Review Article
Mechanisms of resistance in head and neck cancer

Hector Picon[1], Achuta Kumar Guddati[2]

[1]Medical College of Georgia, Augusta University, Augusta, GA 30909, USA; [2]Division of Hematology/Oncology, Georgia Cancer Center, Augusta University, Augusta, GA 30909, USA

Abstract: Resistance to treatment is one of the biggest challenges in combating head and neck squamous cell carcinoma (HNSCC). The concept of resistance, however, is often viewed as a whole without categorization into the two types of resistance: acquired and intrinsic. Comparison of the mechanisms of the two types of resistance can give further insight as to the importance of these resistance pathways, as mechanisms that are common between the two categories are more likely to be integral to cell survival. In this review, a new perspective on resistance is presented in order to identify molecular targets that have potential for wide therapeutic application. Resistance mechanisms are grouped by the primary pathway involved in order to help establish connections between studies and identify the pathways most active in HNSCC resistance. The receptor tyrosine kinase AXL is one of the targets that showed the greatest promise for overcoming resistance to cetuximab, an antibody targeting the epidermal growth factor receptor (EGFR), as it is shown to be upregulated in both acquired and intrinsically cetuximab-resistant cells. Other targets of interest are signal transducer and activator of transcription 3 (STAT3), a downstream transcription factor of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, and TWIST, a marker of epithelial-mesenchymal transition. STAT3 has been shown to be upregulated and more active in cetuximab-resistant HNSCC cell lines, and its inhibition decreased cell growth in cell lines resistant to anti-EGFR therapy. Twist has been shown to have roles in acquired resistance for both cetuximab and cisplatin, a platinum-based therapy that targets dividing cells, which suggests that it also has an integral role in resistance. Other resistance mechanisms are also summarized in this review, but further studies are needed in order to confirm their utility as targets for overcoming resistance in HNSCC.

Keywords: Resistance, cisplatin, cetuximab, EGFR, chemotherapy

Introduction

The worldwide incidence of head and neck squamous cell carcinomas (HNSCC) is roughly 600,000 cases per year, and approximately half of these cases result in death [1, 2]. One reason for this observed mortality is the lack of effective therapies. Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor (EGFR), is one of the few drugs approved by the United States Food and Drug Administration for use in HNSCC, but its response rate is only 13% when used alone [3]. Platinum-based agents, such as cisplatin or 5 fluorouracil, that interfere with cell division are often used in combination with cetuximab and have been shown to raise response rates to as high as 36% [4]. However, HNSCC often recurs after initially responding to treatment.

Resistance to chemotherapy may occur initially or later after the first line of chemotherapy. Resistance to radiation therapy is called radiation resistance and has been observed in HNSCC. Intrinsic resistance refers to the lack tumor regression following treatment and is thought to be the result of mechanisms that existed before initiation of the therapy [5]. Acquired resistance refers to the elimination of an observed response after an initial clinical benefit following treatment [6]. Both types of resistance are prevalent in HNSCC, and the specific mechanisms of each are not fully understood. It is imperative that a better understanding of these mechanisms is gained in order to improve the response to the few treatment options available. In this review, a comparison is made between intrinsic and acquired resistance in HNSCC, especially in regard to

cetuximab and cisplatin, with the dual objective of summarizing recent advancements in the field and identifying targets that are players in both intrinsic and acquired resistance pathways. It can be argued that targets present in both pathways are more likely to be integral cell processes and thus more efficient for inhibition. The hope is that this new way of examining resistance will lead to novel approaches to cancer therapies and better outcomes for HNSCC patients.

Pathways involved in resistance to chemotherapy

Several major pathways have been implicated in mechanisms of resistance for HNSCC. Here, they are grouped by the pathway that is the most involved, though it is important to note that some studies report overlap between pathways. Mechanisms of acquired resistance are detailed first, followed by intrinsic resistance and then the mechanisms common between intrinsic and acquired resistance, if applicable.

AKT/PI3K pathway

Upstream signaling of an extracellular receptor, such as a receptor tyrosine kinase (RTK) or G-protein coupled receptor, by growth factors or cytokines activates this pathway. The signal is transmitted intracellularly by the catalytic activation of phosphatidylinositol (3,4,5)-triphosphate (PIP_3) by phosphoinositide 3-kinase (PI3K). PIP_3 then activates Protein Kinase B (AKT), which phosphorylates a number of other proteins involved in diverse cell processes including cell cycle progression, survival, and growth [7].

In terms of acquired resistance, multiple studies have shown increased AKT signaling in cetuximab resistant cells [8, 9]. More specifically, Nair et al. found that mutations in the extracellular domain of EGFR can cause epidermal growth factor (EGF)-independent signaling, leading to sustained AKT signaling and resistance to cetuximab [10]. Saki et al. found that Ras family proteins, which are small molecules that use guanosine triphosphate (GTP) to transmit intracellular signals, are involved in cetuximab resistance and also radioresistance by autocrine production of EGFR ligands, and they conferred survival via the AKT/PI3K pathway but not the RAS/RAF/MEK/ERK pathway (discussed below) [11]. Another study examined

heregulin, a ligand of the RTK human epidermal growth factor receptor 3 (HER3) of the same family as EGFR. Heregulin was upregulated in HNSCC cells with acquired cetuximab resistance and conferred survival through AKT signaling. However, cell growth was decreased by pan-HER inhibition by the small molecule tyrosine kinase inhibitor (TKI) afatinib, which inhibits EGFR, HER2, HER4, and signal transduction of HER3. The study also provided some evidence that aberrant heregulin expression could be a mechanism of intrinsic cetuximab resistance [12].

