REVIEW

Concomitant EGFR Inhibitors Combined with Radiation for Treatment of Non-small Cell Lung Carcinoma

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Abstract

Epidermal growth factor receptor (EGFR) is considered to be one of the key driver genes in non-small cell lung cancer (NSCLC). Several clinical trials have shown great promise of EGFR tyrosine kinase inhibitors (TKIs) in the first-line treatment of NSCLC. Many advances have been made in the understanding of EGFR signal transduction network and the interaction between EGFR and tumor microenvironment in mediating cancer survival and development. The concomitant targeted therapy and radiation is a new strategy in the treatment of NSCLC. A number of preclinical studies have demonstrated synergistic anti-tumor activity in the combination of EGFR inhibitors and radiotherapy in vitro and in vivo. In the present review, we discuss the rationale of the combination of EGFR inhibitors and radiotherapy in the treatment of NSCLC.

Keywords: Non-small cell lung carcinoma - epidermal growth factor receptor - radiation - combine therapy

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Introduction

Lung cancer is the leading cause of cancer-related death in the world (Jemal et al., 2009). Non-small cell lung cancer (NSCLC) accounts for more than 80% percent of all cases of lung cancer. Surgery, radiotherapy and chemotherapy remain the three main regimens for the treatment of NSCLC. Over the past few years, molecular targeted agents, especially the EGFR inhibitors, have expanded the spectrum of rationale and practices for the treatment of advanced NSCLC. With the initiation of cancer genome project, advances have been made in the identification of oncogenic driver mutations in subsets of NSCLC patients. EGFR is considered to be one of the key members among the driver genes in Non-small cell lung cancer (An et al., 2012). Recent clinical trials such as IPASS, OPTIMAL, WJTOG3405, and NEJGSG002 demonstrated remarkable survival advantage and great promise for EGFR targeted therapeutics as first-line treatment (when used alone) for EGFR mutated NSCLC patients (Mok et al., 2009; Maemondo et al., 2010; Mitsudomi et al., 2010; Zhou et al., 2011). Although the great prospect had been shown in clinical investigations, EGFR inhibitors as the novel therapeutic agents are in themselves not curative by far, and almost all these NSCLC patients eventually develop resistance to small-molecule EGFR kinase inhibitors. Current researches focus on the development of new agents and the exploration of the integration of conventional therapies and targeted therapy. On the one hand, EGFR over expression was found in many cancers, and was shown to be associated with radioresistance. On the other hand, EGFR inhibitors and radiotherapy have distinct cytostatic and cytotoxic effects, which was demonstrating by some synergistic effect and prospect in the combination of radiation and EGFR inhibitors in several preclinical studies. A milestone Phase III clinical study of EGFR antibody Cetuximab plus radiotherapy in the treatment of squamous head and neck carcinomas showed promise in the paradigm of combined modality of radiotherapy and EGFR inhibitors to improve cancer treatment outcomes (Bonner et al., 2006). Based on these studies, extensive exploration of the combined treatment of EGFR TKIs and radiotherapy have been made. Here we discuss the underlying mechanisms of the emerging evidence and advances in this productive field.

EGFR biology and dysregulation of its signal pathway

Epidermal growth factor receptor is a transmembrane glycoprotein as a member of the erbB receptor tyrosine kinase family. The erbB family is composed of four distinct receptor molecules: erbB1/ EGFR, erbB2/ HER2, erbB3/HER3, and erbB4/ HER4. EGFR consists of an extracellular ligand-binding domain, a transmembrane region, and a intracellualr cytoplasmic domain that contains a tyrosine kinase region. EGFR ligands include epidermal growth factor (EGF), transforming growth factor-α (TGF-α), neuregulins (NRGs), amphiregulin (AREG), heparin binding EGF-like growth factor (HB-

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EGF) and so on. Ligand binding causes homodimerization or heterodimerization of EGFR with other erbB family members, which subsequently causes auto-phosphorylation on tyrosine residues of the receptor's intracellular cytoplasmic domain, initiating the EGFR signal transduction cascade. This cascade consists of various major pathways, including PI3K/AKT, Ras/Raf/MEK/MAPK, PLCγ, STATs, and PKC. These intrigued pathways result in catalytic activity of several signal molecules and transcription of target genes that mediate cell cycle, proliferation, differentiation, survival, angiogenesis, and migration (Nicholson et al., 2001; Janmaat et al., 2003; Morgan et al., 2009).

