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ErbB Receptors in the Biology and Pathology of the Aerodigestive Tract

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Abstract

The most common sites of malignancies in the aerodigestive tract include the lung, head and neck and the esophagus. Esophageal adenocarcinomas (EA), esophageal squamous cell carcinomas (ESCC), and squamous cell carcinomas of the head and neck (SCCHN) are the primary focus of this review. Traditional treatment for aerodigestive tract cancers includes primary chemoradiotherapy (CRT) or surgical resection followed by radiation (or CRT). Recent developments in treatment have focused increasingly on molecular targeting strategies including cetuximab (a monoclonal antibody against epidermal growth factor receptor (EGFR)). Cetuximab was FDA approved in 2006 for treatment of SCCHN, underscoring the importance of understanding the biology of these malignancies. EGFR is a member of the ErbB family of growth factor receptor tyrosine kinases. The major pathways activated by ErbB receptors include Ras/Raf/MAPK; PI3K/AKT; PLC γ and STATs, all of which lead to the transcription of target genes that may contribute to aerodigestive tumor progression. This review explores the expression of ErbB receptors in EA, ESCC and SCCHN and the signaling pathways of EGFR in SCCHN.

Introduction

The aerodigestive tract encompasses the lungs, esophagus, oral cavity, nasal cavity, paranasal sinuses, pharynx and larynx. The three most common sites where malignancies arise include the lung, head and neck and esophagus. This review will focus on esophageal adenocarcinomas/squamous cell carcinomas and squamous cell carcinoma of the head and neck (SCCHN). The primary risk factors in SCCHN include tobacco and alcohol use [1,2]. A subset of SCCHN has been shown to be caused by the human papillomavirus, primarily types 16 and 18 [2,3]. There is a high incidence of synchronous and metachronous esophageal squamous cell carcinoma (ESCC) in patients diagnosed with SCCHN, indicating the common biology of these aerodigestive tract neoplasms [4–7]. A recent report noted that ESCC accounts for approximately 38% of esophageal cancers in the United States (1998–2003) [8]. Established risk factors for ESCC also include tobacco and alcohol use [9] with esophagitis/inflammation as a possible contributing variable [10].

Esophageal adenocarcinoma (EA) is responsible for ~56% of esophageal cancers in the United States (1998–2003) [8] and has several established risk factors including Barrett's esophagus [11,12], gastro-esophageal reflux [13,14] and obesity (independent of reflux) [15–17].

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Medications that relax the lower esophageal sphincter may also contribute but the current evidence is inconclusive [18]. Some reports suggest that *helicobacter pylori* and the regular use of non-steroidal anti-inflammatory drugs may contribute to a reduced risk of EA [18].

Treatment for aerodigestive tract cancers including SCCHN and ESCC/EA has traditionally included primary chemoradiotherapy (CRT) or surgical resection followed by radiation (or CRT). Cetuximab is a monoclonal antibody against EGFR that has been shown to reduce patient mortality and increase locoregional control of the tumor when combined with radiotherapy in SCCHN [19]. In 2006 cetuximab became the first molecular targeting strategy approved by the FDA for SCCHN. Preliminary work in ESCC has shown that cetuximab can induce antibody-dependent cell cytotoxicity in ESCC cell lines [20]. A recent phase II clinical trial reported that cetuximab can be safely administered in combination with chemotherapy and radiotherapy in esophageal carcinomas without increased mucosal toxicity [21]. A phase III clinical trial is currently underway to determine if cetuximab in combination with CRT treatment will increase survival compared to CRT alone [21]. The success of this molecular targeting strategy in SCCHN and esophageal carcinomas underscores the importance of understanding the biology of these malignancies.

