Kinase-driven pathways of EGFR in lung carcinomas: perspectives on targeting therapy

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1. ABSTRACT

Despite remarkable advances in oncology medicine, the prognosis of lung cancer patients has not greatly improved over the past few decades. To overcome the current limit, new classes of agents that specifically target particular cascades have been developed. Gefitinib and erlotinib, which are tyrosine kinase inhibitors specific for the epidermal growth factor receptor (EGFR), have provided hope for better survival. The relationship between the sensitivity to gefitinib and the tumors' EGFR mutations have allowed the selective and accelerated use of these therapies. However, their efficacy is still limited, predominantly due to side effects and drug resistance. Further development of rational clinical strategies will require greater clarification of the key signaling factors downstream of EGFR which are potential targets for cancer therapies. In this review, we describe the various observed abnormalities in EGFR, the mechanisms of activation of several critical signaling cascades in lung cancer. Summarizing the data gleaned from preclinical, and clinicopathological aspects, we discuss the molecular mechanisms that may underlie a possible successful response to the blockade of EGFR and/or its downstream signaling.
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2. INTRODUCTION

Lung cancer is the leading cause of cancer death, annually resulting in 1.1 million deaths (17.8% of all cancer deaths) worldwide (1). Five-year survival rates range from less than 20% (Stage IIIB and IV) to 67% (Stage I and II) and the overall cure rate is less than 15% (2-5).

Lung cancers are conventionally divided, initially, into two categories; small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC)(6). SCLC accounts for 15 - 25% of the total cases and exhibits neuroendocrine features, histologically (6). This group of carcinomas is conventionally treated by chemotherapy, except for peripherally localized Stage I tumors. NSCLC accounts for 75 - 85% of all, and is the most common cause of death in male and second next to breast cancer in female (7). NSCLC is further subdivided into three subtypes: squamous cell carcinoma (SCC, -28%), large-cell carcinoma (LCC, -24%) and adenocarcinoma (AC, - 48%)(6, 8). Although these types differ histologically, until recently, little clinical distinction was made among them (9). Treatment of NSCLC is guided by the disease stage. Surgery is the first-line treatment of choice for localized cancers of clinical stage I/II, whereas multimodality therapies are the norm for patients in advanced stage (7). About 40% of patients with NSCLC present at an advanced stage, with metastatic or locally advanced disease, which underscores the importance of identifying a therapeutic strategy. Therefore, treatment for advanced cancers is palliative with the hope of prolonging survival and prevent deterioration in the quality of life (10). Combination chemotherapy, usually platinum-based regimens, and doublet containing platinum with paclitaxel, gemcitabine, docetaxel or vinorelbine are currently the first-line therapy of choice (5, 7, 10, 11).

Along with the refinement of drug combinations, a great effort has been made in the oncology research for innovative therapeutic agents in NSCLC that are more effective than conventional agents (12, 13). Since it was found that many aberrant signaling cascades emanating from growth factor receptors function as oncogenic signaling pathways, they and their effector molecules have been investigated as possible drug targets. Eventually, this effort has spurred the development of more than 500 molecularly targeted pharmacological agents and ushered in the concept of tailored therapy, i.e., matching the appropriate patients with the appropriate therapies (7, 14). One such target is the epidermal growth factor receptor (EGFR)(3, 13). A large number of previous reports have demonstrated oncogenic alterations of EGFR in human cancers, including gene amplifications, deletions and mutations. Consequently, several EGFR-targeted agents have been developed and are in clinical use, including the anti-EGFR monoclonal antibody cetuximab, as well as the tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib (7, 15). However, it has been repeatedly recognized in the history of medical oncology that only a subset, and not a majority of patients derive significant benefit from a particular anticancer agent, presumably, due to the complexity of the signaling pathways involved in each case (14). Indeed, the agents developed against EGFR, either monoclonal antibody or gefitinib, initially did not produce consistent results and a particular group of the patients had been noted to show remarkable response to gefitinib (16). However, one breakthrough discovery in the past decade has been the identification of specific genetic lesions in the tumors of a subset of treated patients who were seen to receive clinical benefit from these anticancer agents. Frequently, this genetic lesion is an activating mutation within the target kinase gene (7, 14). These discoveries have prompted efforts to stratify patients before treatment or after the operation at the time of relapse, and to apply kinase inhibitors based on the specific genomic character of the cancer.

In lung cancers, the discovery of the relation between TKI sensitivity and somatic mutations in the tyrosine kinase domain of EGFR represents a dramatic step in the development of treatment strategies (16). These mutations are typically gain-of-function, transforming mutations that act to enhance EGFR activation, and thus, in most of the cases, markedly increase the sensitivity to EGFR inhibitors (7). However, even with TKI therapy for the patients harboring EGFR mutations, efficacy is not completely satisfactory and results are somewhat heterogeneous (13, 16). Furthermore, even though NSCLC patients with EGFR mutations show an initial dramatic response to EGFR-TKIs, a serious clinical problem is that almost all acquire resistance within 1 year (details in Chapter 6.2.) (16). Thus, therapy targeting EGFR must be further refined in the forthcoming age of lung cancer therapy. This should consist of the identification in advance, of the subpopulation of patients who respond to a particular (adjuvant) therapy so as to derive greater therapeutic benefit and simultaneously to avoid unnecessary treatment of patients who have little probability of benefiting. In parallel with this approach, new modes of combination therapy, including folate-antagonist, pemetrexed or the anti-endothelial growth factor-antibody, bevacizumab have recently been introduced as the regimens for non-squamous cell carcinoma (17, 18). But, impediments similar to those seen with EGFR-TKIs will most likely emerge in clinical application, i.e., limitation of the applicants, drug resistance and side effects.