In addition to these prevailing transduction pathways, EGFR can undergo translocation into the nucleus by induction of EGFR ligand or radiation, where it may activate transcription of genes mediating cell proliferation and modulate proteins associated with DNA damage repair (Yamazaki et al., 2002; Dittmann et al., 2005). EGFR is known to be over expressed in a wide range of cancers. The expression rate is found to be 50% or more in NSCLC, more than 90% in head and neck squamous cell carcinomas, and 30% to 70% in esophageal carcinomas (Ettinger et al., 2006; Karamouzis et al., 2007). High EGFR expression is associated with poor prognosis and resistance to treatment with radiotherapy (Meert et al., 2002; Liang et al., 2003). EGFR mutations can be divided into two main categories: deletion mutations of the extracellular domain and somatic mutations in the TK activity of intracellular domain. EGFRvIII, the type-III mutated variant of the human EGFR, characterized by a deletion in the extracellular domain that leads to constitutive activation of its tyrosine kinase (TK) domain, is the most common EGFR mutation, which accounts for almost 60% of all EGFR mutations (Karamouzis et al., 2007). EGFR somatic mutations, the inframe deletion ΔE746-750 deletion and substitution mutation L858R, exhibits high sensitivity to EGFR TKI Gefitinib (Lynch et al., 2004). Crystallographic study showed that the L858R mutant significantly increased the catalytic activity of the tyrosine kinase enzyme and demonstrated much higher affinity to EGFR TKI Gefitinib than the wild-type enzyme (Yun et al., 2007). Another substitution mutation T790M confers resistance to Gefitinib (Kobayashi et al., 2005). Besides the mutations within EGFR, the MET proto-oncogene can cause ErbB3 driven activation of PI3K, which can lead to the resistance against Gefitinib (Engelman et al., 2007).

There are two major strategies developed for inhibiting EGFR signaling: Gefitinib and Erlotinib are small molecular weighted inhibitors of the EGFR tyrosine kinase activity (TKIs), which target the EGFR intracellular TK domain. And Cetuximab is a monoclonal antibody that blocks the engagement of the extracelluar ligand.

EGFR signal transduction pathway and radiation sensitivity

Ionizing radiation induces multiple cellular and biological effects by either direct DNA damage in forms of double strand breaks (DSBs) and/or single strand breaks, or through the formation of free radicals causing indirect DNA damage. These effects include induction of cell cycle growth arrest, repair of the damaged DNA, modulation of radical-scavenging proteins, induction of gene mutations, transcriptional and translational changes, and cell death (Chung et al., 2005). The biological responses of tumor cells after exposure to radiation can be summarized into the classical 4Rs proposed by Withers, namely, repair of radiation damage, redistribution, reoxygenation, and repopulation, which form the theoretical cornerstone of fractionated radiotherapy. Debucquoy and colleagues (Debucquoy et al., 2010) suggested that the EGFR inhibitors were involved the underlying mechanism of all these four aspects: (1) inhibition on DNA damage repair by various mechanisms, (2) redistribution of tumor cells by blocking cell cycle in the G1 phase and prolongation of a radiation-induced G2 phase, thus decreasing fraction of the S phase cells, (3) inhibition on the downstream pro-survival PI3K/AKT and RAS/RAF/MEK/MAPK pathway by EGFR antagonists, thus curbed cancer cell repopulation, (4) Inhibition on reoxygenation through inhibition on tumor vasculature.

The most intensively investigated aspect is the PI3K/AKT pathway's impact on DNA damage repair. There are two distinct mechanisms for DSB repair complementary to each other in mammalian cells: (1) nonhomologous end-joining (NHEJ), which is the dominant way of the DNA damage mechanism, and (2) homologous recombination repair (HRR), which plays a supportive role to NHEJ. Nonhomologous end-joining is catalyzed by a core complex that consists of a group of proteins, including the DNA dependent protein kinases (DNA/PK), XRCC4, et al. The catalytic subunit of DNA-dependent protein kinase (DNA/PKcs) is the key component in the radiation-induced DNA damage repair process, which initiates series of activity that leads to the stabilization of DNA breaks and physical rejoining of DNA DSBs (Chen et al., 2007). The EGFR/PI3K/AKT signaling is involved in the intracellular distribution of DNA/PK and activation of the catalytic subunit of DNA/PKcs with the mediation on the transcription of DNA repair genes such as Rad51, ATM, XRCC1, et al, is also the important mechanism by which EGFR and downstream effectors' modification on DNA damage repair (Meyn et al., 2009; Schuurbiers et al., 2009; Debucquoy et al., 2010).