Biology of ErbB Receptors in the Aerodigestive Tract

ErbB receptors are members of the ErbB growth factor receptor tyrosine kinase family and are generally found on the cell surface. ErbB receptors contain an extracellular ligand-binding domain, a transmembrane region and an intracellular domain with tyrosine kinase activity (except ErbB3). Upon ligand binding, the receptors dimerize and autophosphorylate thereby initiating a signaling cascade downstream of the dimer. Ligand binding induces a conformation change of the receptor ectodomain (creating an extended and stabilized conformation, except for ErbB2 which constitutively maintains the stabilized conformation and has no known ligand [22]) to facilitate receptor dimerization [23]. ErbB ligands are produced as transmembrane precursors and the ectodomains are processed via proteolysis leading to the shedding of soluble growth factors [24]. In normal tissues this signaling cascade is tightly controlled and regulates processes that include epithelial development and injury response. The major pathways activated by ErbB receptors include Ras/Raf/MAPK; PI3K/AKT; PLC γ and STATs, all of which lead to the transcription of target genes that may contribute to aerodigestive tumor progression [25]. Regulation of ErbB receptor signaling occurs through temporal and spatial expression of receptor ligands and through receptor endocytosis. Endocytic trafficking leads to receptor recycling or ubiquitination and lysosomal degradation of the receptor [26].

EGFR activation can be induced through autocrine or paracrine ligands. There are six major EGFR ligands that are expressed at the mRNA level in some, but not all, SCCHN cell lines including: heparin binding EGF (HB-EGF), transforming growth factor alpha (TGF- α), betacellulin, with amphiregulin (AR), heregulin, and epidermal growth factor (EGF) [27]. TGF- α and AR are the primary ligands implicated in autocrine growth signaling [28]. EGFR can homodimerize or heterodimerize with other members of the ErbB receptor family [29].

ErbB2 has no known exogenous ligands that directly bind to it. If ErbB2 is highly overexpressed it can spontaneously dimerize and autoactivate, but it is most commonly activated *via* heterodimerization with other ErbB family members [22]. ErbB3 has no intrinsic tyrosine kinase activity but is transactivated by EGFR and ErbB2. ErbB3 ligands include neuregulins, heregulin and neu differentiation factor [30]. ErbB4 can homodimerize or heterodimerize with other members of the ErbB receptor family. ErbB4 ligands include neuregulins, epiregulin, heregulin, neu differentiation factor, and betacellulin [30].

ErbB Receptors in Aerodigestive Tract Development

Knockout of each of the ErbB family members in mouse models leads to early stage lethality, limiting the study of ErbBs in the development of the aerodigestive tract. ErbB1 null mice had a generalized epithelial immaturity and multiorgan failure [31]; these mice have a short postnatal survival period in which impaired epithelial development in various organs is observed, including the skin, lungs and gastrointestinal tract. It was noted in EGFR $-/-$ mice that the epithelium of the tongue also appeared to be immature and was thinner and less organized with no fungiform taste papillae, indicating a role for EGFR in mucosal epithelial development [31]. ErbB2 and ErbB4 null mice die mid-gestation due to malformations of the heart and central nervous system [32,33]. Most erbB3 null mice die before birth due to gross malformations of the nervous system and Schwann cells and those that do survive through birth die shortly after from respiratory insufficiency [34]. These cumulative results suggest that ErbB1 likely contributes primarily to epithelial development while ErbB2, ErbB3 and ErbB4 seem to regulate the development of neural and muscle tissues. Interestingly, ErbB receptor null mice phenotypes are distinct depending on the precise genetic background, demonstrating that there are genetic factors that also influence the role of ErbB receptors in development.

ErbBs in Aerodigestive Tract Cancer

ErbB Family Expression

Expression of EGFR, ErbB2, ErbB3 and ErbB4 has been reported in SCCHN [28], although data on ErbB4 are conflicting [35–39]. The entire ErbB receptor family has been found to be expressed at increased levels in invasive carcinoma and ErbB1, ErbB2 and ErbB4 have been identified as overexpressed in *in situ* carcinoma [28]. Most cases of overexpression demonstrated elevated expression of multiple receptors simultaneously, providing indirect evidence for the formation of ErbB receptor heterodimers [40].