For cisplatin resistance, Peng et al. studied microRNAs, small RNA molecules that are known to be involved in diverse cell processes including resistance. They found that expression of microRNA-23a inhibited cisplatin-induced apoptosis by signaling through c-Jun N-terminal kinase (JNK), a downstream target of the MAPK pathway, to increase expression of Twist1. Further, the observed effect was abolished by knockdown of Twist1 [13]. Finally, one interesting study reported that HNSCC cells exhibiting resistance to cisplatin showed decreased growth when treated with a MEK (discussed below) inhibitor and afatinib. The MEK inhibitor blocked the RAS/RAF/MEK/ERK pathway and caused increased AKT signaling, while afatinib synergistically blocked HER family signaling and the AKT pathway [14]. No specific mechanisms of intrinsic resistance via the AKT/PI3K pathway are reported, and no studies were found that detailed an overlapping mechanism for intrinsic and acquired resistance involving the AKT/PI3K pathway.

RAS/RAF/MEK/ERK pathway

This pathway involves the activation of the extracellular RTK which then activates RAS, a small GTPase. Upon hydrolysis of GTP, RAS signals the kinase RAF, which dimerizes and activates to phosphorylate mitogen-activated protein kinase kinase (MEK). MEK in turn phosphorylates extracellular signal-regulated kinase (ERK), which phosphorylates several other proteins involved in cell cycle progression, differentiation, and evasion of apoptosis [15]. Another common name for this pathway is the mitogen activated protein kinase (MAPK) pathway.

This pathway is one of the most well-studied for the topic of HNSCC resistance, especially for anti-EGFR therapy. The fusion protein FGFR3-

TACC3 was found to drive acquired resistance to anti-EGFR and anti-HER3 combination treatment by activating the RAS/RAF/MEK/ERK pathway in HNSCC cells [16]. Schulz et al. found that HNSCC cells with acquired resistance to 5 different inhibitors, including cisplatin and afatinib, signal through ERK1/2 [17]. Other acquired resistance mechanisms center around the receptor tyrosine kinase AXL. Brand et al. found that AXL mediates acquired resistance to cetuximab in HNSCC via MAPK signaling and subsequent activation of EGFR and the transcription factor c-Jun. Further, the development of resistance was associated with AXL hyperactivation and EGFR association in HNSCC patient-derived xenografts (PDXs), which are valued for their ability to preserve the integrity and complexity of human tumors while still allowing for experimentation [18, 19]. Another study also found AXL upregulation in cetuximab-resistant cells [20]. A third study also implicated AXL in anti-EGFR resistance, but it was shown in non-small cell lung cancer cells rather than HNSCC [21].

There have been many mechanisms postulated for intrinsic resistance through the MAPK pathway. The same study referenced above examining AXL activity also found that intrinsic resistance to cetuximab correlated with AXL expression in HNSCC tumor xenografts [18]. Rampias et al. showed that HRAS mutations are quite common in HNSCC (9.44%) and are associated with a lesser response to cetuximab therapy, but this is one of the few times an HNSCC resistance mechanism is attributed to an activating mutation [22]. Further downstream in the pathway, Boeckx et al. studied dual-specificity phosphatases 5 and 6 (DUSP5/6), known negative regulators of ERK1/2, by examining levels of ERK1/2 phosphorylation in cetuximab-resistant cells after treatment with cetuximab. They found that significantly more ERK1/2 phosphorylation was present in cetuximab-resistant cells versus sensitive cells. This implies that DUSP5/6 suppression could be involved in cetuximab resistance. Further evidence was provided by analysis of aurora kinase B (AURKB), a downstream component of the MAPK pathway, which was found to be upregulated in cetuximab-resistant cells. Importantly, cell growth of cetuximab-resistant cells was decreased upon inhibition of ERK1/2 or AURKB [23]. These findings implicate both DUSP5/6

and AURKB in intrinsic cetuximab resistance. Along similar lines, another study using cetuximab-resistant cells showed that the activity transcription factor AP-1, a downstream target of the MAPK pathway, can be decreased by ERK inhibition [24]. The most promising target of this pathway appears to be AXL according to the aforementioned evidence, especially because it is involved in both acquired and intrinsic cetuximab resistance.

JAK/STAT pathway

Another pathway that presents promising targets is the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway. Cytokines or growth factors provide an extracellular stimulus for JAK activation by transphosphorylation, which allows for STAT binding and phosphorylation. Phosphorylated STATs dimerize and then function as transcription factors in the nucleus, where they effect gene transcription for processes involving cell growth, development, differentiation, and survival [25].

This pathway is only reported to be involved in cetuximab resistance rather than cisplatin. One study found that overexpression of microRNA-204 could restore cetuximab sensitivity to HNSCC cells that had acquired cetuximab resistance. It was shown that miR-204 blocks JAK2 directly to inhibit the JAK/STAT pathway [26]. Additionally, Willey et al. found that STAT3 was a likely player in cetuximab resistance given its increased phosphorylation and total expression in two cetuximab-resistance HNSCC cell lines. In fact, knockdown of STAT3 caused increased cell death in both the presence and absence of cetuximab, lending further support to its role in resistance [27]. Similarly, Sen et al. found higher STAT3 activation in HNSCC patient tumors that recurred after cetuximab treatment compared to those that received no treatment [28]. No mechanisms of JAK/STAT intrinsic resistance alone are reported, but Sen et al. also found that inhibition of STAT3 can decrease cell growth for cell lines that have either intrinsic or acquired resistance to anti-EGFR therapy [28]. And lastly, one study found that STAT3 is a downstream effector of EGFR variant III (EGFRvIII), a mutant form of EGFR able to confer cetuximab resistance, that cannot be abrogated by cetuximab treatment in EGFRvIII-expressing HNSCC cells [29]. Taken together, these

findings suggest that STAT3 could be a good target for overcoming cetuximab resistance.