It can be postulated that nearly all these afomentioned responses need the engagement of the EGFR signal transduction pathway. The PI3K/AKT is one of the major downstream targets of the ErbB tyrosine kinase receptor family, which has multiple roles in mediating tumor proliferation, invasion, apotosis, angiogensis (Schuurbiers et al., 2009; Karar et al., 2011). The PI3K/AKT pathway plays a pivotal role in mediating radiation response of cancer cells, which was highlighted by many researchers (McKenna et al., 2003; Rodemann et al., 2007; Schuurbiers et al., 2009). Nevertheless, as far as the complexity of signal transduction network concerned, the activity of the PI3K/AKT pathway can be influenced by various factors. The PI3K/AKT and Ras/Raf/MAPK signal pathway are interlinked pathways, which can be activated by oncogene Ras independent of EGFR.

PTEN, the Phosphatase and Tensin homolog deleted on chromosome Ten, is a tumor suppressor gene and is the major negative regulator of the PI3K/AKT pathway (Castellino et al., 2007).

The Ras gene mutation was found in about 30% of all human tumors in it's different isoforms. And K-ras was the most frequently mutated gene, which presented in 30% lung adenocarcinomas. The occurrence of EGFR and K-ras mutations was found to be mutually exclusive in lung cancers (Shigematsu et al., 2005). K-ras mutation was shown to be correlated with EGFR TKIs resistance in lung adenocarcinomas (Pao et al., 2005). Nevertheless, the correlation between K-ras mutations and response of lung cancer to treatment with EGFR antibody, Cetuximab, is not clear (Lynch et al., 2007; O'byrne et al., 2007). In the study conducted by Gupta and colleagues, inhibition of the RAS/MEK/MAPK pathway by MEK inhibitor PD98059 or the MEK kinase-p38 pathway inhibitor SB203580 didn't lead to radiosensitivity of cells with active Ras (Gupta et al., 2001). In series of studies, Toulany and Minjgee (Toulany et al., 2005) investigated the role of K-ras mediated radioresistance via the PI3K/AKT pathway. Ionizing radiation stimulated the production of EGFR ligands in K-ras mutated cancer cells, such as transforming growth factor-a (TGF-α) and amphiregulin (AREG), which contributed to the formation of the autocrine stimulatory loop of Ras pathway for EGFR signaling cascade. And it was shown that the TGF-α and AREG dependent EGFR activity selectively stimulated PI3K/AKT pathway. EGFR inhibitors (BIBX1382BS and LY294002) could inhibit DNA repair by targeting the PI3K/AKT pathway in K-ras mutated cancer cells. It was demonstrated from their studies that K-ras activity indirectly manipulates the PI3K/AKT pathway through EGFR, thus K-ras mutated cancer cells conferred radioresistance rather through an indirect manner (Toulany et al., 2005; Toulany et al., 2007; Minjgee et al., 2011).

PTEN is a tumor suppressor gene that located in chromosome 10q23, and the PTEN homozygous deletion and mutation was frequently found in various cell lines (Li et al., 1997). PTEN dephosphorylates phosphatidylinositol-3,4,5-triphosphate (PIP3) to phosphatidylinositol-4,5-triphosphate (PIP2), as the result of loss of PTEN, increased PIP3 levels lead to sustained PI3K/AKT signaling. Thus by removal of the lipid second messenger PIP3, PTEN negatively regulates the PI3K/AKT pathway activity (Castellino et al., 2007). PTEN transcription is mediated by several pathways that involve many transcription factors, one of which is p53. PTEN promoter contains a unique p53 binding site, and PTEN expression is regulated at the transcriptional level by interplay and autoregulatory mechanisms through interaction with p53. The p53 induction causes elevated PTEN gene expression and down regulates the PI3K/AKT pathway (Stambolic et al., 2001; Wang et al., 2005; Tang et al., 2006). Researchers have revealed that reduced PTEN or loss of PTEN expression conferred EGFR TKIs resistance in various cell lines (Kokubo et al., 2005; Sos et al., 2005; Yamamoto et al., 2010), and clinically survival was longer in those with high PTEN expression than in those with low expression of PTEN in lung cancer patients who had recurrent disease after surgical resection treated with Gefitinib (Endoh et al., 2006). Besides loss of PTEN, the PTEN gene function can be disturbed by mutation, or silenced by epigenetic mechanisms (Yu et al., 2008; Jung et al., 2010). High PTEN expression or restoring PTEN gene expression by gene therapy can exert sensitizing effect to radiation and EGFR antibodies in tumor cells (Tomioka et al., 2008; Bouali et al., 2009; Jung et al., 2010). And a recent study showed that PTEN elevation was unexpectedly resulted in a reprogramming of tumor metabolism and led to a tumor-suppressive anti-Warburg state through the modulation of both PI3K-dependent and -independent pathways, thus modulating PTEN gene function provides a new insight of curbing cancer from the perspective of modifying cancer cell metabolism model (Garcia et al., 2012).