EGFR—EGFR is the most well studied of the ErbB receptors in cancers of the aerodigestive tract. Overexpression of EGFR in oral dysplasias compared to normal mucosa has been demonstrated [41,42]. However, EGFR upregulation in the normal-appearing epithelium adjacent to malignant tissue in SCCHN has also been reported, supporting the idea of field cancerization in SCCHN [43]. EGFR expression has been demonstrated to be lower in laryngeal tumors as compared to tumors of the pharynx and oral cavity [44] indicating that EGFR expression may differ between SCCHN anatomic sites [28]. In SCCHN tumors, 92% have elevated EGFR mRNA levels and 87% have elevated mRNA for its ligand TGF- α [43]. Additionally, EGFR protein overexpression has been reported in over 80% of SCCHN cases [28] and EGFR gene amplification has been demonstrated in up to 15% of SCCHN tumors [45]. In ESCC, EGFR protein overexpression has been detected in 60–70% [46,47]. Gene amplification in ESCC was evaluated using FISH and found to be present in approximately 28% of tumors [46]. In EA EGFR is frequently expressed and may contribute to the progression of Barrett's metaplasia to EA [48,49]. A recent study in esophageal carcinomas indicates that TGF- α and EGFR are both expressed in 88% of tumors [50]. High levels of EGFR protein expression have been correlated with lower patient survival in esophageal carcinomas as well as SCCHN [47,51–53]. EGFR overexpression in ESCC has also been significantly correlated with increased tumor invasion [46].

EGFR dysregulation appears to result from several potential mechanisms, including gene amplification and transcriptional activation [54–56]. Additionally, EGFR mRNA overexpression may result from dysregulated p53, which directly increases EGFR gene transcription in SCCHN [57]. Abnormalities in dinucleotide repeats in the first intron of the EGFR gene have also been implicated in altering the efficiency of EGFR gene transcription. In 12 SCCHN cell lines, those with lower numbers of dinucleotide repeats had increased levels

of EGFR mRNA and protein [56,58]. Transcriptional activation appears to be a common cause of EGFR overexpression in SCCHN, although the precise mechanisms leading to increased gene transcription are incompletely understood [59].

Another possible mechanism of cellular dysregulation by EGFR is through nuclear localization of EGFR where it can act as a transcription factor. Nuclear localization of EGFR in SCCHN has been reported and is associated with STAT3 interaction and transcriptional activation of inducible nitric oxide synthase in SCCHN [28]. While the precise role of nuclear EGFR is incompletely understood, localization of EGFR to the nucleus suggests that genes may be transactivated by EGFR independent of direct EGFR downstream signaling.

ErbB2—ErbB2 is overexpressed in SCCHN and in esophageal cancers compared to levels detected in corresponding normal mucosa. Studies have shown that ErbB2 is overexpressed in ~20–40% of tumors of SCCHN with gene amplification present in approximately 5–10% of cases [37,60–64]. In ESCC, reports of IHC staining indicate that ErbB2 is overexpressed in ~26–64% of cases [65–69]. Increased expression of ErbB2 in EA is considered to be more common with ~10–70% of patients showing overexpression [70,71]. The large range of incidence of ErbB2 overexpression in these studies is likely due to the variety of methods used to detect ErbB2 that are currently in use. While there is no consensus regarding the optimal assay for ErbB2 detection, immunohistochemistry (IHC) and FISH have both been approved by the FDA and are commonly used [70]. IHC is only semi-quantitative and does not always accurately reflect ErbB2 status. ErbB2 gene amplification is found in few cases of ESCC (~5%) but is more common in EA (~15%) [72].