Another important issue is that, although we may be able to identify a subpopulation of patients for therapy by the presence of specific genomic aberrations, the larger population of the patients await more suitable and reasonable chemotherapeutics. There is a large body of work describing abnormalities in EGFR protein expression and in gene copy numbers (Chapter 3), however the majority of “abnormal EGFRs” which play a role in oncogenesis are of the apparent wild-type variety. These “abnormal wild-type EGFRs” function by activating myriad downstream effectors. Herein, the question arises as to whether targeting a specific single molecule is indeed hitting an “Achilles heel” or would effective treatments require targeting multiple points by single or multiple agents. In general, it is unlikely that any one signaling
pathway drives the oncogenic behavior of tumors, rather, the process of malignant transformation and/or progression is driven by complex signaling cascades and accumulated multiple gene aberrations (12). Therefore, with respect to the therapeutic intervention, inhibiting EGFR alone may not be sufficient for substantial inhibition of tumor cell growth and/or proliferation depending on the cases, and multipoint intervention may be highlighted. In such cases, further precise and comprehensive analyses are required to identify molecules critical to individual cases and to develop the most effective drug combinations. In this review, we describe the known abnormalities in the cascade from EGFR to its effector downstream molecules and discuss future potential therapeutic strategies.

3. EGFR ABNORMALITIES IN LUNG CANCER

The EGFR gene, located on chromosome 7p12, encodes a 170 kD membrane glycoprotein. Upon binding of specific ligands, the receptor dimerizes, autophosphorylates and activates downstream effectors (Figure 1A). This results in changes in gene and protein expression crucial to tumor proliferation and/or progression (21). Traditionally, EGF or EGF-related growth factors, produced either by cancer cells themselves or by surrounding stromal cells, have been described as causing constitutive EGFR activation via autocrine or paracrine mechanisms (12). Progress in molecular biology and pathology and the development of multiple tools to investigate EGFR abnormalities, including immunohistochemistry (IHC), fluorescence in situ hybridization analysis (FISH) and sequencing techniques have clarified further the precise profiles of EGFR abnormalities (22).

We and others worked and have focused on, (i) the frequency of EGFR protein overexpression (Chapters 3.1., 3.2.), (ii) the frequency of EGFR gene amplification and mutations as well as predilection in various human cancers (Chapters 3.3, 3.4.), and (iii) the correlation between the status of the gene, protein and clinicopathological features (Chapter 3.5.). Accordingly, abnormalities in EGFR are introduced in the following sections in this order.

3.1. Overexpression of EGFR protein

EGFR overexpression is readily detectable in NSCLC, but the reported frequencies have varied ranging from 32%–80% (23-26). These significant variations in the prevalence of overexpression may result in part from the historical background of different scoring systems in IHC, in contrast to genes, such as HER-2, for which scoring has been standardized worldwide. However, results have been converging more closely in recent reports, ranging from 53.1–69.7% (Table 1), probably due to the standardization of antibodies and criteria of evaluation in IHC (26-32). We evaluated EGFR expression by IHC using a 4-tier-system (0, 1+, 2+ and 3+) following the criteria for HER-2 expression. We defined 2+ and 3+ staining as “overexpression”, since only those cases showing 2+ or 3+
positive IHC were associated with gene amplification (26, 33). Although the frequency of EGFR overexpression was definitely rare in SCLC (7%), there was no significant difference in positive frequency among histological types in NSCLC, with slight predominance in SCC than in AC (26, 34, 35). Even in the carcinomas scored as positive for overexpression, heterogeneity of EGFR staining was observed within a single tumor nodule, suggesting that EGFR overexpression is not a clonal phenomenon in most of the cases (26).

3.2. Activation of EGFR protein

The activated EGFR is phosphorylated, and recently this phosphorylated form has become more amenable to efficient analysis by direct immunoblotting or even by IHC with anti-phospho-EGFR-specific antibodies. By these efficient methods, EGFR activation was observed in up to 47% of NSCLC cases by IHC and IB (Table 1) (32, 35). Among the phosphorylated EGFR (p-EGFR)-positive NSCLC cases in our series, 80% were accompanied by EGFR overexpression. Therefore, overexpression is not prerequisite for activation and low level expression of activated EGFR exists. In contrast, only 7% of SCLC showed EGFR activation (35).

3.3. Amplification of EGFR gene

In gliomas, EGFR amplification is often accompanied by structural rearrangements that cause in-frame deletions in the extracellular domain, the most frequent being the EGFRvIII variant (36). On the other hand, in NSCLC, amplification with rearrangement is extremely rare (37). The frequency of EGFR amplification in NSCLC was variously described in past literature, as ranging from 6% to 40%, and more frequent in SCC (Table 1) (26, 28, 30). But higher frequencies (up to 50%) of EGFR amplification or polysomy with high copy number have been reported in patients with advanced NSCLC (2, 34, 38).

We found EGFR amplification in 23% of the NSCLC cases, consisting of high level amplification in 10% and low level amplification in 13% (26). Among the tumors showing protein overexpression, the frequency of EGFR gene amplification was found to be 74%. Moreover, it is noteworthy that, 95% of 3+ cases and 60% of 2+ cases were associated with gene amplification. Nowadays, gene amplification has been recognized as a main cause of EGFR overexpression in a number of studies (12, 26, 39). In tumors exhibiting EGFR gene amplification, EGFR protein expression was found to be markedly enhanced, activated and moreover, its downstream signal transducer and activator of transcription protein-3 (Stat-3) was frequently activated (details in Chapter 4, (20)).

With regards to the pathobiological significance of these aberrations, one of the in vitro studies demonstrated that amplification readily disappeared in response to chemotherapy (40), validating the use of chemotherapy against tumors with EGFR amplification. This treatment may cause downregulation of EGFR and subsequently abrogate the EGFR-mediated intracellular signal cascade. However, the number of human cancers fitting this particular model of “EGFR addiction” is not very large (41).

Other mechanisms of EGFR overexpression include increased EGFR gene copy number by chromosome 7 polysomy in the absence of amplification, however, this accounts for only a minor proportion of cases (28, 33). EGFR protein overexpression is also caused by transcriptional or post-transcriptional activation without an increase in gene copy number, and various theories have been proposed to explain the mechanisms underlying this phenomenon: p53 directly activates EGFR expression at the transcriptional level (42), in addition, EGFR transcription is modulated by polymorphic CA repeats (43) and by a 140 bp enhancer region (44), both of which reside within intron 1.

