TANG HUMANITAS MEDICINE

Review

Alternative drug therapies are superior to epidermal growth factor receptor -targeted chemotherapeutic drug responses in non-small cell lung cancer

Sourav Sikdar, Anisur Rahman Khuda-Bukhsh*

Department of Zoology, University of Kalyani, Kalyani-741235, India

ABSTRACT

Cancer is one of the major dreaded diseases causing high mortality. Lung cancer is second in position of all cancer related deaths and mainly divided into two morphologic sub-types: small-cell lung cancer and non-small cell lung cancer (NSCLC). NSCLC is an aggressive neoplasm which hardly responds to any conventional chemotherapy. Epidermal growth factor receptor (EGFR) belongs to the ErbB family of receptor tyrosine kinase that is mainly over-expressed in NSCLC. EGFR is mainly involved in the pathogenesis and progression of different carcinoma. *In vivo* and *in vitro* studies suggest that EGFR and EGF like peptides are often over-expressed in human NSCLC and these proteins are able to induce cell transformation. The conventional therapies mostly inhibit the EGFR activity and expression level in human NSCLC with the use of some EGFR-inhibitors like HKI-272, EKB569, CL-387785 etc. and some synthetic chemotherapeutic drugs like erlotinib, gefitinib, plumbagin, docetaxel, cisplatin etc., alone or in combination of two or more drugs. These therapies selectively act by competitive inhibition of the binding of adenosine triphosphate to the tyrosine kinase domain of the EGFR, resulting in inhibition of the EGFR signaling pathway. But these chemotherapeutic drugs have some cytotoxic activities to the normal cells and have some adverse side-effects. Recent studies on some traditional alternative therapies including some herbal and plant extracts, active ingredients like curcumin, different homeopathic drugs, etc. can target EGFR-signalling in NSCLC with less toxic side-effects are being currently developed.

Keywords lung cancer, non small cell lung cancer, chemotherapies, EGFR, alternative therapies

INTRODUCTION

Cancer, almost as old as mankind, is globally a major health problem and one of the major causes of mortality today. A recent report suggests that nearly 13% of all human deaths occurred by cancer and more than ten million people are diagnosed with cancer annually (Lentini et al., 2010). Cancer, after its initiation, spreads rapidly to other distant sites within the body from its origin and thus becomes difficult to cure (Misra et al., 2010). Cancer can affect people of all ages, but the risk of cancer progression increases with the age (Aaujo et al., 2007).

Lung cancer is one of the most common cancer related deaths worldwide in both men and women accounting for one third of all deaths from cancer (nearly 31% mortality in men and 26% in women) (Fukuoka et al., 2003; Herbst et al., 2005; Hirsch et al., 2007; Gomathinayagam et al., 2008; Sharma et al., 2007). Although there has been a gradual decline in the incidence of lung cancer in men, it continues to increase in women. More than 213,000 new cases were diagnosed in the U.S. in 2007 (Ramalingam et al., 2008). Today lung cancer remains the leading cause of cancer-related deaths mainly in the United States, Western countries, North America and Japan (Ceppi et al., 2006; Mitsudomi et al., 2005). Currently, lung cancer is the most common type of cancer in Europe also (381,500 new cases in 2004). The American Cancer Society projected 159,390 deaths from lung cancer in 2009, accounting for about 28% of all cancer deaths.

Tobacco/cigarette smoke is the major cause of 80% lung cancer cases (Lee et al., 2011). However, lung cancer is far more common in never-smokers than smokers in some countries, especially within the Pacific Rim, particularly in females (Herbst et al., 2002). It has been estimated that lung cancer will become a relevant social disease in India and China in the next 20 years based on the current trends of cigarette consumption (Aaujo et al., 2007). Fig. 1 represents the population-based distribution of lung cancer patients diagnosed in 2010 by Local Health Integration Network (LHIN).