Studies in SCCHN indicate that ErbB2 overexpression may correlate with survival. IHC staining has shown that ErbB2 overexpression is significantly correlated with a decrease in disease free survival indicating a possible prognostic value of ErbB2 expression in this cancer [64]. Gene amplification was also studied using a semi-quantitative PCR technique, but genomic amplifications of ErbB2 are not common and did not significantly correlate with patient survival in SCCHN [73]. ErbB2 may also be involved in resistance to 5-fluorouracil, cisplatin and the EGFR inhibitor gefitinib [36,74]. However, ErbB2 targeting strategies have not demonstrated clinical efficacy to date in SCCHN [75–77]. While ErbB2 may heterodimerize with other ErbB family members and contribute to SCCHN tumor progression, the role of heterodimers in SCCHN is incompletely understood [36].

A correlation between patient survival and ErbB2 overexpression or gene amplification has not been clearly elucidated in esophageal carcinomas. In ESCC, some reports indicate an association between ErbB2 overexpression and poor prognosis [69,78,79] while others have indicated no correlation [65–67]. Results of one study indicated a possible association between ErbB2 overexpression and chemoradioresistance in ESCC [68]. Studies in EA are also inconclusive regarding the correlation of ErbB2 expression and/or amplification with patient prognosis [80,81].

ErbB3—ErbB3 is overexpressed at the mRNA and protein levels in a subset of SCCHN cell lines [82] and is overexpressed in 21–81% of SCCHN tumors [37,60–62]. However, studies vary and are limited by the poor quality of the antibodies available for IHC and immunoblotting. To date, ErbB3 gene amplification has not been detected in SCCHN [83]. ErbB3 appears to be related to the malignant progression of SCCHN in clinical specimens [82] and ErbB3 overexpression in SCCHN is correlated with survival and metastasis in several cohorts [84]. ErbB3 expression and signaling has been correlated with resistance to the EGFR inhibitor gefitinib in lung cancer [36,85] and the limited SCCHN data suggests that a similar mechanism may also be important in this cancer [36].

ErbB3 has not been studied specifically in ESCC and data in EA is limited. Some results suggest that ErbB3 is upregulated in ~40–50% of EA compared to normal tissue as detected by both quantitative RT-PCR and IHC [86]. Further investigation of ErbB3 biology in esophageal carcinomas is warranted.

ErbB4—Expression of ErbB4 is detected in SCCHN (26–69%) [37,39,60,61] but the role of this ErbB family member in these cancers is unclear. Expression of ErbB4 did not correlate with invasion, angiogenesis, metastasis or SCCHN tumor progression [87], although expression of all four ErbB receptors in oral SCC was significantly associated with decreased patient survival [88]. Other evidence indicates that ErbB4 expression may be lost *in vitro* [39]. These findings provide indirect evidence of cooperation among ErbB receptors in SCCHN cancer progression.

In ESCC membranous, cytoplasmic and nuclear staining are noted in more than 80% of ESCC samples demonstrating ErbB4 expression in both the cell membrane and cytoplasm [89]. Cytoplasmic and nuclear staining of ErbB4 expressing cells may result from ErbB4 cleavage by tumor necrosis factor alpha converting enzyme (TACE) and γ -secretase producing an intracellular domain fragment that can be translocated to the nucleus where it functions as a transcription factor [90–92]. In ESCC, the full-length membrane spanning ErbB4 receptor may contribute to inhibition of tumor progression while the transcription factor ErbB4, when localized in the nucleus, may contribute to tumor progression [89].