3.4. EGFR gene mutation spectrum and associated amplification

Given the apparent clustering of EGFR mutations (45, 46), many groups screened mutations in the ATP-binding domain (exons 18 through 21), and revealed the presence of mutations in 3 to 40% of NSCLC and 13 to 65% of AC, depending on the patients’ ethnicity (30, 47, 48). The scientific consensus is that mutations are more frequent in adenocarcinomas (21% in average) compared to other histologies (SCC and LC, 2%), more frequent in females (20%) than males (9%), and in Asians (26%) compared to Caucasians (2%) (35, 47-49).

The most frequently encountered mutation is a leucine to arginine substitution at amino acid position 858 (L858R) of exon 21, found in 40 to 50% of the cases harboring mutations (45, 46, 50). Other common mutations include in-frame deletions within exon 19, removing a region of amino acids from 746 through 753, with or without the generation of a novel codon at the deletion breakpoint, in 40 to 45% of mutants. A glycine to serine/cysteine/glutamine substitution at codon 719 (G719S/C/D) of exon 18 was found in up to 5% of the case, and half of these are TKI-sensitive (7). A threonine to methionine substitution at codon 790 (T790M) of exon 20,
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which was found in 5 to 10%, causes resistance by sterically blocking binding of tyrosine kinase inhibitors (51). However, the precise underlying mechanism is unclear since it is still sensitive to structurally similar irreversible inhibitors (7). Other rare mutations include S695R (exon 18) in AC as well as Y727H (exon 18), V843I (exon 21) in SCC and G729R (exon 19) in “undifferentiated carcinoma” and several others (20, 49).

Among the cases exhibiting genetic mutation, an association with EGFR amplification was reported as 15% to 100%, depending on the scoring system for FISH that was used (30, 34, 52). Our FISH analysis revealed that amplification was observed in 22% of NSCLC cases harboring mutation, and the increase of EGFR (amplification and polysomy) was in about 43% of the cases (35). Therefore, genetic mutations could be more or less associated with an increase in gene copy numbers, probably reflecting the instability of the EGFR gene in cancer cells.

3.5. Clinicopathological significance of EGFR abnormalities

A large number of reports have analyzed the correlations between EGFR abnormalities and clinicopathological factors and patients’ survival, but these have often produced contradictory results. Again, these results could be partially due to the significant difference in the rates of overexpression and of amplification caused by the different scoring systems.

3.5.1. Overexpression

Although EGFR overexpression has been well recognized to be important in the development and progression of lung cancers, its prognostic significance is still unclear. Representave reports could be summarized as below.

i) A correlation was found between EGFR expression and lymph node metastasis, tumor invasiveness as mediastinal involvement, the more advanced pathological Stage (p-Stage) (26), and thus, worse survival (53).

However, contradictory results were also reported.

ii) No significant difference in lymph node metastasis or overall survival was found in correlation with EGFR overexpression, not only by qualitative, but also quantitative analysis for expression of EGFR mRNA and/or protein (23, 54).

The latter seems credible since recent successive reports described similar results probably due to the standardization of technique and criteria for evaluation of “overexpression” (55).

3.5.2. Amplification

A clinicopathological correlation with amplification has also been variably reported.

i) The presence of high level amplification in AC was associated not only with nodal metastasis, but also with a higher histological grade, invasive growth and higher p-Stage (26, 52). Consistently, amplification is rare in bronchioalveolar carcinoma or its precursor lesion, atypical adenomatous hyperplasia (52).

ii) EGFR amplification correlated with a response to gefitinib, but not with overall survival when NSCLC were viewed as a whole (52, 56). Capuzzo et al. showed that 33% of FISH-positive cases (increase in EGFR gene copy number) had a higher response rate to gefitinib than the FISH negative cases (30).

3.5.3. Mutations

The broad consensus is that carcinomas harboring L858R in exon 21 or deletions in exon 19 predict responsiveness to TKIs (45, 46), whereas T790M predicts resistance (7, 51). Other mutations correlated to a minor extent with sensitivity or were not clarified. TKIs have been utilized for defined patients whose tumors were confirmed to exhibit EGFR mutation (approximately 15 to 20% of the total). These patients show a remarkable response of about 50% (30), although the overall survival even in this particular group has still remained at a plateau (56). However, recent multi-institutional study in Japan reported the prolonged progression-free survival by the first-line gefitinib, instead of conventional regimens, for patients with advanced NSCLC harboring EGFR mutations (57).

Collectively, there was no significant difference in survival rates between two groups (positive and negative) when divided with respect to protein overexpression, gene amplification or mutation.

4. SIGNALING CASCADE OF EGFR

As mentioned in the earlier chapters, EGFR status per se can not predict a definitive therapeutic response and clinical course in NSCLC, except for the presence of mutations. One reason for this is that there is not a completely direct or linear correlation among abnormality of the EGFR gene, protein overexpression/activation, and downstream activation (20, 58). Therefore, the functional profile for activation of downstream effector proteins that indicates the critical biochemical effectors in individual cases need to be better clarified in order to obtain the most efficient therapeutic effect.

A number of downstream signaling intermediates of EGFR have been implicated as transmitters of the oncogenic proliferation signal (19, 20). There are three main pathways activated through EGFR; the Stat (signal transducers and activators of transcription), the extracellular signal-regulated kinase 1/2 (Erk1/2) and the phosphatidylinositol 3-kinase (PI3K)–Akt pathways (Figure 1A) (12, 36). A significant body of evidence has already shown that these three downstream cascades are actively involved in the survival, proliferation and/or progression of tumors in association with activation not only of EGFR, but also of other growth factor receptors (12, 19, 36).
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Figure 2. Immunohistochemical staining for proteins of the Akt/mTOR pathway in a case of lung adenocarcinoma. A. phosphorylated-Akt (p-Akt) was observed in the nuclei and the cytoplasm. B-D. Activation of mTOR (B), S6K (C) and rS6 (D) was observed as the positive staining in the cytoplasm of cancer cells forming acinar structure. Asterisk in (B) showed non-neoplastic bronchial mucosa. Original magnification, x200.