Lung cancer is divided into two morphologic sub-types: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). SCLC is a tumour of neural crest origin and it initially responds well to chemotherapy. NSCLC is further subdivided into adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma, which hardly responds to conventional chemotherapy (Mitsudomi et al., 2005). So, in the present review, our main focus will be on the conventional and recent therapeutic approaches including traditional therapies against NSCLC that have little or less side-effects. The pie-chart shows the distribution of different types of NSCLC in between smokers and non-smokers (Fig. 2).

NSCLC is an aggressive neoplasm, responsible for more lung cancer deaths each year in the United States than colon, breast, pancreas, and prostate cancers combined. NSCLC is thought to originate in lung epithelial cells (Sharma et al.,

*Correspondence: Anisur Rahman Khuda-Bukhsh
E-mail: prof_arkb@yahoo.co.in
Received December 24, 2012; **Accepted** May 15, 2013; **Published** May 31, 2013
doi: http://dx.doi.org/10.5667/tang.2012.0048

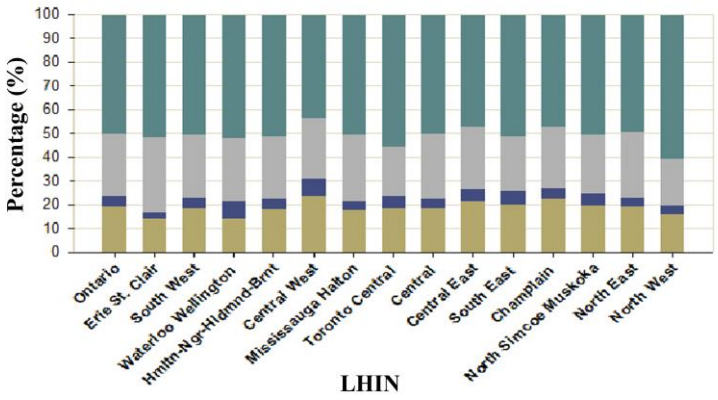

Fig. 1. Population-based distribution of lung cancer patients diagonosed in 2010 by LHIN.

2007). It is mainly encountered in smokers and has a different spectrum of molecular abnormalities like molecular etiology, pathogenesis, and possibly prognosis than that of the non-smokers. Patients with later stages of NSCLC are often symptomatic, with specific pulmonary problems like cough, breathlessness, hemotypsis and general symptoms like fatigue, weight loss that can cause extreme distress to the patient, even death (Fukuoka et al., 2003; Eberhard et al., 2005). An overall survey revealed that 15% of NSCLC patients with poor prognosis die within 5 years (Hirsch et al., 2007). Recently researchers reported that the five-year survivality of NSCLC patients hovers at approximately 15%, which certainly drops dramatically at advanced stage (Kelly et al., 2008).

Cell proliferation is mainly responsible for the growth and differentiation of cancer cells. Proliferative activity of NSCLC cells is maintained through several mechanisms, including autocrine loops, not completely dependent upon exogenously supplied growth factors. The ability of cancer cells to produce high levels of their own peptide growth factors depends on the activation of cellular proto-oncogenes are mostly responsible for cancer cell proliferation (Aaujo et al., 2007). So, if there is any possibility to inhibit the proliferative activity of NSCLC by any exogenous inhibitors, it would be helpful in modern cancer therapy.

Detection and diagnosis of lung cancer during the early stages is difficult due to the absence of characteristic symptoms. Recent treatment options, such as chemotherapy and radiation therapy have limited therapeutic success in lung cancer, particularly in NSCLC, as they develop resistance to therapy (Gomathinayagam et al., 2008). Now a days, surgery and systemic chemotherapy with some synthetic agents are followed depending on the extent of the signs and symptoms of the disease. Nevertheless, despite the advances in chemotherapy, and improvements in the delivery of radio- and chemo-therapies, the long-term survivality of NSCLC patients remains poor (Kelly et al., 2008). Surgery is the most effective therapeutic modality with curative intent in early-stage NSCLC, but post-operative survival remains unsatisfactory, with a 5-year survival rate less than 70% even in pathologic stage I disease. Even with the use of combination chemotherapy and chemoradiation, survival has improved very little over the past few decades. Although chemotherapy has been definitely proven to be active in locally advanced or metastatic NSCLC, its activity should be still considered suboptimal (Ceppi et al., 2006).