ErbB Alterations

Unlike non-small cell lung carcinoma, SCCHN is not characterized by mutations of the EGFR kinase domain [93]. In esophageal carcinomas, EGFR mutations remain relatively uncharacterized with a few reports indicating that EGFR tyrosine kinase mutations are rare in North America [94–96]. Expression of the EGFR variant III has been identified in up to 40% of SCCHN tumors [97] but has not been studied, to date, in esophageal carcinomas. EGFRvIII was originally characterized in glioblastoma where it was found to be a common somatic mutation associated with EGFR gene amplification leading to gene rearrangement [98,99]. EGFRvIII lacks exons 2–7 with a novel glycine residue at the exon 1/8 junction. Exons 2–7 comprise the majority of the extracellular ligand binding domain so that cells that express EGFRvIII are likely to bind to EGFR monoclonal antibodies with reduced affinity. Thus, EGFRvIII represents a possible mechanism of cetuximab resistance [97]. EGFRvIII expression is lost *in vitro*; consequently SCCHN cells must be stably transfected with an EGFRvIII construct to establish a model for preclinical investigations. We have shown that EGFRvIII expression in SCCHN cell lines leads to increased cell proliferation *in vitro* and increased tumorigenicity *in vivo* as compared to vector-transfected control cells [97].

ErbB Dimerization

Heterodimerization of ErbB receptors allows for potent transduction of various signals. Heterodimerization of other members of the ErbB family with EGFR is implicated in early SCCHN carcinogenesis where co-expression has been detected in premalignant lesions [100]. ErbB2 heterodimers have increased signaling potency compared to any other ErbB dimers. This is caused by an increased ligand affinity through a decelerated rate of ligand dissociation, efficient coupling to signaling pathways and a decreased rate of receptor downregulation through endocytosis [101]. ErbB2-ErbB3 heterodimers have been shown to induce the PI3K pathway, most likely because ErbB3 can directly bind the PI3K p85 subunit [83].

ErbB Signaling

Upon ErbB receptor autophosphorylation, a variety of protein signaling molecules are recruited to the plasma membrane including growth factor receptor bound protein 2 (Grb2) and Shc. Activation of these proteins initiates the ErbB signaling cascades that lead to transcriptional regulation of target genes. In SCCHN members of the EGFR signaling pathway have been found at increased levels, including MAPK, AKT, STAT3 and STAT5 (figure 1) [28]. Studies in esophageal carcinomas are more limited but indicate that the MAPK, AKT and STAT pathways are involved in the oncogenic signaling through EGFR overexpression [102–107]. More mechanistic studies are needed to clearly define the signaling cascades involved with ErbB signal transduction and esophageal carcinoma progression.

MAPK—Following EGFR activation, Grb2 and Sos (adaptor proteins) bind EGFR directly at Y1092 and Y1110 or through Shc (which binds at EGFR Y1172 and Y1197) [108]. This activates Raf-1 initiating a cascade that results in phosphorylated MAPK, which is then translocated to the nucleus where it activates cell proliferation transcription factors [23]. The Ras-Raf-MEK-ERK pathway is the primary MAPK pathway downstream of ErbBs in SCCHN and leads to upregulation of cyclin D1, which induces cell cycle progression (figure 1) [83]. Activated MAPK in SCCHN was found to correlate with EGFR and TGF- α overexpression [109]. The formation of an E-cadherin-EGFR intercellular complex between tumor cells is thought to contribute to SCCHN invasion and metastasis. This complex leads to ligand independent activation of EGFR, which activates the MAPK pathway and transcription of Bcl2 allowing the cell to escape apoptosis induced by loss of the extra cellular matrix [110].

In esophageal carcinomas, MAPK has been implicated as a key mediator in the downstream signaling of EGFR activation. Use of the pan ErbB tyrosine kinase inhibitor CI-1033 in ESCC lines resulted in the inhibition of phosphorylation of MAPK and inhibition of cell proliferation [111]. A separate study combined radiation treatment with the MEK inhibitor PD98059 and found synergistic effects on cell killing in esophageal cancer cell lines indicating that MAPK signaling may be a contributing factor in cell survival in esophageal cancer [106].

STATs—STAT1, STAT3, and STAT5 [100,112,113] are activated in SCCHN and contribute to the transduction of EGFR signaling in the cell. STAT3 and STAT5 are transcription factors and oncogenes where to help to regulate cell cycle progression, angiogenesis and apoptosis inhibition through their target genes. STATs can be activated by interacting directly with EGFR through SH2 domains or indirectly through Src-mediated EGFR signaling [114]. After activation, STATs dimerize and are then translocated to the nucleus where they induce the transcription of target genes (figure 1) [114].