EGFR mutations and radiosensitivity

Two main categories of EGFR mutations have been identified: deletion mutations of the extracellular domain and somatic mutations in the intracellular domain (Karamouzis et al., 2007). EGFRvIII, characterized by a deletion in the extracellular domain that leads to constitutive activation of it's tyrosine kinase (TK) domain, is the most common EGFR mutation, which accounts for almost 60% of all EGFR mutations. This kind of mutation has been observed in different malignancies including NSCLC. It was found that EGFRvIII was prevalent in about 40% of non-small cell lung cancer tumor species (Okamoto et al., 2003; SASAKI et al., 2007). Other kinds of EGFR somatic mutations are inframe deletion ΔE746-750 and substitution mutation L858R, which exhibit high sensitivity to EGFR TKI Gefitinib (Lynch et al., 2004). And another substitution mutation T790M confers resistance to Gefitinib (Kobayashi et al., 2005). Studies have shown that NSCLCs, which harbored either the L858R or the ΔE746-E750 mutations in the tyrosine kinase domain of EGFR, exhibited enhanced sensitivity to radiation. In one study conducted by Das etal exhibited that NSCLC cell lines harbored these two drug sensitive mutations ΔE746-750 and L858R exhibited predominantly radiosensitivity (Das et al., 2003). Cell lines harbored somatic mutations were shown to be correlated with incomplete DSB repair, failure to halt DNA synthesis or mitosis, and either induction of apoptosis or development of micronuclei in response to radiation. Particularly one cell line which contained the T790M drug resistance mutation still exhibited enhanced radiosensitivity. It indicated that the mechanism by which TKD mutations in the EGFR might contributed to radiosensitivity was due to the amplification of IR-induced DNA damage through abrogation of IR-induced cell cycle checkpoints or the interference in the repair of IR-induced (Das et al., 2006). In a subsequent study, authors from the same group further revealed that the underlying mechanism of the radiosensitivity in somatic mutation EGFR cell lines was associated with the defect in radiation induced translocation to the nucleus and failure to bind the catalytic and regulatory subunits of the DNA-dependent protein kinase (DNA/PKcs) (Das et al., 2007). In a recent

retrospect study, researchers observed that EGFR-mutant patients with locally advanced NSCLC treated with RT had lower rates of local recurrence rate (LRR) than wild-type EGFR patients. The study population was largely treated without incorporation with EGFR TKIs, among all the subjects, 25% patients had EGFR mutations (59%exon 19 deletions, 17% L858R substitution and 24% other missense mutations) (Mak et al., 2011). They found that the presence of an EGFR mutation was associated with a lower LRR rate, with 2-year LRR rate of 17.8% among EGFR-mutant patients and of 41.7% among wild type EGFR patients (p=0.005), and a higher OS rate (2-year OS rate, 92.6% versus 69.0%; p =0 .04) (Mak et al., 2011). Further studies are needed to elucidate the relationship between EGFR mutations and radiosensitivity. Clinical investigation exploring the effects of combination of radiotherapy and EGFR inhibitors on EGFR mutant patients are ongoing (NCT01391260, NCT01091376).

EGFRvIII that lacks the extracellular domain is incapable of binding EGF or TGF-α, however, EGFRvIII is constitutively activated and undergoes tyrosine autophosphorylation, stimulates cell proliferation independently of ligand interaction8. EGFRvIII expression conferred significant radioresistance in vitro and in vivo studies (Lammering et al., 2003; Lammering et al., 2004; Lammering et al., 2004). Mukherjee et al revealed that the enhanced radioresistance was due to accelerated repair of DNA double strand breaks (DSB) in EGFRvIII-expressing models (Mukherjee et al., 2009). They concluded that EGFRvIII-mediated radioresistance was associated with hyperactivation of DNA/PKcs and enhanced DSB repair kinetics, which was possibly transduced via the PI3K/AKT pathway (Mukherjee et al., 2009). EGFRvIII expression was also shown to be resistant to EGFR inhibitors in preclinical studies. Sok etal indicated that EGFRvIII expression was found in HNSCC cells. In vitro and in vivo studies using EGFRvIII-expressing HNSCC cells showed a decreased response to the EGFR mAb Cetuximab when compared with EGFR wild type overexpression cells (Sok et al., 2006). In one study conducted by Ji et al, they constructed a Ba/F3 cell model with infected EGFRvIII, EGFR-L858R,EGFRL858R-T790M mutations, and the results showed that the Ba/F3 cells transformed with EGFRvIII were more resistant to gefitinib and erlotinib compared with the EGFR-L858R mutants (Ji et al., 2006).