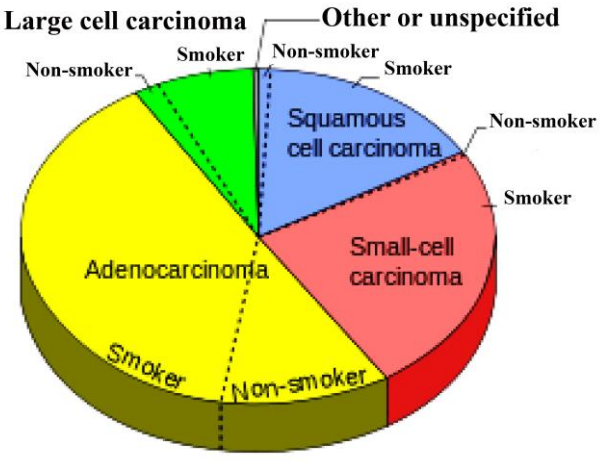

Fig. 2. Represents the distribution of different types of NSCLC in smokers and non-smokers. The respective colours denote the amount in percentage of different types of NSCLC.

Epidermal growth factor receptor (EGFR) and epidermal growth factor (EGF) like peptides are often over-expressed in human NSCLC and these proteins are able to induce cell proliferation and transformation, evidenced from the *in vitro* and *in vivo* studies. Recently EGFR-targeted chemotherapies come forward for the NSCLC prevention. EGFR-inhibition by certain synthetic drugs is now developed to reduce the NSCLC proliferation and damage.

Recently, it has become generally accepted that systemic chemotherapy and proliferative gene targeted therapy are beneficial in advanced or last stage of NSCLC patients in terms of improved survival and quality of life. So, effective, palliative, low-toxicity treatments for patients with advanced NSCLC are needed (Fukuoka et al., 2003). Although chemotherapy has produced modest survival benefits in patients with advanced stage NSCLC, standard two-drug combinations generate considerable toxicity (Cappuzzo et al., 2005). Despite the advances recently achieved in the NSCLC treatment, the management of malignancies still remains a difficult task for the oncologist. Molecular-targeted therapy has now emerged as an alternative treatment for NSCLC patients, but variable responsiveness rates and the development of drug resistance have created challenges in clinical practice. Thus, the development of more effective therapeutic modalities based on the molecular mechanisms including inhibition of some proliferative genes involved in cancer cell progression and generation of apoptosis by using some alternative drugs with less side-effect is of utmost important today.

Chemotherapeutic approaches in modern lung cancer therapy, especially NSCLC

In lung cancer therapy, however, several technical and practical obstacles make the road extremely difficult to move from research findings to clinical practice. On the basis of "chemotherapy efficacy plateau" for the lung cancer treatment, molecular-targeted drugs have entered the therapeutic arena in recent years. There are various synthetic drugs and/or chemotherapeutic agents commonly used against NSCLC. Erlotinib, gefitinib etc. are the common chemotherapeutic agents recently used against lung cancer. A study on erlotinib therapy in 57 patients with relapsed NSCLC demonstrated an objective response rate of 12.3%, and gefitinib provided an overall response rate of 10.4% in European patients and 27.5% in Japanese patients. In phase III studies of patients with untreated advanced NSCLC, adding gefitinib or erlotinib to chemotherapy did not significantly improve outcome over chemotherapy alone (Eberhard et al., 2005). The anticancer and