4.1. Stats

Activation of Stat-3, Erk1/2 and Akt can be detected immunohistochemically using phosphoprotein-specific antibodies which have become commercially available and validated by recent studies (20, 59-61). Therefore, the topographical localization of the phosphorylated forms of these effector proteins within the tumor and their involvement in various kinds of human cancers have been described successively (58, 62-64).

Stats are transcriptional factors that can be activated by interaction with phosphorylated receptors, including the phosphotyrosine residues of EGFR, leading to the modulation of cell proliferation and differentiation (61). Constitutive activation of Stats, in particular Stat-3, is often found in human malignant tumors, including those of the head and neck (65), the breast (66), and the lungs (20, 62, 63). Histologically, phosphorylated Stat-3 (p-Stat-3) signal was confined to the nucleus, and was observed in 38 to 71 % of NSCLC (20, 62, 63). In particular, in tumors exhibiting EGFR gene amplification, Stat-3 was frequently activated (20).

4.2. Erk1/2

Erk1/2 was shown to be activated by a variety of mitogenic agents, and is a ubiquitous component of signal transduction pathways through ras/raf, regulating cell proliferation, survival and transformation (67). Constitutive activation of Erk1/2 was observed in both the nuclei and the cytoplasm, in up to 28 % of lung cancers (20, 68), but Erk1/2 activation did not correlate with any specific aberrations of EGFR (20).

4.3. Akt

Akt is a serine/threonine kinase that acts downstream of many growth factor receptors, including EGFR, which itself is activated by phosphorylation at amino acids Thr308 and Ser473 through the PI3K pathway. Akt plays a critical role in various cellular actions, including glucose metabolism and anti-apoptotic signaling (69). Constitutive activation of Akt can oncogenically transform cells, and indeed, the PI3K/Akt pathway is frequently activated in a variety of human malignancies (Table 1)(64, 69, 70). In particular, in cases of NSCLC harboring mutations in EGFR, the level of EGFR phosphorylation was enhanced and hyperphosphorylated Akt was detected in 44–96% (Figure 1B)(35, 64, 69, 71). The importance of Akt activation has been further strengthened by the finding that gefitinib responsiveness could subsequently be predicted by Akt activation (3, 16, 20, 72). Hence, the PI3K/Akt pathway plays a critical role downstream of EGFR, particularly in the population of NSCLC harboring EGFR mutations, and Akt is, at least, one of the determinants of gefitinib sensitivity.

p-Akt was histologically observed in both the cytoplasm and the nucleus. However, intense nuclear p-Akt staining was often observed in carcinomas harboring EGFR mutation (Figure 2A).

4.4. Possible correlations between EGFR status and downstream activation

The previous reports mentioned in Chapters 3. and 4. can be summarized as below.

i) Carcinomas harboring EGFR gene amplification revealed a higher level of EGFR
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expression/phosphorylation compared with those harboring EGFR in disomy. In those cases, Stat-3 was often activated. This suggests that persistent Stat-3 activation may be a critical event downstream of EGFR that has been overexpressed due to gene amplification (20).

ii) On the other hand, carcinomas harboring the EGFR mutation may persistently activate a cascade via Akt. In these cases, both EGFR expression and EGFR phosphorylation was enhanced, and Akt was activated at a high frequency (20). However, in one report using cultured cells transfected with mutant EGFR, both the PI3K-Akt and Stat pathways were activated by EGF stimulation (46). Thus, there may be a discrepancy between the experimental model and actual human specimens.

iii) In those cases not showing gene amplification or mutation, specific correlation of EGFR overexpression/activation with activation of downstream molecules was not observed (20).

The diversity of the activation patterns in these molecules may be the evidence of mechanism in the maintenance of homeostasis in established cancer cells, and the particular signaling cascade that is predominantly activated may differ depending on the genotype, the stage of the tumor and their environment.

5. AKT-MEDIATED PATHWAY

5.1. Akt, as a potential key molecule and its significance in human cancer

Akt is a central node in a signaling pathway consisting of many components that have been implicated in tumorigenesis, such as upstream PI3K, PTEN (Phosphatase and Tensin homologue deleted on chromosome Ten) and downstream tuberous sclerosis gene product complex (TSC)(Figure 1B) (73). In human malignant tumors, constitutive Akt activation is noted in a wide variety of cancers; ovarian, lung, renal, breast carcinoma, lymphoma and sarcomas (73-75). Therefore, not only Akt, but also the components of this pathway have emerged as the focus of cancer research and therapy.

In addition to an association between activated Akt pathway and gefitinib sensitivity in NSCLC (72), numerous reports have described correlations between Akt activity and clinicopathologic parameters in human cancers. In ovarian carcinomas, elevated Akt activity has been shown to be particularly prevalent in high grade, late stage and/or metastatic tumors, and linked to reduced patient survival (73). In NSCLC, alterations in Akt have been variously described.

i) Akt expression correlated with worse clinical profiles; lymph node metastasis, resistance against chemotherapy and radiation (20, 69). As one of the possible underlying mechanisms in the lymph node metastasis, Akt is known to reduce E-cadherin expression in the cultured cell lines (76).

ii) Phosphorylation of Akt was a poor prognostic factor for NSCLC patients with lymph node involvement (77).

iii) Conversely, Akt activation was associated with a better prognosis (64).

iv) The remaining reports show that elevated Akt activity did not correlate with tumor stage, grade or survival rate (20, 35, 71, 73, 74).