Constitutive activation of STAT3 has been reported in SCCHN [114] and STAT3 has been shown to be an oncogene and a mediator of cellular transformation [23,115]. STAT3 is likely activated in SCCHN through autocrine activation of EGFR by TGF- α [114]. The identification of constitutive STAT3 activity in normal mucosa indicates that STAT3 activation may have an early role in SCCHN progression. STAT3 has been demonstrated to be upregulated both with and independent of EGFR upregulation [114]. In SCCHN, targeting STAT3 inhibited cell growth *in vivo* and *in vitro* [116,117]. Additionally, combined targeting of STAT3 and EGFR produces enhanced antitumor effects *in vitro* and *in vivo* as compared to EGFR targeting alone [118]. These findings indicate that STAT3 overexpression may contribute to cancer progression. STAT5 is also overexpressed and activated in SCCHN and an antisense blockade of STAT5b inhibited tumor growth [119].

In esophageal carcinoma cell lines, proliferation via autocrine signaling of EGFR was attributed to TGF- α stimulation leading to STAT3 activation [120]. In esophageal keratinocytes, EGFR stimulation leads to STAT1 phosphorylation and dimerization with

STAT3 and subsequent formation of the STAT-JAK complex. This pathway results in keratinocyte migration as well as MMP1 activity [103].

Src Family Kinases—Src family kinases (SFKs) are involved in cell proliferation, migration, adhesion, angiogenesis, and immune function [23]. Src is a signal transducer of EGFR signaling and is also independently activated and leads to the activation of many pathways including STATs and PI3K [23]. In SCCHN cell lines Src family kinases are activated by TGF- α stimulation via direct binding to EGFR at Y915 and Y944 [108,121]. Corresponding normal epithelial cells did not show activation of the SFKs (cSrc, cYes, Fyn, Lyn) [121]. Activated levels of STAT3 and STAT5 were highly correlated with Src phosphotyrosine (activation) levels and coimmunoprecipitation of STAT3 or STAT5 showed interaction with cSrc. cSrc blockade demonstrated reduced STAT3 and STAT5 activation in addition to reduced cell growth in SCCHN cell lines. This indicates that SFKs mediate STAT growth pathways in SCCHN [121]. In SCCHN Src can be activated independently of EGFR and transphosphorylate EGFR at Y845, leading to EGFR receptor activation [122].

PLC γ -1—PLC γ -1 likely contributes to SCCHN invasion and migration. PLC γ -1 can interact directly with EGFR and hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol 1,3,5-triphosphate (involved in intracellular calcium release) and 1,2-diacylglycerol (a cofactor in PKC activation). PKC activates Raf, which leads to MAPK activation (figure 1) [23,123]. PLC γ -1 is downstream of EGFR and may mediate the invasive and metastatic mechanisms of SCCHN [124]. PLC γ -1 contributes to tumor cell invasion in *in vitro* SCCHN experiments when activated by EGFR [125]. Activation of PLC γ through EGFR stimulation with EGF promotes SCCHN migration [125]. In human SCCHN tumor samples, IHC staining showed that the tumor stained higher for phosphorylated PLC γ -1 than the normal mucosa [125] demonstrating an increase in PLC γ -1 activity in SCCHN. Other *in vitro* experiments on SCCHN cells demonstrated that chemotaxis and invasion of metastatic SCCHN cells were dependent on PI3K and its substrate PLC γ -1 although this pathway can be activated through EGFR or chemokine receptor 7 [126].