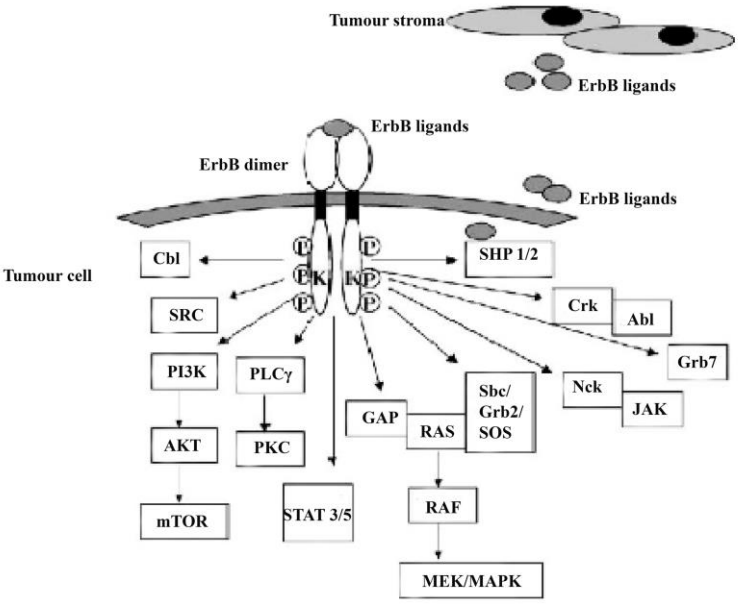

Fig. 3. Mechanisms of action of ErbB receptors in lung tumor cells. ErbB receptors are activated by binding to specific ligands that are produced by either tumor cells or by surrounding stromal cells.

anti-proliferative properties of plumbagin have been also reported in various cancer cell lines, *in vitro*. Plumbagin, a napthoquinonoid compound has been reported to induce apoptosis in several cancer cell lines including those of the breast, ovary and lung (Gomathinayagam et al., 2008). Both Erlotinib and gefitinib displayed a trend toward longer survival during the maintenance phase of the study (Ramalingam et al., 2008). Currently, docetaxel, having demonstrated survival benefits of NSCLC patients, reported as the approved treatment in the United States and the European Union for patients who have been failed by previous platinum-based chemotherapy (Cappuzzo et al., 2005). Cisplatin also used as anti-NSCLC agent that cause monoadducts and intrastrand or interstrand cross-links in DNA. Yun et al., (2007) reported the 50% growth inhibition in H460 and A549 NSCLC cell lines and in HN5 tumor models by the combination chemotherapy including paclitaxel and cisplatin (Yun et al., 2007). Cisplatin/gemcitabine is a widely used combination therapy in any stage of NSCLC (Ceppi et al., 2006).

Although chemotherapy has produced modest survival benefits in patients with advanced stage disease, standard two-drug combinations generate considerable toxicity and require intravenous administration involving Akt in lung cancer cell lines (Cappuzzo et al., 2005; Dziadziuszko et al., 2006). Synthetic anilinoquinazoline compound, approved in Japan and the United States for relapsing patients with advanced NSCLC, acts selectively by competitive inhibition of the binding of adenosine triphosphate to the tyrosine kinase (TK) domain of the EGFR receptor, resulting in inhibition of the EGFR signaling pathway (Marchetti et al., 2005). Recent works by several groups have suggested that never-smokers are more likely to harbour mutations in the TE domain of EGFR than smokers. Pao et al. (2005) reported such mutations in seven of fifteen unselected early-stage never-smokers with adenocarcinoma (Pao et al., 2005). In never-smokers, it would be important to investigate prospectively the value of combining erlotinib with chemotherapy versus erlotinib alone. The side-effects of rash and diarrhoea associated with erlotinib seemed to be characteristic of these types of chemotherapeutic synthetic drugs. Current first line chemotherapy options for patients with advanced NSCLC is the use of combination of a platinum-based agent with paclitaxel, gemcitabine, vinorelbine, or docetaxel which have substantial toxicity and side-effects (Herbst et al., 2005).