EGFR nuclear translocation and radiosentivitiy

Besides the classical membrane-bound EGFR signal transduction activity induced by radiation and other extracellular stimulus, accumulating evidences have shown that EGFR can be translocated into nucleus where it may regulate tumor response to therapy by interaction with various factors. Both the EGFR family members and the mutative EGFRvIII can shuttle from the cell surface to the nucleus.And the life cycle of the trafficking including:(1) Receptor endocytosis to early endosomal compartment in clathrin , lipid raft- and caveolin-dependent manner; (2)Recycling of the endosomal trafficking internalized EGFR to the cell surface , or undergoing degradation through sorting into lysosomes from the late endosomes, which mechanism is mediated by the Rab family proteins or ubiquitination of the EGFR proteins;(3)Shuttling into the nucleus with the association of nuclear localization signals (NLSs) within the receptors and nuclear transport receptors such as importins α/β (Lo et al., 2006; Wang et al., 2010).

The nuclear EGFR function can be summarized into three key aspects: (1) Regulation of the expression of several genes including cyclin D1, inducible nitric oxide synthase (iNOS), B-Myb, aurora A ,C-Myc ,cyclooxygenase-2 (COX-2) and breast cancer resistance protein, (BCRP); (2) Kinase function leading to tyrosine phosphorylation of target proteins, such as nuclear EGFR phosphorylates proliferating cell nuclear antigen (PCNA) to promote cell proliferation and DNA repair; (3) Modulation of DNA repair (Dittmann et al., 2010; Lo et al., 2010; Han et al., 2012).

Nuclear EGFR has been shown to be associated with relative poor clinical prognosis in different cancer types including breast cancer (Lo et al., 2005), ovarian cancer (Xia et al., 2009), esophageal squamous cell carcinoma (Hoshino et al., 2007) and et al. Nuclear EGFR was reported to be associated with resistance to molecular therapeutic agents. A study conducted by Li et al revealed that NSCLS cells that had acquired resistance to Cetuximab expressed increased levels of nuclear EGFR, and the expression of a nuclear localization sequence-tagged EGFR which was reintroduced by retrovirus vector rendered resistance to Cetuximab, both in vitro and in vivo (Li et al., 2009). Cetuximb was developed to target the extracellular ligand-binding domain of the EGFR and thereby blocked natural EGFR ligand binding. Investigations with respect to the effect of Cetuximb on EGFR translocation generated contradicting results. Dittmann and colleagues found that ionizing radiation could induce EGFR translocation into the nucleus; and more importantly, Cetuximab blocked the EGFR transportation to the nucleus, resulted in an inhibition of the double strand break repair, thus led to increased cellular sensitivity to ionizing radiation (Dittmann et al., 2005; Dittmann et al., 2005). On the contrary, Liao et al showed that Cetuximab could activate EGFR nuclear transport by promoting receptor endocytosis and activating receptor intracellular trafficking to the endoplasmic reticulum72. The antibody induced EGFR translocation could be blocked by the potent, orally inhibitor of various tyrosine kinases Dasatinib (Li et al., 2010). Another orally small molecule targeted therapeutics which was approved by FDA in the treatment of breast cancer, is the dual EGFR and HER2 tyrosine kinase inhibitor, Lapatinib. Lapatinib was shown to be capable of inhibiting the nuclear translocation of EGFR and HER2 and downregulating thymidylate synthase, thus sensitized cancer cells to fluoropyrimidine (Kim et al., 2009).

Researchers have shown that NSCLC cell lines harboring somatic activating mutations in the tyrosine kinase domain (TKD) of the EGFR exhibit radiosensitivity to ionizing radiation. They also indicated that the underlying mechanism of the mutant EGFR-associated radiosensitivity was partly due to the abrogation of the radiation-induced nuclear translocation (Das et al.,

2006; Das et al., 2007). A recent study showed that EGFR nuclear expression in transfected EGFR-null cells modulated repair of DNA damage through the DNA/PK pathway. However, the constructed L858R-expressing cells showed impaired EGFR nuclear localization following either cisplatin or ionizing radiation treatment, compared with increased DNA/PK activity observed in wtEGFR and EGFRvIII cells following radiation, was not significant changed in DNA/PK activity in L858R expressing cells (Liccardi et al., 2011).