Epithelial-mesenchymal transition

Epithelial-mesenchymal transition (EMT) is the process by which epithelial cells lose their adhesive properties in order to migrate and invade other tissues. It is a physiological process necessary for important functions like embryonic development and wound healing, but is used by cancer cells as a mechanism for metastasis to distant tissues. Markers of EMT include downregulation of E-cadherin, a protein responsible for cell-cell adhesions, and upregulation of Zing finger E-box binding homeobox 1/2 (ZEB1/2), vimentin, TWIST, and SNAIL, among others. After migration to a secondary site, cancer cells can undergo mesenchymal-epithelial transition (MET) in order to revert to a phenotype capable of proliferation and generation of a secondary tumor. While studies involving EMT often concern its role in metastasis, it has also been implicated in drug resistance [30].

Acquired resistance, but not intrinsic, was found to be relevant to EMT. Keysar et al. found that cells that acquired resistance to EGFR inhibition showed upregulation of the transcription factor GLI1. In addition to upregulating the mesenchymal marker vimentin, GLI1 controls transcription of target genes of the Hedgehog pathway, which also has been shown to result in EMT [31]. Further, cetuximab sensitive cells treated with cetuximab were found to express higher levels of ZEB2, TWIST1, and SNAIL, which suggest that the treatment selects for a mesenchymal phenotype [31]. The role of TWIST1 in acquired resistance to cisplatin-induced apoptosis was discussed in the AKT/PI3K section, but it is relevant to EMT as well because TWIST1 is a known regulator of EMT [13]. The fact that TWIST has been implicated in resistance to both cisplatin and cetuximab indicates that it should be explored further as a potential target for overcoming acquired resistance.

Cancer stem cells

The cancer stem cell (CSC) theory states that a tumor contains a few cells that have the important abilities of differentiation, self-renewal, repopulation of a tumor, and mobility via EMT, and increased resistance [32]. They are often

in a quiescent state, which enables them to resist traditional therapies that target dividing cells. Thus, it is challenging to eradicate CSCs because they must be targeted very specifically. Markers that have been confirmed in HNSCC include cell surface antigen CD44, the enzyme aldehyde dehydrogenase 1 (ALDH1), and transcription factors octamer-binding transcription factor 4 (OCT4) and sex determining region Y-box 2 (SOX2) [32, 33].

Cancer stem cells (CSCs) are thought to play an important role in drug resistance. It is postulated that these cells possess or acquire mechanisms that allow them to survive a chemotherapy treatment and then proliferate, thereby conferring resistance to the entire tumor. Little evidence of cetuximab resistance in relation to CSCs has been found, but Kulsum et al. showed that sensitivity to cisplatin can be restored by inhibition of the stemness marker ALDH1A1 in cisplatin-resistant HNSCC cells. Additionally, HNSCC explants treated with cisplatin plus an ALDH1A1 inhibitor showed significantly less proliferation than those treated with cisplatin alone [34]. Long non-coding RNA molecules are known to be involved in regulation of cell processes and have been shown to be involved in resistance [35]. Lee et al. examined the role of long non-coding RNA molecules and found that the long-noncoding RNA LINC00963 regulates the known multidrug resistance protein ABCB5, which leads to resistance to cisplatin treatment in oral squamous cell carcinoma cell lines. Suppression of LINC00963 was also found to decrease activity of stemness marker ALDH1, and TCGA analysis showed a positive correlation between LINC00963 and stemness markers SOX2 and CD44 [36]. Lastly, Li et al. reviewed the role of RNA-binding protein LIN28 in HNSCC and concluded that its role in induction of stemness markers SOX2 and OCT4 suggests that it has a role in resistance as well [37]. These results indicate that acquired resistance to cisplatin seems to be related to stemness properties of cancer stem cells.

HNSCC cells that acquired resistance to afatinib showed increased expression of stemness markers CD44 and OCT4, indicating that stemness could play a role in this resistance mechanism as well [17]. Lathia et al. recently reviewed the mechanisms of intrinsic resistance in cancer and writes that mechanisms of innate resistance in cancer stem cells include ATP binding

cassette (ABC) transporters, low reactive oxygen species levels, overexpression of anti-apoptotic proteins, enhanced DNA repair, and the phenomenon of multiple pathways for stemness [38]. Of these, evidence of ABC transporters was found in another study, but only as it relates to acquired resistance. In non-HNSCC cell lines, ABC transporters were found to cause acquired drug resistance after activation by never in mitosis-related kinase 2 (NEK2), a kinase that has a role in chromosomal instability [39]. Moreover, Xu et al. found that expression of NEK2 predicts acquired resistance to cisplatin therapy in nasopharyngeal carcinoma, and sensitivity to cisplatin can be restored by NEK2 knockdown [40]. This indicates that ABC transporters could be relevant to both intrinsic and acquired resistance via NEK2 in HNSCC cells, but further investigation is needed in order to confidently state this connection.