EGFR is a growth factor receptor that is mainly over-expressed in NSCLC in most cases. Today's conventional therapies are mostly developed to inhibit the EGFR-activity and expression level. So, in the present work, the main objective is the EGFR-targeted therapy by both chemotherapeutic drugs and natural agents.

EGFR-targeted chemotherapeutics in NSCLC

General account on EGFR

The role of growth factor-driven signalling in the pathogenesis of human cancer has long been established. According to the previous report (Larco and Todaro, 1978), cancer cells generally exhibit a reduced requirement for exogenously supplied growth factors to maintain high rate of cell proliferation. This relaxation in growth factor dependency is due to the ability of tumor cells to produce high levels of peptide growth factors (Normanno et al., 2006). EGFR is a trans-membrane glycoprotein with an extracellular ligand-binding domain and an intracellular domain possessing intrinsic tyrosine kinase (TK) activity. After ligand- binding, receptor dimerization leads to both activation of the TK domain and recruitment and phosphorylation of intracellular substrates, which drive normal cell growth and differentiation (Marchetti et al., 2005). Phosphorylated tyrosines serve as the binding sites for several signal transducers that initiate multiple signaling pathways resulting in cell proliferation, migration, and metastasis, evasion from apoptosis, or angiogenesis, all of which are associated with cancer phenotypes (Kosaka et al., 2004). Upon growth-factor binding, EGFR and other ErbB family members activate their cytoplasmic TK domains to initiate intracellular signaling (Yarden and Sliwkowski, 2001; Schlessinger et al., 2004). Overexpression or mutational activation of the EGFR is implicated in the development and progression of numerous human malignancies, and a number of small-molecule tyrosine kinase inhibitors (TKIs) have been developed to target the ATP-binding cleft of the EGFR (Hynes and Lane, 2005). Despite broad enthusiasm regarding the potential value of EGFR target modulation in cancer therapy, the field rests at an important crossroads in the light of negative results from several large-scale phase III clinical trials in lung cancer reported in 2002 - 2003 (Huang et al., 2004). Mitogen-activated protein kinase (MAPK) pathway is one of the main downstream effectors of the EGFR, including the extracellular signal-regulated kinase (ERK), the c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK), and the p38 MAPK (Shan et al., 2009). Furthermore, EGFR over-expression has been associated with a poor prognosis in lung cancer patients in several studies. So EGFR-targeted cancer therapies are currently being developed (Fukuoka et al., 2003; Dougherty et al., 2008).

EGFR family receptors and ligands

EGFR superfamily, including the four distinct receptors EGFR/ErbB-1, HER2/ErbB-2, HER3/ErbB-3, and HER4/ErbB-4, were identified as a potential therapeutic target in solid tumors. After ligand-binding, these receptors become homo- and hetero-dimerized and their TK-domain is activated, initiating a cascade of events implicated in the development

and progression of cancer through effects on cell cycle progression, apoptosis, angiogenesis, and metastasis (Cappuzzo et al., 2005). Normanno et al., (2006) diagrammatically depicts the EGFR-receptor binding represented in figure 3. Table 1 represents the EGFR-ligand mutation and the affected organs involved.

EGFR Polymorphisms

Overexpression of EGFR has been linked to the amplifications of the EGFR gene located at 7p13-12. At intron 1 of EGFR, a highly polymorphic repeat called CA repeat was identified. Recent *in vitro* and *in vivo* studies showed that transcriptional activity of EGFR is reduced with more CA repeats. Thus, the length of this (CA) dinucleotide polymorphism is inversely related to the transcriptional activity of the gene. Depending on the association between the expression of the gene and the polymorphic (CA) repeat, the response to therapy with EGFR inhibitors may vary among patients, due to their genotypic differences. The highest response rate to these therapies was observed in the Japanese population and may be due to a reduced expression of EGFR associated with the longer sequence of allele 20 (the most frequent allele in Asians) unlike the shorter sequence of 16 repeats associated with white and black ancestry (Aaujo et al., 2007).