PI3K—PI3K can be activated by EGFR through EGFR heterodimerization with ErbB3, which contains a docking site for the p85 subunit of PI3K, or alternately through Gab-1 binding EGFR. Once the p85 subunit is docked, the p110 subunit of PI3K (containing catalytic activity) generates phosphatidylinositol 3,4,5-triphosphate (PIP3), which phosphorylates and activates AKT. Activated AKT was found to be overexpressed in 57–81% of SCCHN tumors [125]. This pathway is involved in resistance to apoptosis, cell growth, invasion and migration [23]. Cell migration may also be mediated by the Rho family of GTP-binding proteins [127] and PI3K through activation of Ras and Rac [128]. Other non-receptor tyrosine kinases appear to be possible mediators downstream of EGFR and upstream of PI3K. The non-receptor tyrosine kinase Syk may operate downstream of EGFR to participate in mediating signaling through the PI3K and PLC γ pathways in SCCHN causing increased cell motility [129].

The PI3K pathway downstream of the ErbB receptors is also a major mechanism of apoptosis evasion in head and neck cancer. PI3K catalyzes the conversion of PIP2 to a lipid second messenger PIP3, which results in recruiting and activating of PKB and AKT through PDK. In head and neck cancers EGFR can lead to PI3K activation directly or through Ras. PI3K then induces downstream amplification of PDK1, which in turn phosphorylates AKT. In SCCHN, AKT regulates cell survival by affecting several downstream targets including the FOXO family of forkhead transcription factors, Bad, caspase 9 and by activating NF- κ B. Studies in SCCHN have shown that PTEN mutations are extremely rare indicating that PI3K activation through loss of PTEN function is not a major factor in dysregulated PI3K signaling in SCCHN [83].

The PI3K/AKT pathway appears to also be involved in esophageal neoplasms. Esophageal cancer cells stimulated with EGF showed induction of AKT phosphorylation with differential activation between AKT isoforms [107]. In primary and immortalized esophageal organotypic cultures, AKT overexpression and activation is permissive for differentiation of primary and immortalized esophageal epithelial cells [104]. These results indicate a role for the PI3K/AKT pathway in the context of EGFR stimulation. Irradiation combined with the inhibitor of PI3K LY294002 resulted in a synergistic increase in radiation-induced cell killing as compared to radiation alone in esophageal cancer cell lines [106]. These studies indicate that the PI3K pathway is involved in esophageal cancer cell survival and may be an appropriate future target for molecular therapeutics.

ErbB Crosstalk

EGFR can be transactivated by G-protein-coupled receptors (GPCRs) through ligand-dependent and independent pathways. GPCRs can directly activate the tyrosine kinase domain of EGFR or can induce cleavage of EGFR ligand precursors. Several GPCRs have been implicated in EGFR activation in SCCHN including gastrin-releasing peptide (GRP), prostaglandin E2 (PGE2), bradykinin, thrombin and lysophosphatidic acid (LPA) [130]. GPCR-EGFR crosstalk has been shown to contribute to lung and head and neck cancer progression [130]. GPCR ligands induce EGFR phosphorylation and downstream MAPK activation through cleavage of EGFR proligands, including TGF- α and amphiregulin, by TNF α converting enzyme (TACE) (figure 1) [131]. EGFR and MAPK appear to be key mediators in the mitogenic effects of GPCR as their inhibition eliminated SCCHN proliferation induced by GPCR [132]. Further investigation demonstrated a role for Src family kinases and the PDK1 subunit of PI3K in this process in SCCHN [133,134]. Combined blockade of GPCR and EGFR pathways significantly inhibited proliferation, invasion and colony formation of SCCHN cell lines but not immortalized mucosal epithelial cells [135]. GPCRs can also activate EGFR via Src-mediated phosphorylation of EGFR at Y845 in SCCHN [28].