Other mechanisms

There are other important mechanisms involving noncanonical pathways to chemoresistance. For intrinsic resistance, it has been shown that there are high levels of nuclear EGFR in intrinsically cetuximab-resistant HNSCC cells. Experiments using non-HNSCC cells showed that this process of internalization of EGFR is mediated by HER family ligands and Src family kinases [41]. Another study implicates dysregulation of EGFR internalization in acquired cetuximab resistance as well [9]. For both acquired and intrinsic resistance, Leonard et al. achieved promising results. Instead of downstream signaling, they focused on regulation of alternative RTKs, such as HER3, AXL, and MET, an RTK known to respond to hepatocyte growth factor rather than EGF. They found that bromodomain-containing protein-4 (BRD4) is an important regulator of both intrinsic and acquired resistance to cetuximab. Joint inhibition of BRD4 and EGFR with cetuximab delays acquired resistance and delays increase in expression of other RTKs [20]. Also, these results lend further support to the aforementioned idea that AXL is important for both intrinsic and acquired resistance. Lida et al. also targeted multiple RTKs at once via pan-HER inhibition of EGFR, HER2, and HER3 in HNSCC PDXs. They found that this treatment resulted in growth delay for both intrinsic and acquired cetuximab resistant models, indicating that these alternative RTKs

are an important mechanism of resistance [42]. Along similar lines, De Pauw et al. examined resistance to cetuximab using afatinib. They found that afatinib was able to overcome both acquired and intrinsic resistance to cetuximab using multiple HNSCC cell lines, but they could not rule out the possibility of cross-resistance between afatinib and cetuximab [43]. However, a different study found evidence for a lack of cross-resistance between the two in a phase II clinical trial [44]. Therefore, the importance of HER family ligands as well as alternative RTKs is underscored.

Lastly, analysis of acquired resistance to afatinib indicated that the proteins granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-8 (IL-8), tissue inhibitor of metalloproteinase-1 (TIMP-1), and vascular endothelial growth factor (VEGF) may be involved in resistance to EGFR TKIs including afatinib. These proteins are involved in tumor angiogenesis and invasion, which could explain the aggressiveness of recurrent HNSCC [45]. There exists limited research concerning afatinib resistance in HNSCC, but it is important to study given that, there are few therapeutic options for patients with HNSCC who develop resistance to chemotherapy. A summary of important pathways involved in the development of resistance in HNSCC is depicted in **Figure 1** which shows three predominant pathways: 1) As EGFR is inactivated by cetuximab treatment, AXL is upregulated and signals through the MAPK pathway to provide both acquired and intrinsic resistance to cetuximab. 2) Cetuximab treatment causes increased expression and phosphorylation of STAT3 and leads to resistance to anti-EGFR therapy. 3) miR-23a activates JNK which in turn activates the transcription factor Twist to provide acquired resistance to cisplatin. Similarly, cetuximab treatment causes increased expression of TWIST, which then leads to acquired cetuximab resistance.

Strategies for rescue therapy

There has been a considerable amount of research into the reversal of cetuximab resistance. Many agents have shown success in their ability to rescue cetuximab from acquired or intrinsic resistance; a summary of these agents, including the effector that they act
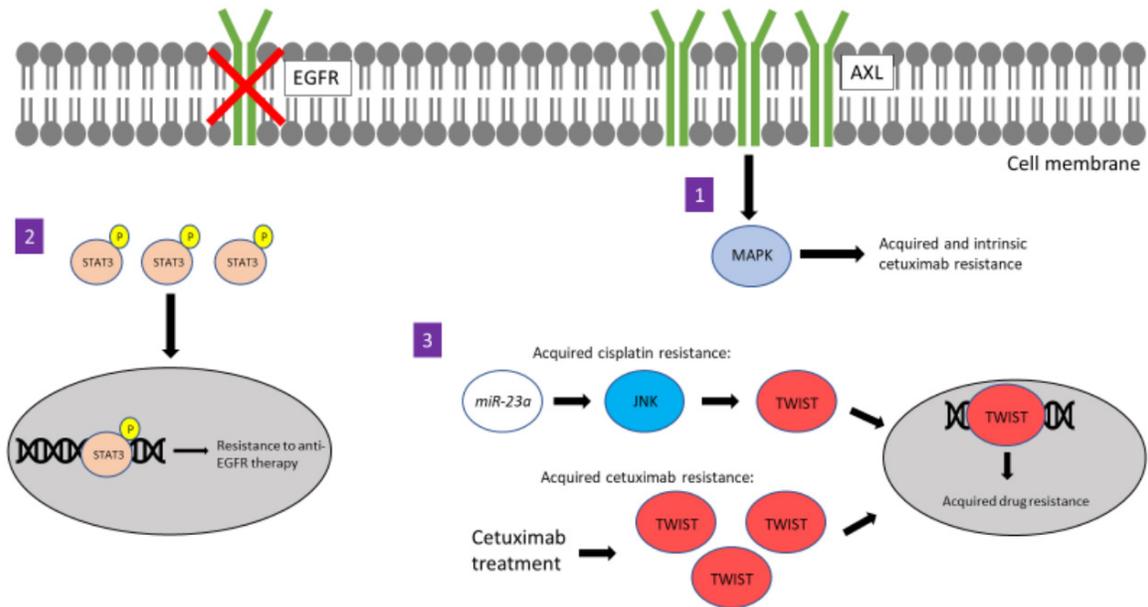
Figure 1. The picture provides a summary of the resistance mechanisms for the major targets identified in this review.