EGFR signal pathway, Tumor microenvironment and radiosensitivity

As tumor cells are more than a bunch of isolated malignant cells, the interaction between tumor cells and their "compartment" is gaining more and more attention. Tumors cells are located in a surrounding environment that is composed of structural, soluble and cellular components which consist the tumor microenvironment (Zhong et al. 2008). The tumor microenvironment is an "integrated ecosystem" consists of extracelluar matrix, adhesion molecules, chemokines, cytokines, fibroblasts, migratory haematopoietic cells, and other factors. The dynamic interaction between tumor cells and these structural is essential in tumor survival, progression, metastasis and metabolism (Liotta et al., 2001; Pollard et al., 2004; Burger et al., 2006). One of the mostly investigated field of the tumor microenvironment is tumor vascularization. The induction of tumor angiogenesis is crucial for the development and growth of most solid tumor in their early stage, and it also facilitates dissemination of cancer cells through blood vessels. Folkman proposed that tumor growth depends on angiogenesis and that blocking of nutrition supply could inhibit or eliminate tumors (Sherwood et al., 1971). According to Folkman and colleagues'study, tumor vasculature is regulated by a defined set of pro-angiogenic and anti-angiogenic factors, and the tumor often expresses and secretes excessive pro-angiogenic factors and breaks the balance between above mentioned factors, thus turns the "angiogenic switch" towards an angiogenic phenotype, and their ingenious efforts lead to the discovery of vascular endothelial growth factor (VEGF) (Cao et al., 2008). Tumors can engulf existing blood vessels or form new blood vessels, however, the resulted new vessels are often both structurally and functionally abnormally. This vasculature malfunction as well as increased oxygen consumption of the proliferating tumor cells could lead to the altered tumor microenvironment, which Is characterized by hypoxia, acidosis and increased interstitial fluid pressure (Milosevic et al., 2004). It was well established that tumors are more radiation resistant in hypoxia conditions. Under the condition of hypoxia, hypoxia-inducible factors-1 (HIF-1) is activated in tumor cells responding to that altered tumor microenvironment (TME). HIF-1 can activate or transactivate a number of genes including VEGF, PKM2,GLUT1 et al. This is a significant factor engaged in many crucial aspects of cancer biology including angiogenesis, stem cell maintenance, metabolism, autocrine growth factor signaling, epithelial-mesenchymal transition, invasion, metastasis, and resistance to radiotherapy or chemotherapy. And a growing number of HIF-1 inhibitors are being evaluated in preclinical and clinical studies (Semenza et al., 2003; Semenza et al., 2012).

HIF-1 is a heterodimeric transcriptional activator composed of HIF-1α and HIF-1β subunits. HIF-1α and HIF-2α are closely related isoforms. HIF-1 can regulate the transcription of a wide range of genes including EGFR mainly through the activation of HIF-1α, and the regulation of the HIF-1 in an oxygen dependent or oxygen independent manner (Semenza et al., 2003). Zhong and colleagues showed that EGFR induced activation of the PI3K/AKT/FRAP pathway by results in increased expression of HIF-1α protein, HIF-1 transcriptional activity, and VEGF protein expression in prostate cancer cells (Zhong et al., 2000). Jiang and colleagues found that activation of PI3K led to increased levels of HIF-1α protein and positively regulates HIF-1α-mediated transcription of VEGF in diverse tumor cell lines (Jiang et al., 2001). Peng and colleagues' study demonstrated that activation of the EGFR signaling pathway leads to the up-regulation of HIF-1α through the PI3K/AKT pathway, and that HIF-1α directly bind to the survivin promoter to activate gene transcription in normoxic condition (Peng et al., 2006). These studies demonstrated a role of EGFR in regulating HIF-1 in an oxygen independent manner. Franovic and colleagues revealed that the hypoxia microenvironment mediated the up-regulation of EGFR protein levels through the activation of HIF-2α, provided one non-mutational explanation for the EGFR over-expression that commonly observed in human cancers (Franovic et al., 2007). Wang and Schneider showed that under moderate hypoxia HIF-2α induced EGFR pathway activation in HNSCC cells overexpressed EGFR levels. And immunohistochemical study demonstrated that EGFR phosphorylation occurs in the vicinity of HIF-2α expression within hypoxic foci as evidenced by HIF-2α immunostaining (Wang et al., 2010).