5.2. Akt/mtOR cascade

Signaling through Akt is transduced to a myriad variety of molecules one of which is mammalian target of rapamycin (mTOR). mTOR is a 289 kD serine/threonine protein kinase and a member of the PI3-K-related kinase (PIKK) family. The mTOR pathway is highly conserved and mediates signals from nutrients and oxygen, as well as from growth factor receptors (78, 79). mTOR activation positively regulates cell proliferation by promoting entry into the G1 phase of the cell cycle, via phosphorylation of substrates that cooperate in ribosomal biogenesis and translational initiation (Figure 1B) (79, 80). The current model of Akt signaling to mTOR involves direct activation of mTOR through phosphorylation of the Ser4248 residue and indirect activation as well (81). Indirect activation involves Akt phosphorylation of a complex formed by the tumor suppressor proteins TSC 1 and 2 (TSC1/2), also known as hamartin and tuberin, which normally suppress mTOR activity (80). mTOR phosphorylates its downstream targets p70S6-kinase (S6K) and eukaryotic initiation factor 4E (eIF4E) binding proteins 1, 2, and 3 (4E-BPs 1-3)(58, 80, 82). The former, S6K phosphorylates the ribosomal protein S6 (rS6) to increase translation of mRNAs with 5'-terminal oligopoly pyrimidine (5'TOP) tracts (80). The latter, 4E-BP1, dimerizes with the initiation factor elF4E and blocks cap-dependent translation (58). Phosphorylation of 4E-BP1 releases elF4E to promote translation (80). Involvement of mTOR, p-S6K as well as p-4E-BP1 in cancer have been suggested by their activation in ovarian, endometrial and breast carcinomas and also by the correlation with poor prognosis (58, 82, 83).

In NSCLC harboring mutant EGFR, the Akt-mediated pathway was constitutively and frequently activated although no significant correlation was observed between EGFR mutations and Akt activation in several studies (35, 84). This notion has added further weight to the idea that this kinase-driven pathway leading to activation of Akt/mTOR and its downstream effectors constitute another critical mechanism underlying the pathology of a subset of NSCLC.

5.3. Involvement of mTOR in cancer

Since it is evident that mTOR plays a role in lung cancers, its activation profiles in NSCLC has recently been examined by IHC. We observed that, in non-neoplastic tissue, activated mTOR staining was weakly observed in the cytoplasm of bronchial epithelial cells (Figure 2B). In lung carcinomas, intense p-mTOR signal has been observed in 51 to 74% (Table 1)(71, 85, 86). However, the activation patterns of mTOR vary among...
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Histological types. In AC, approximately 90% of the tumors revealed mTOR activation, but this incidence was only 40% in SCC and much less in SCLC (10.0%) (35, 71). In AC, the frequency of p-mTOR staining correlated with the degree of morphological differentiation, i.e., p-mTOR staining was observed predominantly in the cytoplasm of the tumor cells in the well-formed acinar structure (Figure 2B). This suggests that mTOR has a dual function in human lung carcinomas: mTOR may not only promote the proliferation of tumor cells as an effector molecule downstream of growth factor receptors, but also may function in the differentiation and, possibly, acinar morphogenesis of AC. S6K and rS6 are likely to participate downstream in acinar morphogenesis in AC, since they were also activated more frequently in AC. Studies in the literature report that enhanced p-rS6 signal has been observed throughout the lung epithelium after birth, but not in the interstitial tissue. This specific localization of p-rS6 in the epithelium may imply that the S6K-rS6 cascade has an essential function in the morphogenesis and/or acinar differentiation of lung epithelial structure (87). Along the same lines, elevated signaling of the mTOR-S6K-S6 axis may be an evidence of their involvement critical in the morphogenesis of well differentiated AC.

However, this histology-dependent mTOR activation was observed only in vivo, and not in cultured cells, i.e., SCLC or SCC-derived cultured cells also exhibit an equivalent level of mTOR phosphorylation as AC-derived cells (our unpublished data).

In our study, we found that among NSCLC cases exhibiting Akt activation, 63% revealed mTOR activation (Figure 3) (35). On the other hand, among p-mTOR positive cancers, 66% were associated with Akt activation. mTOR activation that appeared to be EGFR-Akt pathway-dependent (i.e., positive for p-EGFR, p-Akt and p-mTOR) comprised 18% of total NSCLC cases (Figure 3). Hence, although not the principal effector, mTOR is one of the critical downstream modulators in the EGFR-Akt cascade.

**5.4. Downstream of mTOR and specific correlations between intermediate effectors**

The idea of targeting signaling through the Akt/mTOR pathway [Akt, mTOR, S6K, rS6 and 4E-BP1] downstream of EGFR in lung carcinomas is a tantalizing one. The involvement of downstream effectors of mTOR signaling, S6K-rS6 proteins as well as 4E-BP1, have been studied in parallel with mTOR in cancer.

In the literature, S6K was reported to be activated in B-cell lymphoma, breast, ovary, gastric cancers and sarcomas (88-90). Positive signal of p-S6K in non-neoplastic tissue was seen in the smooth muscle cells of vascular walls and more weakly in the bronchial epithelial cells. In NSCLC, S6K activation has been observed in up to 58%, but up to 73% in AC (35, 71) (Table 1 and Figure 2C). Approximately, 50% of those positive cases showed nuclear staining, suggesting that S6K shuttles between the cytoplasm and the nucleus.

p-rS6 was observed in 50 to 56%, but up to 67 to 73% in AC, while the 32% in SCLC (35, 59, 71, 91) (Table 1 and Figure 2D). Notably, NSCLC cases with EGF mutations were associated with the higher levels of p-S6K and, p-rS6 proteins (up to 54% of all NSCLC cases and up to 90% of the cases harboring EGF mutations) (35, 91). The positive p-rS6 signal was almost exclusively cytoplasmic (Figures 2D). Among p-rS6-positive NSCLC cases, 70% were p-S6K dependent and 26% were activated through the EGFR/Akt/mTOR/S6K cascade (35).

4E-BP1 was activated in 25% of NSCLC without any particular prevalence among histological types in our series (Table 1) (35). The positive signal was almost exclusively cytoplasmic. Among p-4E-BP1-positive NSCLC cases, 14% were activated through the EGFR/Akt/mTOR cascade (35).

**5.5 Constitutive activation of the EGFR-Akt-mTOR cascade**

Next, we examined clinical cases of lung carcinomas to dissect the mode of signal transduction from the EGFR/Akt/mTOR cascade to S6K-rS6/4E-BP1. This involved immunohistochemical staining for all of these proteins simultaneously in each case and evaluation of the activation pattern of effector molecules in a sample group. EGFR activation (p-EGFR positive) was observed in 45% of NSCLC, and 56% of these p-EGFR positive cases (i.e. 25% of all cases) revealed Akt activation (Figure 3). Although activation of the Akt pathway is not completely dependent on the EGFR axis, more than 50% of the cases with Akt-activation were EGFR-dependent.