Table 1. Drugs that have shown the ability to rescue cisplatin or cetuximab resistance

Therapy	Agent	CAS RN	Downstream Effector	Ref
Cisplatin	Decitabine	2353-33-5	Gene methylation	[46]
Cisplatin	LY2109761	700874-71-1	TGFβRII	[47]
Cisplatin	LY364947	396129-53-6	TGFβRI in HPV-negative cells	[48]
Cisplatin	N^G-amino-L-arginine	1031799-40-2	Nitrous oxide synthases	[49]
Cisplatin	EGF	62253-63-8	EGFR	[50]
Cetuximab	JQ1	1268524-70-4	BET protein family	[20]
Cetuximab	Apigenin	520-36-5	ERK1/2	[23]
Cetuximab	Barasertib	722543-31-9	AURKB	[23]
Cetuximab	Afatinib	439081-18-2	EGFR, HER2, HER4	[43]
Cetuximab	PKI-587	1197160-78-3	PI3K and mTOR	[51]
Cetuximab	Dorsomorphin	866405-64-3	AMPK	[52]
Cetuximab	Oxamate	565-73-1	Lactate dehydrogenase A	[53]
Cetuximab	Dichloroacetic acid	79-43-6	Pyruvate dehydrogenase kinase-1	[54]
Cetuximab	Bortezomib	179324-69-7	NF-κB	[55]
Cetuximab	Parthenolide	20554-84-1	NF-κB	[55]
Cetuximab	Dasatinib	302962-49-8	Src family kinase	[56]
Cetuximab	R428	1037624-75-1	AXL	[57]
Cetuximab	PPY-A	875634-01-8	c-ABL kinase	[57]
Cetuximab	R763	871357-89-0	Aurora kinases	[58]
Cetuximab	TAK242	243984-11-4	Toll-like receptor 4	[59]
Cetuximab	MEHD7945A	unknown	EGFR and HER3	[60]

The Chemical Abstracts Service Registry Number is included (CAS RN) is included for identification purposes. TGFβR = transforming growth factor β receptor; HPV = human papillomavirus; BET = bromodomain and extra terminal; mTOR = mammalian target of rapamycin; AMPK = 5'-adenosine monophosphate-activated protein kinase.

upon, is shown in **Table 1**. This list is by no means comprehensive and is only meant to provide an overview of the research that has been conducted. Note that the N^G-amino-L-

arginine was shown using ovarian cancer rather than HNSCC, but it is included because of the strong similarities between the two types of cancer.

Conclusion

While there are many different postulated mechanisms to drug resistance in HNSCC, it is necessary to consider how different theories can complement each other. Such an approach is utilized here to suggest some of those connections by categorizing mechanisms by pathway and by type of resistance, acquired or intrinsic. As a result, evidence is presented on mechanisms common to both acquired and intrinsic resistance pathways, namely AXL, STAT3, and TWIST1. Proteins active in both pathways are more likely to be integral to cell survival and thus have a great potential for therapeutic use to improve outcomes for HNSCC patients.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Achuta Kumar Guddati, Division of Hematology/Oncology, Georgia Cancer Center, Augusta University, Augusta, GA 30909, USA. Tel: 312-404-8928; E-mail: aguddati@augusta.edu

References

[1] Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 2015; 517: 576-582.

[2] Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.

[3] Vermorken JB, Trigo J, Hitt R, Koralewski P, Diaz-Rubio E, Rolland F, Knecht R, Amellal N, Schueler A and Baselga J. Open-label, uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to respond to platinum-based therapy. J Clin Oncol 2007; 25: 2171-2177.

[4] Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, Erfan J, Zabolotnyy D, Kienzer HR, Cupissol D, Peyrade F, Benasso M, Vynnychenko I, De Raucourt D, Bokemeyer C, Schueler A, Amellal N and Hitt R. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med 2008; 359: 1116-1127.

[5] Wang J, Wang B, Chu H and Yao Y. Intrinsic resistance to EGFR tyrosine kinase inhibitors in advanced non-small-cell lung cancer with activating EGFR mutations. Onco Targets Ther 2016; 9: 3711-3726.

[6] Jackman D, Pao W, Riely GJ, Engelman JA, Kris MG, Janne PA, Lynch T, Johnson BE and Miller VA. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. J Clin Oncol 2010; 28: 357-360.

[7] Cai Y, Dodhia S and Su GH. Dysregulations in the PI3K pathway and targeted therapies for head and neck squamous cell carcinoma. Oncotarget 2017; 8: 22203-22217.

[8] Silva-Oliveira RJ, Melendez M, Martinho O, Zanon MF, de Souza Viana L, Carvalho AL and Reis RM. AKT can modulate the in vitro response of HNSCC cells to irreversible EGFR inhibitors. Oncotarget 2017; 8: 53288-53301.

[9] Wheeler DL, Huang S, Kruser TJ, Nechrebecki MM, Armstrong EA, Benavente S, Gondi V, Hsu KT and Harari PM. Mechanisms of acquired resistance to cetuximab: role of HER (ErbB) family members. Oncogene 2008; 27: 3944-3956.

[10] Nair S, Trummell HQ, Rajbhandari R, Thudi NK, Nozell SE, Warram JM, Willey CD, Yang ES, Placzek WJ, Bonner JA and Bredel M. Novel EGFR ectodomain mutations associated with ligand-independent activation and cetuximab resistance in head and neck cancer. PLoS One 2020; 15: e0229077.