Transactivation of EGFR by cell adhesion molecules such as E-cadherin from neighboring tumor cells has been proposed to prevent apoptosis in the early stages of metastasis as the tumor cell detaches from the extracellular matrix [110]. Cell adhesion molecules such as E-cadherin and integrins in SCCHN can form a complex with EGFR and transactivate EGFR (figure 1) [83,110]. This transactivation of EGFR by E-cadherin leads to activation of the MAPK pathway which leads to transcription of the anti-apoptotic protein Bcl-2 [110] allowing for SCCHN tumor cell survival in the absence of the extracellular matrix. EGFR activation can also alter the expression of integrins and E-cadherin by phosphorylating beta-catenin which leads to the internalization of E-cadherin [83] allowing decreased tumor cell aggregation and increased motility on extra cellular matrix proteins in SCCHN. In esophageal carcinomas, an inverse correlation between E-cadherin and EGFR has been reported where expression of these proteins may serve as prognostic markers in this malignancy [136–138].

In SCCHN, EGFR has also been shown to be involved in crosstalk with platelet-derived growth factor receptor and hormone receptors [26]. Overexpression of the urokinase-type plasminogen activator receptor can also lead to ligand independent activation of EGFR [23]. Insulin-like growth factor 1 receptor can transactivate EGFR [83], while EGFR is known to transactivate cMET in SCCHN. These cumulative results suggest that there are several potential pathways to transactivate EGFR in this cancer.

Concluding remarks and future directions

Overexpression of EGFR is relatively common in both SCCHN and esophageal carcinomas where expression levels have been correlated with decreased survival [47,51–53]. Preclinical targeting of EGFR inhibited tumor growth in SCCHN and esophageal carcinomas leading to

the FDA-approval of the EGFR monoclonal antibody cetuximab in SCCHN in 2006. EGFR tyrosine kinase inhibitors are under investigation and have shown efficacy in phase II studies in SCCHN [139] and advanced esophageal carcinomas [140] but phase III data are lacking. EGFR signaling mechanisms likely contribute to the response to EGFR targeting agents. Increased understanding of the role of the other ErbB family members in aerodigestive tract cancers and the downstream signaling consequences of EGFR homodimerization and heterodimerization with other ErbB family members may improve our ability to therapeutically target ErbB signaling in aerodigestive tract cancers by identifying those patients who are most likely to respond.

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Figure 1. EGFR signaling in SCCHN

EGFR is activated by ligand binding and subsequent receptor heterodimerization or homodimerization which leads to receptor autophosphorylation. EGFR can be transactivated by G-protein-coupled receptors (GPCR). GPCR activates Src leading to activation of PDK1 and PI3K, whereby TACE cleaves EGFR proligand and activates EGFR. EGFR can also be transactivated by cell adhesion molecules such as E-cadherin from neighboring tumor cells. The EGFR-E-cadherin complex activates EGFR and leads to MAPK signaling allowing for cell survival during the early stages of metastasis. EGFR activation leads to five primary signaling cascades. 1) MAPK: EGFR phosphorylation recruits Grb2 and Sos (adaptor proteins) that bind EGFR at Y1092 and Y1110 or alternately Grb2 and Sos bind via Shc at Y1172 and Y1197. Raf-1 is activated and the MAPK signaling cascade is activated resulting in cell survival, proliferation and differentiation; 2) STATs: STATs can be activated by interacting directly with EGFR or through Src-mediated EGFR signaling. Once phosphorylated STATs homodimerize or heterodimerize and are translocated to the nucleus where they induce the transcription of target genes that lead to cell cycle progression, angiogenesis, and apoptotic inhibition; 3) Src: Can be activated by binding directly to EGFR at Y915 and Y944 and can also activate EGFR by phosphorylating EGFR at Y845. Downstream of EGFR, Src can activate the STAT pathway or the PI3K pathway. Activation of Src is implicated in cell proliferation, migration, adhesion, angiogenesis and immune function; 4) PLC γ : Binds directly to EGFR and activates the MAPK pathway through PKC and the PI3K pathway leading to AKT activation. This results in migration and invasion; and 5) PI3K: Involved in many pathways and results in activation of AKT that leads to apoptotic resistance, cell growth, invasion and migration.