Constitutive activation of all the intermediates between EGFR through rS6 was found in only 12% with a slight preponderance in AC (Figure 3). However, this does not necessarily imply the minimal involvement of this cascade in lung carcinoma since there remains the possibility that some of these proteins could be transiently activated, then dephosphorylated, and thus not all phosphorylated proteins may be visualized simultaneously by IHC.

Among the tumors harboring EGF gene mutations, constitutive activation of the entire cascade from EGFR through rS6 was found in approximately 50%. This correlation between EGF mutation and activation of this cascade was observed even in SCC cases although the numbers of SCC cases with mutated EGF were much smaller (35).

**5.6. Clinicopathological analysis**

In previous IHC studies on NSCLC, no consistent conclusion could be drawn regarding the correlation between activation of effector proteins downstream of EGFR and clinicopathological features, which probably reflects large differences in IHC data. The positive correlations among the activation of mTOR cassette proteins and various clinicopathological factors and profiles are as follows.
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Figure 3. Prevalence of activation in EGFR and intermediate effectors in Akt/mTOR-mediated pathway evaluated by immunohistochemistry. The values indicate positive ratios in total cases of non-small cell carcinoma. Abbreviations: p-EGFR, phosphorylated EGFR; mTOR, mammalian target of rapamycin; S6K, p70S6 kinase; rS6, S6 ribosomal protein.

i) There was the positive correlation between nodal metastasis and mTOR phosphorylation in SCC, but not in AC (71).

ii) S6K and 4E-BP1 activation was a determinant of cisplatin resistance in NSCLC (92).

iii) Elevated p-S6 is associated with lymph node metastasis in lung AC and exhibited significantly shorter time-to metastasis compared with p-rS6 negative tumors (59).

iv) There was no predictive value for nodal metastasis or prognostic value for overall survival in p-4E-BP1 or p-S6K in lung cancer (35, 71).

Collectively, Akt/mTOR-mediated signaling confers clinical aggressiveness, and in particular, the signals which lead to S6 activation may be involved in nodal involvement in NSCLC.

6. MOLECULAR TARGETED THERAPY IN LUNG CANCER

6.1. Development of EGFR-targeted therapy

The ultimate goal of current efforts in anticancer drug development is to selectively kill cancer cells using agents that are specific and toxic just for tumor cells with fewer side effects. The highly conserved ATP–binding site within the catalytic domain of most kinases was initially viewed as a suitable target for the development of selective and potent small-molecule kinase inhibitors. The clinical success of such kinase inhibitors as imatinib (Abl inhibitor) and gefitinib has validated this strategy and promoted a explosion in the identification of additional kinase inhibitors (13). So far, at least 30 distinct selective kinase inhibitors have been introduced, and many more are following as cancer genome projects continue (Table 2) (13, 14, 93).

In lung carcinoma, EGFR has been most intensively pursued as a therapeutic target, and two major classes of EGFR therapeutics have been found: ectodomain-binding antibodies and small-molecule TKIs (Table 2) (7, 12, 14, 16). The former includes cetuximab (IMC-C225, Erbitux™), a chimeric monoclonal antibody (IgG1 subtype) that competitively binds the extracellular domain of EGFR. Cetuximab was initially approved for the treatment of metastatic colorectal cancer and is now well established in various lines of treatment (94). A number of novel antibodies with different spectra of activity or mechanisms of receptor inhibition are currently in development. The representative one is panitumumab (Vectibix®), a fully human monoclonal antibody of the immunoglobulin subclass IgGκ (7). In preclinical experiments, it was shown that the antitumor effect of EGFR-targeted monoclonal antibodies was strengthened when combined with the DNA-crosslinking drug, cisplatin (95).

The latter group is TKIs.

i) Gefitinib is the first small molecule TKI approved for NSCLC, and is an orally active, selective, and
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Table 2. Molecularly targeted inhibitors

<table>
<thead>
<tr>
<th>Agent / Compound</th>
<th>Trade Name</th>
<th>Target</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>cetuximab/IMC-C225</td>
<td>Erbitux®</td>
<td>EGFR</td>
<td>16, 135</td>
</tr>
<tr>
<td>panitumumab</td>
<td>Vectibix®</td>
<td>EGFR</td>
<td>7, 16</td>
</tr>
</tbody>
</table>

Tyrosine kinase Inhibitors

Reversible inhibitors

- gefitinib / ZD1839 
  Iressa® 
  EGFR | 13, 14, 96 |
- erlotinib / OSI-774 
  Tarceva® 
  EGFR | 13, 14, 36, 97 |
- lapatinib / GW572016 
  Tykerb® 
  EGFR/HER-2 | 98, 99 |
- vandetanib / ZD6474 
  ZACTIMA™ 
  EGFR/RET/VEGFR2 | 7, 16 |

Irreversible inhibitors

- canertinib / CI-1033 
  Pan-ErbB | 16, 104 |
- neratinib / HKI-272 
  Pan-ErbB | 103 |
- PF00299804 | EGFR/HER2/HER4 | 16 |
- BIIB-2992 
  Tovoka® | EGFR/HER2 | 105 |
- foretinib/GSK136089, XL880 | Met/VEGFR2 | 100 |
- pemexetrexed | Alimta® | Folate | 13 |
- berenacumab | Avastin™ | VEGF | 18 |
- PR2341066 | Met/ALK | 108 |
- ARQ197 | Met/Focal Adhesion Kinase | 16 |
- XL184 | Met/VEGFR2 | 16 |
- PL-15 | PI3-K/mTOR | 130 |
- lactacystin | Akt | 109 |
- tamoxifen | Akt | 109 |
- KPI32-1 | Akt | 111 |
- perifosine | Akt/mTOR | 129 |
- sirolimus | mTOR | 13, 122, 123, 124 |
- temsirolimus / CCI-779 
  Torisel® | mTOR | 124 |
- everolimus / RAD-001 
  Afinitor® | mTOR | 66, 124 |
- AP-23573 | mTOR | 124 |
- cucurbitacin-I | Sirt-3 | 112 |
- tyrophostin AG490 | Sirt-3 | 123 |