The VEGF expression is regulated by various factors in the tumor microenvironment including hypoxia, low pH and nutrient deprivation, growth factors, stress incitation including chemotherapy and radiotherapy. Bevacizumab, the anti-VEGF monocolonal antibody was approved by FDA for the treatment of colorectal and lung cancer. The endogenous angiogenesis inhibitor Endostatin inhibits endothelial cell proliferation, migration, invasion and tube formation (Abdollahi et al., 2005). Endostar, the recombinant human Endostatin(RHES), was shown to have synergistic effect on tumor growth and angiogenesis when combined with radiotherapy in preclinical studies (Jiang et al., 2011; Zhang et al., 2011). In a clinical study which enrolled 50 NSCLC patients, the RHES combined with radiotherapy was shown to have better short-term therapeutic effects and local control rates, but failed to show a 3 year survival advantage (Jiang et al., 2011). The mechanisms for VEGF-targeted therapy is not quite exclusive. Jain proposed that anti-VEGF agents could normalize the abnormal vasculature, resulting in more efficient delivery of drugs and oxygen to the targeted cells, thus enhanced the outcome of chemotherapy

and radiotherapy (Jain et al., 2005). According to Jain, tumors often expressed high levels of VEGF, leading to chaotic, torturous, and leaky vasculature that provided inadequate blood delivery to tumors. A partial decrease in VEGF function by using an anti-VEGF agent could reduce interstitial fluid pressure and "normalize" blood vessel morphology and increase blood flow. The increased drug delivery and oxygenation as a result of the vascular normalization might improve the chemotherapy and radiotherapy treatment outcomes (Jain et al., 2005).

The EGFR signal cascade is involved in tumor angiogenesis. EGFR and VEGFR share the common downstream Ras/Raf/MAPK and PI3K/AKT signaling pathways. EGFR plays an important role in modulating VEGF independent of HIF-1α. Maity and colleagues' study revealed that EGFR could transcriptionally up-regulate VEGF in glioblastoma cell models via a pathway involving Ras and PI3K, which was distinct from the pathway induced by hypoxia (Maity et al., 2000). The mammalian target of rapamycin (mTOR) is one of the downstream component which is phosphorylated by AKT, the PI3K/AKT/mTOR pathway plays an important part in mediating VEGF secretion both in a HIF-1 dependent and independent manner (Karar et al., 2011). It is shown that anti-EGFR agents could mediate the tumor microenvironment through the direct or indirect impact on angiogenesis. Hirata and colleagues observed that Gefitinib treatment could inhibit the EGF-induced migration of the human microvascular endothelial cells (MVEC) and formation of tube-like structures by microvascular endothelial cells in vitro (Hirata et al., 2002; Hirata et al., 2004). They observed a marked decrease in the number of vascular endothelial cells with phosphorylated EGFR in response to Gefitinib, but almost no effect on VEGF induced neo-vascularization was observed. The authors suggested that the anti-angiogenic effect of Gefitinib in the vascular endothelial cells of neo-vasculature was partly attributable to direct inhibition of EGFR activation (Hirata et al., 2002; Hirata et al., 2004). When combined with radiation, EGFR inhibitors were shown as radiosensitize to cancer cells, and this effect could be partially attributed to the down regulation of VEGF expression (Huang et al., 2000; Solomon et al., 2003). Recent findings provided further evidences for EGFR inhibitors' effect on tumor microenvironment(TME). Pore et al demonstrated that Gefitinib could inhibit the VEGF expression of head and neck squmaous carcinomas in two distinct ways, either by decreasing VEGF promoter activity independently of the HIF-1 binding site or by down-regulation HIF-1α protein translation hence suppressed HIF-1α induced VEGF expression (Pore et al., 2006). In a subsequent study, Cerniglia and colleagues further indicated that Erlotinib treatment altered tumor vessel morphology and permeability, increased tumor blood flow, and decreased hypoxia and increased SO_2 (Pore et al., 2006). However, their study didn't show synergist anti-tumor effect on SQ20B cells when Erlotinib combined with radiation using the standard clonogenic survival assay. These studies demonstrated the indirect role of EGFR on angiogenesis. And we can discern from the studies that EGFR inhibitors' anti-tumor effect could partly be attributed to the modulation on the TME via vascular normalization (Cerniglia et al., 2009).

