
Vandetanib (Zactima) VEGF, EGFR	VEGF, EGFR	I/II
XL880 VEGF, Tyrosine kinases	VEGF, Tyrosine kinases	II
Perifosine (KRX-0401) Akt	Akt	II
Bortezomib (Velcade) NF-kB, Tyrosine kinases	NF-kB, Tyrosine kinases	I/II
Lonafarnib (Serasar) Farnesyl transferase	Farnesyl transferase	I/II
Tanespimycin (KOS-953) Hsp90	Hsp90	I/II
AZD0530 Src/ Abl kinase	Src/ Abl kinase	II
Erlotinib (Tarceva) EGFR	EGFR	I/II/III
Panitumumab (Vectibix) EGFR	EGFR	I/II/III
BIBW 2992 (Tovok) EGFR, HER-2/neu	EGFR, HER-2/neu	II
Zalutumumab (HuMax-EGFr) EGFR	EGFR	I/II/III
Trastuzumab (Herceptin) HER-2/neu	HER-2/neu	II
Lapatinib (Tykerb) EGFR, HER-2/neu	EGFR, HER-2/neu	I/II/III
Cediranib (Recentin) VEGF	VEGF	I/II
Sorafenib (Nexavar) Raf, VEGF	Raf, VEGF	I/II
Semaxanib VEGF	VEGF	I/II
Pazopanib VEGF	VEGF	II

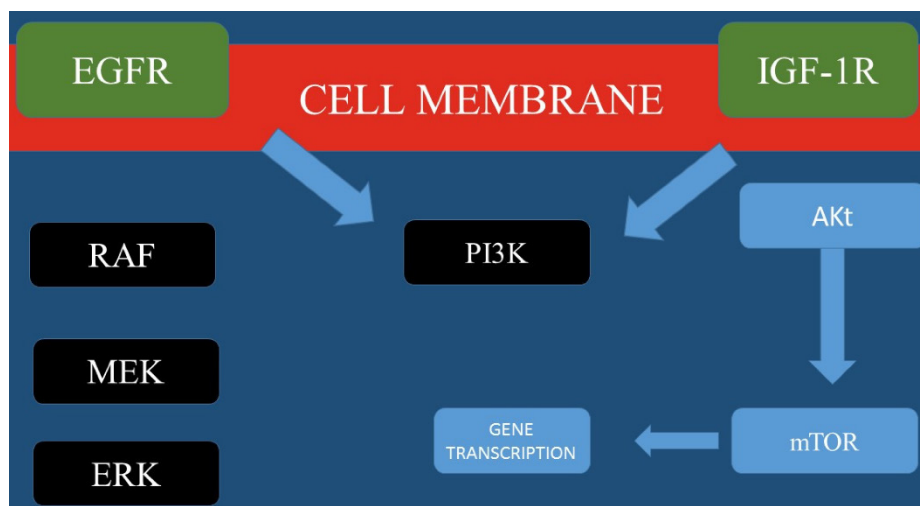


Figure 1. Most important molecular pathways for Head and Neck cancer.

In conclusion, currently there are many novel pathways and treatment approaches which are being explored for head and neck cancer. These pathways include EGFR activity and methods to overcome resistance and targeting PI3K signaling. Moreover; identifying new candidate treatment targets such as ALK1, NOTCH, and hedgehog signaling.

Competing Interests

None to declare.

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