Reversible EGFR-TKI that chemically belongs to the class of anilinoquinazolines (96). Although as a single agent in vitro, the effects of gefitinib were mainly cytostatic, cytotoxic effects have also been observed (96) as suggested by its proapoptotic effects involving Bcl-2 family members (96). Erlotinib (OSI-774) is the second TKI, clinically introduced for NSCLC, and is an EGFR-specific quinazoline derivative. An initial report showed that erlotinib induced apoptosis and growth inhibition in several tumor cell lines in vitro, which was, in part, associated with the blockade in the G1 phase of the cell cycle (16, 97). In tumor biopsy specimens, phosphorylated forms of EGFR, Akt, and ERK were significantly downregulated after treatment with erlotinib (36).

ii) The second category includes a small molecule dual inhibitor of EGFR/HER2, lapatinib (Tykerb®) (7, 98). Lapatinib is currently approved in combination with capcitabine (Xeloda®) for the treatment of HER-2-overexpressing chemorefractory breast cancer patients and is in clinical investigation for multiple solid tumors, including NSCLC (99). Another example is foretinib (XL880/GSK136089) targeting c-Met/VEGFR2 (vascular endothelial growth factor receptor 2) (100).

iii) Inhibitors of triple or more TKs are vandetanib (ZACTIMA™, ZD6474) for EGFR/RET/VEGFR2 and AEE788 for EGFR/HER2/VEGFR (101). BMS-690514 (Bristol-Myers Squibb) is an oral selective inhibitor of EGFR, HER-2, HER-4, VEGFR1-3 and Flt-3 (7) and pazopanib (GW786034) is an oral inhibitor of VEGFR1-3, PDGFRα, β and c-Kit (102).

iv) The last category consists of the irreversible inhibitors, canertinib (pan-ErbB receptor tyrosine kinase inhibitor, CI-1033) and neratinib (HK272, an irreversible pan ErbB inhibitor) (16, 103, 104). Moreover, the anilino-quinazoline derivative BIIB-2992 (Tovoka®), an oral irreversible dual TKI of the EGFR and HER-2 (105), has been currently in clinical trials for NSCLC with T790M mutation (7, 16). These exhibit potent irreversibility in target binding, and simultaneous inhibition of EGFR and other oncogenic pathways.

Several of these agents exhibit activity in tumors refractory to gefitinib or erlotinib, and thus, vandetanib and neratinib have, in particular, undergone the extensive preclinical testing (7).

6.2. Resistance against TKIs

One serious therapeutic obstacle to effective therapy is drug resistance, and this has supplied the impetus to continuously search for new therapies. A secondary point mutation in exon 20 of EGFR that substitutes methionine for threonine at amino acid position 790 (T790M) was identified in at least half of the NSCLC patients who developed acquired resistance to gefitinib or erlotinib (51). Almost all patients acquire resistance to these drugs within 1 year, posing a serious clinical problem. Another 20% of the patients exhibited amplification of the MET gene, which causes activation of ERBB3 and subsequently of Akt. This results in the evasion of apoptosis and resistance against TKI (106, 107).

New strategies to minimize the risk of acquired resistance to EGFR inhibition have been employed in the development of next-generation EGFR tyrosine kinase inhibitors. Within this class, irreversible EGFR-TKIs...
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(Chapter 6.1.4) are currently in clinical trials for NSCLC with TKI resistance. Given that NSCLC-derived cell lines harboring MET amplification demonstrate sensitivity to MET kinase inhibitors, regimens with an EGFR-TKI plus MET inhibitor (ARQ197, XL184, foretinib) are also under investigation (100, 107, 108).

On the other hand, tumors having mutated EGFR were found to signal predominantly through Akt (Chapters 3.4., 4.3.). Therefore, in these types of tumors, resistance against TKIs might be treated with an Akt antagonist (lactoquinomycin, pyranonaphthoquinones, perifosine and KP372-1) (109, 110) or PI3K inhibitors (LY294002, wortmannin), which may still be effective in suppressing Akt-mediated signaling (111).

6.3. Targeting cancers with wild type EGFR

Although clarification of the relation between EGFR mutation and TKI sensitivity has prompted clinicians to stratify patients to optimize clinical outcomes, this stratification excludes a majority of the patients (3, 13, 84). The serious issue here is that in more of the NSCLC cases, EGFR signaling functions through wild type EGFR. Thus, we may need to focus on targeting downstream effector molecules as an alternative strategy.

First, since in tumors with amplification of the EGFR gene, the enhanced EGFR signal is frequently mediated by Stat-3 activation (Chapters 3.3., 4.1.), these tumors could be subjects for treatment with Stat-3 inhibitors, e.g. tyrphostin AG490, cucurbitacin etc. In addition, cetuximab may contribute to efficacy as it inhibits the action of overexpressed EGFR protein (112) (113).

Second, data from past studies have identified patients whose tumors did not harbor EGFR mutations, but who nonetheless benefited from gefitinib. This suggests that additional genetic or biochemical factors contribute to the gefitinib response (114). Although other possible genomic changes were variously described to be linked to TKI sensitivity, including HER2 mutations, K-Ras mutation and PTEN disruption (115-117), these correlations have not been established in lung cancer. Several studies have shown that EGFR amplification is associated with sensitivity to gefitinib/erlotinib and with better survival (Chapter 3.5.2.) (7, 30, 34). In addition, high expression of the ErbB family members may result in the constitutive activation of Akt and subsequently sensitize cells to TKIs (114). This suggests that elevated Akt may solely be a predictive marker for TKI sensitivity.

Thus, we should attempt to identify downstream molecules that are specifically activated in cases that do not exhibit obvious EGFR aberration. This kind of precise screening for the phenotypes could identify which patients may have a good response to targeting therapy and eventually determine the appropriate therapeutic strategy for each patient.