EGFR inhibitors

EGFR related to survival progress in lung cancer biology led to the development of small molecule inhibitors of target proteins involved in proliferation, apoptosis, and angiogenesis. The established irreversible inhibitors of the EGFR kinase, mainly HKI-272, EKB569, and CL-387785, are reported to maintain activity against the T790M mutation (Kwak et al., 2005; Tsou et al., 2005). Several of these inhibitors are currently in clinical trials or have been approved for clinical use, including the 4-anilinoquinazolines gefitinib (Iressa) (Wakeling et al., 2002), erlotinib (Tarceva) (Pollack et al., 1999), lapatinib (Rusnak et al., 2001), and pyrolopyrimidine AEE788 (Traxler et al., 2004; Tsou et al., 2005).

EGFR targeted therapies

Chemotherapy, the mainstay of treatment in advanced disease, is only marginally effective, but gefitinib and erlotinib, which target the EGFR show promising activity in the treatment of NSCLC including its metastatic stage. Response rates are 10 to 20 percent when these ATP-competitive anilinoquinazoline inhibitors are used as second- or third-line treatment for advanced diseases (Kobayashi et al., 2005). Several other molecular targets are under evaluation and observation as monotherapy or in combination with systemic chemotherapy against EGFR in NSCLC. The most common of these mutant EGF receptors, EGFR VIII, is one in which amino acids 6 to 273 of the extracellular domain are deleted. This specific mutation has been shown to promote aggressive growth of tumors, *in vivo* (Marchetti et al., 2005).

Table 1. Ligand mutation and consequent organ defect

Mutation target	Localization of relevant defects
EGFR	Epidermis, mammary gland, lung, pancreas, intestine, central nervous system
ErbB-2	Mammary gland, central nervous system
ErbB-3	Heart, central nervous system
ErbB-4	Mammary gland, heart, central nervous system
EGF	Prostate, central and periferal nervous system
TGF-α	Epidermis, prostate, eye
HB-EGF	Central nervous system
AR/EGF/ TGF-α	Gastrointestinal tract

Inhibition of EGFR by specific blocking agents induces apoptosis and reduces proliferation of tumor growth in different experimental models. Thus, EGFR-inhibitors represent new promising antineoplastic drugs against NSCLC (Marchetti et al., 2005). Objective responses to EGFR-TKIs targeted therapy were reported to succeed in 10% - 27% of NSCLC patients after failure to conventional chemotherapy (Hirsch et al., 2007).

Agents that target the EGFR have proven to be efficacious in the management of NSCLC. Erlotinib is an oral EGFR-TKI. Gefitinib is an orally active, selective EGFR-TKI that blocks signal transduction pathways implicated in the proliferation and survival of cancer cells but failed to demonstrate any survival advantage in a phase III study (Ramalingam et al., 2008; Kosaka et al., 2004). Cappuzzo et al., (2005) have reported on the basis of their clinical trial that the relationship between EGFR gene copy number, EGFR protein expression, EGFR mutations, and Akt activation status as predictive markers for gefitinib therapy in advanced NSCLC (Cappuzzo et al., 2005). In these trials, responses were seen across the gefitinib dose range 150 to 800 mg/day, while the majority of dose interruptions and reductions due to toxicity were required in patients receiving more than 600 mg/day. From these data, two doses (250 and 500 mg/day) were selected for investigation in phase II and phase III trials. The 250 mg/day dose is higher than the lowest dose level at which objective tumor regression was seen, while 500 mg/day is the highest dose that was well tolerated when taken over an extended period in phase I trials. The results suggest that gefitinib is an effective treatment for previously treated patients with advanced NSCLC (Fukuoka et al., 2003). The efficacy of first-line gefitinib was superior to that of standard chemotherapy, with acceptable toxicity, in patients with advanced NSCLC harbouring sensitive EGFR mutations (Maemondo et al., 2010). Gene amplification is another molecular mechanism responsible for oncogene overexpression, and this phenomenon has been associated with mutations in the EGFR gene in glioblastoma (Cappuzzo et al., 2005).