[11] Saki M, Toulany M and Rodemann HP. Acquired resistance to cetuximab is associated with the overexpression of Ras family members and the loss of radiosensitization in head and neck cancer cells. Radiother Oncol 2013; 108: 473-478.

[12] Yonesaka K, Tanaka K, Kitano M, Kawakami H, Hayashi H, Takeda M, Sakai K, Nishio K, Doi K and Nakagawa K. Aberrant HER3 ligand heregulin-expressing head and neck squamous cell carcinoma is resistant to anti-EGFR antibody cetuximab, but not second-generation EGFR-TKI. Oncogenesis 2019; 8: 54.

[13] Peng F, Zhang H, Du Y and Tan P. miR-23a promotes cisplatin chemoresistance and protects against cisplatin-induced apoptosis in tongue squamous cell carcinoma cells through Twist. Oncol Rep 2015; 33: 942-950.

[14] Lin X, Liao J, Yang Z, Fan X, Cullen KJ, Chen L and Dan H. Inhibition of cisplatin-resistant head and neck squamous cell carcinoma by combination of Afatinib with PD0325901, a

MEK inhibitor. Am J Cancer Res 2019; 9: 1282-1292.

[15] Samatar AA and Poulikakos PI. Targeting RAS-ERK signalling in cancer: promises and challenges. Nat Rev Drug Discov 2014; 13: 928-942.

[16] Daly C, Castanaro C, Zhang W, Zhang Q, Wei Y, Ni M, Young TM, Zhang L, Burova E and Thurston G. FGFR3-TACC3 fusion proteins act as naturally occurring drivers of tumor resistance by functionally substituting for EGFR/ERK signaling. Oncogene 2017; 36: 471-481.

[17] Schulz D, Wirth M, Piontek G, Buchberger AM, Schlegel J, Reiter R, Multhoff G and Pickhard A. HNSCC cells resistant to EGFR pathway inhibitors are hypermutated and sensitive to DNA damaging substances. Am J Cancer Res 2016; 6: 1963-1975.

[18] Brand TM, Iida M, Stein AP, Corrigan KL, Braverman CM, Luthar N, Toulany M, Gill PS, Salgia R, Kimple RJ and Wheeler DL. AXL mediates resistance to cetuximab therapy. Cancer Res 2014; 74: 5152-5164.

[19] Nitschinsk K, Idris A and McMillan N. Patient derived xenografts as models for head and neck cancer. Cancer Lett 2018; 434: 114-119.

[20] Leonard B, Brand TM, O'Keefe RA, Lee ED, Zeng Y, Kemmer JD, Li H, Grandis JR and Bhola NE. BET inhibition overcomes receptor tyrosine kinase-mediated cetuximab resistance in HNSCC. Cancer Res 2018; 78: 4331-4343.

[21] Byers LA, Diao L, Wang J, Saintigny P, Girard L, Peyton M, Shen L, Fan Y, Giri U, Tumula PK, Nilsson MB, Gudikote J, Tran H, Cardnell RJ, Bearss DJ, Warner SL, Foulks JM, Kanner SB, Gandhi V, Krett N, Rosen ST, Kim ES, Herbst RS, Blumenschein GR, Lee JJ, Lippman SM, Ang KK, Mills GB, Hong WK, Weinstein JN, Wistuba II, Coombes KR, Minna JD and Heymach JV. An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. Clin Cancer Res 2013; 19: 279-290.

[22] Rampias T, Giagini A, Siolos S, Matsuzaki H, Sasaki C, Scorilas A and Psyrri A. RAS/PI3K crosstalk and cetuximab resistance in head and neck squamous cell carcinoma. Clin Cancer Res 2014; 20: 2933-2946.

[23] Boeckx C, Op de Beeck K, Wouters A, Deschoolmeester V, Limame R, Zwaenepoel K, Specenier P, Pauwels P, Vermorken JB, Peeters M, Van Camp G, Baay M and Lardon F. Overcoming cetuximab resistance in HNSCC: the role of AURKB and DUSP proteins. Cancer Lett 2014; 354: 365-377.

[24] Boeckx C, Blockx L, de Beeck KO, Limame R, Camp GV, Peeters M, Vermorken JB, Specenier P, Wouters A, Baay M and Lardon F. Establishment and characterization of cetuximab resistant head and neck squamous cell carcinoma cell lines: focus on the contribution of the AP-1 transcription factor. Am J Cancer Res 2015; 5: 1921-1938.

[25] Lai SY and Johnson FM. Defining the role of the JAK-STAT pathway in head and neck and thoracic malignancies: implications for future therapeutic approaches. Drug Resist Updat 2010; 13: 67-78.

[26] Wu Q, Zhao Y and Wang P. miR-204 inhibits angiogenesis and promotes sensitivity to cetuximab in head and neck squamous cell carcinoma cells by blocking JAK2-STAT3 signaling. Biomed Pharmacother 2018; 99: 278-285.

[27] Willey CD, Anderson JC, Trummell HQ, Naji F, de Wijn R, Yang ES, Bredel M, Thudi NK and Bonner JA. Differential escape mechanisms in cetuximab-resistant head and neck cancer cells. Biochem Biophys Res Commun 2019; 517: 36-42.

[28] Sen M, Joyce S, Panahandeh M, Li C, Thomas SM, Maxwell J, Wang L, Gooding WE, Johnson DE and Grandis JR. Targeting Stat3 abrogates EGFR inhibitor resistance in cancer. Clin Cancer Res 2012; 18: 4986-4996.