6.4. Rationale for targeting Akt/mTOR signaling in human cancers

As mentioned in the Chapters 5.3. to 5.5., components of the kinase-driven pathway leading to activation of Akt/mTOR are reasonable targets for therapeutic intervention. Blocking this pathway could impede the proliferation of tumor cells by inducing apoptosis or sensitizing tumors to other cytotoxic agents (73, 118, 119). mTOR is one of the most appealing therapeutic target, considering that mTOR interacts via a number of circuitous routes with many signaling elements (78, 79, 120, 121) and it already has an identified inhibitor in clinical use, rapamycin. Rapamycin is a bacterial macrolide agent, and a well-known immunosuppressant, that functions as an allosteric inhibitor of mTOR (79, 122, 123). In addition to rapamycin (sirolimus), its derivatives temsirolimus (CCI-779), everolimus (RAD-001) and AP-23573 are being currently evaluated in clinical trials (124, 125). Some of these exhibit potent activity even as a monotherapy, with minor toxicity against a wide panel of cancers, including renal cell carcinoma, SCLC, sarcoma, glioblastoma, and lymphoma (120), and everolimus has been approved for the treatment of renal cell carcinomas (119, 126). Recent promising experiments looking at the combined inhibition of EGFR, mTOR and PI3-K have shown to have an additive effect on suppressing cell growth and motility in cultured cancer cells (122). Furthermore, everolimus has been shown to enhance the effects of conventional platinum-based therapies in cultured NSCLC cells, by promoting apoptosis (123). Similarly, mitomycin-C and etoposide cause the dephosphorylation of S6K and 4E-BP1, resulting in synergy with rapamycin (73, 123, 127). Therefore, it may be possible to superimpose inhibition of the entire Akt/mTOR/S6K/4E-BP1 axis by rapamycin with conventional regimens against NSCLC regardless of EGFR status. In this sense, rapamycin could play a central role in combination with diverse regimens.

However, suppression of the mTOR signaling is not necessarily beneficial due to the presence of redundant negative feedback (119, 128). Constitutive activation of the Akt/mTOR pathway in cancer cells, in part, induces upstream feedback inhibition of signaling via EGFR. This feedback regulation has garnered significant interest over the past decade, since it had been noted that, despite the potent activity in experimental systems, inhibitors of mTOR clinically exhibit more modest antitumor activity (121, 128). The underlying mechanism is now interpreted, at least partially, as stemming from the fact that inhibition of mTOR abrogates this feedback inhibition of the pathway and causes activation of Akt as a result (128). As a countermeasure, it may be possible to utilize combined treatments involving conventional regimens, rapamycin and the Akt inhibitors (Chapter V-2), which may abrogate rapamycin-induced Akt kinase activity. One of the Akt inhibitors, perifosine, was found not only to remarkably reduce the levels of Akt, but also to inhibit the mTOR axis by two mechanisms. One is the inhibition of the assembly of both mTOR/raptor and mTOR/rictor complexes, and the other is downregulation of its major components, mTOR, raptor, rictor, S6K and 4E-BP1 by promoting their degradation through a GSK3/FBW7-dependent pathway (129).
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Additionally, PI-103, a dual-kinase inhibitor of PI3K and mTOR, holds considerable promise in preclinical studies either as a monotherapy or in combination with targeted EGFR inhibitors (130).

6.5. Multitargeted therapies

With the exception of rare cancers in which growth can depend upon a single factor, lung cancer, in general, is a heterogeneous disease with multiple gene aberrations (12). This implies that selective targeted agents may have limited activity. Furthermore, the efficacy of a single agent is often hindered by resistance. In addition to the T790M mutation and MET amplification, treatment with EGFR-tyrosine kinase inhibitor alone has been reported to increase the levels of EGFR/IGF-IR heterodimer expression, which activates its downstream signaling mediators, and stimulates mTOR-mediated de novo protein synthesis of EGFR in NSCLC cells (131). Another mechanism is the compensatory pathways to confer resistance as bypassing the effects of an EGFR-directed TKI through activation of other ERBBs (132). Persistent activation of signaling cascade caused by aberrations downstream (ex. PI3-K, Akt, etc.) may also have a role in the resistance to EGFR-directed inhibitors (36, 133). These observations highlight the growing consensus that inhibition of multiple targets may be required for successful cancer therapy (12, 130, 134). Thus, multitargeted agents represent the next generation of targeted therapies.

One straightforward strategy towards this end is the development of drugs with a somewhat broader selectivity, such as the use of TKIs targeting many different ERBB receptors. Simultaneous targeted inhibition of multiple signaling pathways has been, indeed, more effective than inhibiting a single pathway specifically in NSCLC (12, 93, 135). In this line, dual and triple TKIs, including irreversible TKIs have been extensively investigated (Chapter 6.1.4.).

Despite of the progress in pursuing in this strategy, for most patients with NSCLC, targeted therapies have not dramatically changed clinical outcome. The molecular complexity of lung cancer is at the root of these unsuccessful results and illustrates the need for optimizing treatment by applying a more tailored therapeutic approach in each case (7, 8, 14).

7. CONCLUSIONS AND PERSPECTIVE

Cancer research has pursued the development of rational clinical strategies based on the understanding of cancer biology. With recent advances in our understanding of the relationship between tumor genotypes and sensitivity to TKIs, together with improved technologies for genotyping and phenotyping tumor samples, the implementation of tailored treatments with new classes of inhibitors have become a reality. In the process of identifying targets for therapy, our knowledge about the molecular pathways involved in cancers has increased, and this knowledge has been translated into clinical trials of drugs that have clearly changed the treatment landscape. In addition, results of clinical trials of specific inhibitors that were developed from basic research using in vitro models, have provided valuable feedback to the laboratory for future development of better agents.

In the future, we need to further elucidate the factors that underlie the clinical response to molecularly-targeted therapeutics by collaboration between basic research laboratories and the clinics. The continued translation of knowledge about lung cancers that emerges from the field of signal transduction, including EGFR/Akt/mTOR cascade, should contribute to the development of novel therapeutics, and their application to the patients in the clinic.

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9. REFERENCES


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Key Words: Lung Carcinoma, EGFR, Akt; mTOR, p70S6-kinase, Molecular Targeted Therapy, Review

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