Tumor cells often have elevated rates of glucose uptake but reduced rates of oxidative phosphorylation which is remarkably different from non-malignant cells. The concept of aerobic glycolysis, also known as the Warburg effect, defines the phenomenon that tumors produce high levels of lactate even in the presence of oxygen (Christofk et al., 2008). EGFR plays an important role in mediating tumor metabolism, which can be reflected from the clinical observation. In these studies, NSCLC patients with sensitizing mutant EGFR showed significant reduction of SUVs in early FDG-PET scanning after treatment with Erlotinib (Zander et al., 2011). The sodium/glucose cotransporter 1 (SGLT1) is a plasma membrane-bound protein that is essential to glucose uptaking of cells. Weihua et al reported that the extracellular domain of EGFR could associate with and stabilize the SGLT1 to promote glucose uptake into cancer cells, down regulation of EGFR led to loss of SGLT1 expression and lower intracellular glucose levels. And the authors found that this phenomenon was independent of EGFR's tyrosine kinase activity (Weihua et al., 2008). Since the EGFR antibody Cetuximab could inhibit the EGFR activity by binding to EGFR extracellular domain, this study may shed new light on the anti-tumor mechanism of EGFR antibody, but this may need further exploration. The pyruvate kinase is the glycolytic enzyme that catalyzes the final step of glycolysis. It was demonstrated that the switch from the isoform M1 to the M2 of pyruvate kinase in tumour cells was necessary for the shift in cellular metabolism to aerobic glycolysis which could promote tumorigenesis, and knocking down of the pyruvate kinase M2 in human cancer cell lines. And reversal of the Warburg effect could be detected by using short hairpin RNA and replacing it with pyruvate kinase M1 (Christofk et al., 2008). A recent study showed that PTEN elevation resulted in a reprogramming of tumor metabolism and led to a tumor-suppressive anti-Warburg state through the modulation of both PI3K-dependent and -independent pathways (Garcia et al., 2012). The researchers constructed transgenic mice carrying additional copies of PTEN gene that maintained high level of PTEN expression, they found that the so-called Super-PTEN cells took up less glucose, and redirected a greater fraction of glycolytic products into mitochondrial oxidative phosphorylation, and this could be partially due to the reduced levels of PKM2 and decreased PK activity in Super-PTEN cells. They further indicated that decreased activity of mTORC1, the downstream target of the PI3K/AkT pathway accounted for the reduced levels of PKM2 in Super-PTEN cells. Apart from the metabolic role of PKM2, Yang et al revealed that PKM2 had a non-metabolic function in directly promoting cell cycle progression by transactivation of β-catenin and, while this process required the direct involvement of EGFR. They also demonstrated that EGFR induced nuclear translocation of PKM2 and c-SRC-mediated phosphorylation of β-catenin, PKM2 could bind to phosphorylated β-catenin within the nucleus (Yang et al., 2011). The synthesis of HIF-1α could be stimulated by PI3K activity which is the

downstream pathway component of EGFR, and in turn the activation of HIF-1 could mediate the transcription of several genes triggered tumor metabolism including GLUT1, PKM2,PGK1, GAPDH, et al (Semenza et al., 2003; 2012). Together with the above, direct involvement of EGFR in mediating metabolic genes could be proposed, and these evidences highlighted the close relationship between EGFR and tumor metabolism.

Conclusion

EGFR is one of the driver genes of non-small cell lung cancer, and the EGFR signaling pathway is one of the most extensively investigated pathways in human cancers. A number of preclinical studies have shown that EGFR inhibitors combined with radiation have synergistic anti-tumor effect. In this review, we have discussed that this effect can be influenced by many factors including EGFR downstream signal cascade components, EGFR mutations, EGFR nuclear translocation, crosstalks with oncogene and/or tumor supperssor gene, interactions with the tumor microenvironment, et al. Although significant advances have been made in the study of the EGFR signal pathway, one might not get a thorough understanding of the mechanisms of the EGFR inhibitor's radiosensitizing effect without taking into account the complex and intricate signaling network of tumor cells, and interactions between tumor cells and their microenvironment to that issue. While further interpretation of EGFR signaling pathway in the context of tumor cell signaling network and the interaction with the tumor microenvironment will help us to devise new strategies and develop new targeted agents for the treatment of lung cancer patients. To date, many novel molecular therapeutic drugs such as agents targeting PI3K/AKT, mTOR, HIF-1, et al, are under development or being tested in clinical trials (Koh et al., 2011). The combination of different targeted agents with radiation and may have the potential to benefit lung cancer patients. Evidences have shown the close relationship between EGFR and tumor metabolism, more efforts are needed to elucidate the role of EGFR inhibitors in tumor metabolism, and by clarifying that might help us find ways to enhance radiosensitivity or to curb cancer through modification of tumor metabolism. Despite the abundance of evidences that EGFR inhibitors have numerous effects that could lead to increased radiosensitization, and the milestone Phase III study of the combined Cetuximab and radiotherapy in head and neck squamous cancinomas showed significant survival advantage. However, no proven benefit of the EGFR TKIs in combination with radiotherapy have been observed in NSCLC (Koh et al., 2011). So, the synergistic effect of EGFR inhibitors and radiotherapy observed in experimental studies can be translated into clinical advantage needs urther clinical validation. Many unanswered questions needed to be addressed towards the optimization of EGFR inhibitors in combination with radiotherapy such as patient selection based on biological markers and the best schedule for EGFR inhibitors intervention during the course of conventional radiotherapy or chemoradiotherapy treatment.

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