[29] Wheeler SE, Suzuki S, Thomas SM, Sen M, Leeman-Neill RJ, Chiosea SI, Kuan CT, Bigner DD, Gooding WE, Lai SY and Grandis JR. Epidermal growth factor receptor variant III mediates head and neck cancer cell invasion via STAT3 activation. Oncogene 2010; 29: 5135-5145.

[30] Scanlon CS, Van Tubergen EA, Inglehart RC and D'Silva NJ. Biomarkers of epithelial-mesenchymal transition in squamous cell carcinoma. J Dent Res 2013; 92: 114-121.

[31] Keysar SB, Le PN, Anderson RT, Morton JJ, Bowles DW, Paylor JJ, Vogler BW, Thorburn J, Fernandez P, Glogowska MJ, Takimoto SM, Sehrt DB, Gan GN, Eagles-Soukup JR, Serracino H, Hirsch FR, Lucia MS, Thorburn A, Song JI, Wang XJ and Jimeno A. Hedgehog signaling alters reliance on EGF receptor signaling and mediates anti-EGFR therapeutic resistance in head and neck cancer. Cancer Res 2013; 73: 3381-3392.

[32] Sayed SI, Dwivedi RC, Katna R, Garg A, Pathak KA, Nutting CM, Rhys-Evans P, Harrington KJ and Kazi R. Implications of understanding cancer stem cell (CSC) biology in head and neck squamous cell cancer. Oral Oncol 2011; 47: 237-243.

[33] Reid PA, Wilson P, Li Y, Marcu LG and Bezak E. Current understanding of cancer stem cells: review of their radiobiology and role in head and neck cancers. Head Neck 2017; 39: 1920-1932.

[34] Kulsum S, Sudheendra HV, Pandian R, Ravindra DR, Siddappa G, R N, Chevour P, Ramachandran B, Sagar M, Jayaprakash A, Mehta A, Kekatpure V, Hedne N, Kuriakose MA and Suresh A. Cancer stem cell mediated acquired chemoresistance in head and neck cancer can be abrogated by aldehyde dehydrogenase 1 A1 inhibition. Mol Carcinog 2017; 56: 694-711.

[35] Kolenda T, Guglas K, Rys M, Bogaczynska M, Teresiak A, Blizniak R, Lasinska I, Mackiewicz J and Lamperska KM. Biological role of long non-coding RNA in head and neck cancers. Rep Pract Oncol Radiother 2017; 22: 378-388.

[36] Lee SP, Hsieh PL, Fang CY, Chu PM, Liao YW, Yu CH, Yu CC and Tsai LL. LINC00963 promotes cancer stemness, metastasis, and drug resistance in head and neck carcinomas via ABCB5 regulation. Cancers (Basel) 2020; 12: 1073.

[37] Li M, Chen H and Wu T. LIN28: a cancer stem cell promoter for immunotherapy in head and neck squamous cell carcinoma. Oral Oncol 2019; 98: 92-95.

[38] Lathia J, Liu H and Matei D. The clinical impact of cancer stem cells. Oncologist 2020; 25: 123-131.

[39] Zhou W, Yang Y, Xia J, Wang H, Salama ME, Xiong W, Xu H, Shetty S, Chen T, Zeng Z, Shi L, Zangari M, Miles R, Bearss D, Tricot G and Zhan F. NEK2 induces drug resistance mainly through activation of efflux drug pumps and is associated with poor prognosis in myeloma and other cancers. Cancer Cell 2013; 23: 48-62.

[40] Xu H, Zeng L, Guan Y, Feng X, Zhu Y, Lu Y, Shi C, Chen S, Xia J, Guo J, Kuang C, Li W, Jin F and Zhou W. High NEK2 confers to poor prognosis and contributes to cisplatin-based chemotherapy resistance in nasopharyngeal carcinoma. J Cell Biochem 2019; 120: 3547-3558.

[41] Li C, Iida M, Dunn EF, Ghia AJ and Wheeler DL. Nuclear EGFR contributes to acquired resistance to cetuximab. Oncogene 2009; 28: 3801-3813.

[42] Iida M, Bahrar H, Brand TM, Pearson HE, Coan JP, Orbuch RA, Flanigan BG, Swick AD, Prabakaran PJ, Lantto J, Horak ID, Kragh M, Salgia R, Kimple RJ and Wheeler DL. Targeting the HER family with Pan-HER effectively overcomes resistance to cetuximab. Mol Cancer Ther 2016; 15: 2175-2186.

[43] De Pauw I, Lardon F, Van den Bossche J, Baysal H, Fransen E, Deschoolmeester V, Pauwels P, Peeters M, Vermorken JB and Wouters A. Simultaneous targeting of EGFR, HER2, and HER4 by afatinib overcomes intrinsic and acquired cetuximab resistance in head and neck squamous cell carcinoma cell lines. Mol Oncol 2018; 12: 830-854.

[44] Seiwert TY, Fayette J, Cupissol D, Del Campo JM, Clement PM, Hitt R, Degardin M, Zhang W, Blackman A, Ehrnrooth E and Cohen EE. A randomized, phase II study of afatinib versus cetuximab in metastatic or recurrent squamous cell carcinoma of the head and neck. Ann Oncol 2014; 25: 1813-1820.

[45] Box C, Mendiola M, Gowan S, Box GM, Valenti M, Brandon Ade H, Al-Lazikani B, Rogers SJ, Wilkins A, Harrington KJ and Eccles SA. A novel serum protein signature associated with resistance to epidermal growth factor receptor tyrosine kinase inhibitors in head and neck squamous cell carcinoma. Eur J Cancer 2013; 49: 2512-2521.

[46] Viet CT, Dang D, Achdjian S, Ye Y, Katz SG and Schmidt BL. Decitabine rescues cisplatin resistance in head and neck squamous cell carcinoma. PLoS One 2014; 9: e112880.

[47] Rodrigues-Junior DM, Tan SS, Lim SK, Leong HS, Melendez ME, Ramos CRN, Viana LS, Tan DSW, Carvalho AL, Iyer NG and Vettore AL. Circulating extracellular vesicle-associated TGFbeta3 modulates response to cytotoxic therapy in head and neck squamous cell carcinoma. Carcinogenesis 2019; 40: 1452-1461.

[48] Liu Q, Ma L, Jones T, Palomero L, Pujana MA, Martinez-Ruiz H, Ha PK, Murnane J, Cuartas I, Seoane J, Baumann M, Linge A and Barcellos-Hoff MH. Subjugation of TGFbeta signaling by human papilloma virus in head and neck squamous cell carcinoma shifts DNA repair from homologous recombination to alternative end joining. Clin Cancer Res 2018; 24: 6001-6014.

[49] Leung EL, Fraser M, Fiscus RR and Tsang BK. Cisplatin alters nitric oxide synthase levels in human ovarian cancer cells: involvement in p53 regulation and cisplatin resistance. Br J Cancer 2008; 98: 1803-1809.

[50] Mandic R, Rodgarkia-Dara CJ, Krohn V, Wiegand S, Grenman R and Werner JA. Cisplatin resistance of the HNSCC cell line UT-SCC-26A can be overcome by stimulation of the EGF-receptor. Anticancer Res 2009; 29: 1181-1187.

[51] D'Amato V, Rosa R, D'Amato C, Formisano L, Marciano R, Nappi L, Raimondo L, Di Mauro C, Servetto A, Fusciello C, Veneziani BM, De Placido S and Bianco R. The dual PI3K/mTOR inhibitor PKI-587 enhances sensitivity to cetuximab in EGFR-resistant human head and neck cancer models. Br J Cancer 2014; 110: 2887-2895.

[52] Li X, Lu Y, Lu H, Luo J, Hong Y and Fan Z. AMPK-mediated energy homeostasis and associated metabolic effects on cancer cell response and resistance to cetuximab. Oncotarget 2015; 6: 11507-11518.

[53] Lu H, Li X, Luo Z, Liu J and Fan Z. Cetuximab reverses the Warburg effect by inhibiting HIF-1-regulated LDH-A. Mol Cancer Ther 2013; 12: 2187-2199.

[54] Lu H, Lu Y, Xie Y, Qiu S, Li X and Fan Z. Rational combination with PDK1 inhibition overcomes cetuximab resistance in head and neck squamous cell carcinoma. JCI Insight 2019; 4: e131106.

[55] Hsu DS, Hwang WL, Yuh CH, Chu CH, Ho YH, Chen PB, Lin HS, Lin HK, Wu SP, Lin CY, Hsu WH, Lan HY, Wang HJ, Tai SK, Hung MC and Yang MH. Lymphotoxin-beta interacts with methylated EGFR to mediate acquired resistance to cetuximab in head and neck cancer. Clin Cancer Res 2017; 23: 4388-4401.

[56] Stabile LP, Egloff AM, Gibson MK, Gooding WE, Ohr J, Zhou P, Rothenberger NJ, Wang L, Geiger JL, Flaherty JT, Grandis JR and Bauman JE. IL6 is associated with response to dasatinib and cetuximab: phase II clinical trial with mechanistic correlatives in cetuximab-resistant head and neck cancer. Oral Oncol 2017; 69: 38-45.

[57] McDaniel NK, Iida M, Nickel KP, Longhurst CA, Fischbach SR, Rodems TS, Kranjac CA, Bo AY, Luo Q, Gallagher MM, Welke NB, Mitchell KR, Schulz AE, Eckers JC, Hu R, Salgia R, Hong S, Bruce JY, Kimple RJ and Wheeler DL. AXL mediates cetuximab and radiation resistance through tyrosine 821 and the c-ABL kinase pathway in head and neck cancer. Clin Cancer Res 2020; 26: 4349-4359.

[58] Hoellein A, Pickhard A, von Keitz F, Schoeffmann S, Piontek G, Rudelius M, Baumgart A, Wagenpfeil S, Peschel C, Dechow T, Bier H and Keller U. Aurora kinase inhibition overcomes cetuximab resistance in squamous cell cancer of the head and neck. Oncotarget 2011; 2: 599-609.

[59] Ju H, Hu Z, Lu Y, Wu Y, Zhang L, Wei D, Guo W, Xia W, Liu S, Ren G and Hu J. TLR4 activation leads to anti-EGFR therapy resistance in head and neck squamous cell carcinoma. Am J Cancer Res 2020; 10: 454-472.

[60] Huang S, Li C, Armstrong EA, Peet CR, Saker J, Amler LC, Sliwkowski MX and Harari PM. Dual targeting of EGFR and HER3 with MEHD7945A overcomes acquired resistance to EGFR inhibitors and radiation. Cancer Res 2013; 73: 824-833.