Although these EGFR TKIs are well established in the treatment of NSCLC, to date, the biologic activity of these in this NSCLC tumor type has remained poorly defined. Subsequently, several randomized phase III trials of chemotherapy alone versus combination of two or more drugs were initiated. But the results of these types of study suggest that these trial designs may prove to be suboptimal for demonstrating the efficacy of combination-chemotherapy (Hirsch et al., 2008).

But these chemopreventive drugs and therapies targeting EGFR have several side-effects described already. So there will be an urgent need to search for alternative treatments targeting EGFR with little or less side-effects and with very small amount of recommended dose.

Alternative therapies targeting EGFR against NSCLC

In the last several decades, a great amount of effort has been made to examine phytochemicals found in fruits and vegetables used as complementary and alternative medicines (CAM) for their cancer chemopreventive activities. Indeed, a number of promising natural products have come forward in the modern cancer prevention.

In November 2007, the American Institute for Cancer Research published a list of recommendations that people can follow to help to reduce their risk of developing cancer, including dietary guidelines, such as the reduction of food and drink intake that promote weight gain (i.e. energy-dense foods and sugary drinks), the limited intake of red meat, salt,

alcoholic beverages, and the preferential consumption of plant-derived food (Lentini et al., 2010). The presence of smoking-related co-morbid illness and the inherent molecular heterogeneity have all been impediments to research efforts to improve outcomes for patients with lung cancer (Ramalingam et al., 2008). There has been recent development in molecular targeted therapies, an available option in addition to conventional cancer treatments. Molecular-targeted therapeutic drugs can interfere with and block specific molecular pathways involved in cancer cell growth and progression (Chang et al., 2011). By screening the 598 herbal and natural compounds containing the range of alkaloids, sesquiterpenes, diterpenes, pentacyclic triterpenes, sterols and many other diverse representatives are now tested to the different gefitinib-resistant NSCLC cell lines, *in vitro* through cell proliferation assay.

Now-a-days, many natural products and their derivatives like vinca alkaloids, taxanes, camptothecins etc. are being used for cancer therapy, both in orthodox and traditional medicines. In recent years, the potential of natural products from plants, notably from medicinal plants used in traditional Chinese medicine (TCM), has been recognized by the scientific community in the Western world. Increased knowledge of the molecular mechanisms of TCM-derived drugs and recent developments in their applications demonstrate that the combination of TCM with modern cutting-edge technologies provide an attractive strategy for the development of novel and improved cancer therapeutics (Efferth et al., 2007).

Curcumin (diferuloylmethane) is one of the ingredients used abundantly in Indian traditional systems of medicine like Ayurveda and Unani. Curcumin has been tested as a potential anti-cancer and anti-tumour agent. It could inhibit cell cycle progression; induce cell apoptosis and anti-metastasis by regulating various mechanisms in different cell types related to NSCLC. Thus, these results indicated curcumin may be an alternative therapeutic agent to combine with gefitinib for altering cyclin D1 gefitinib-resistant NSCLC. It was also invented from the above study that curcumin combined with low dose gefitinib (1mM) exhibited more anticancer activities as compared to a single therapy of a high dose of gefitinib (10 - 20 mM) in inhibiting cell proliferation, decreasing colony formation ability and inducing apoptosis of these gefitinib-resistant lung adenocarcinoma cell lines, *in vitro*. This is the first report that indicates the potential of curcumin as an effective agent which can combine with gefitinib enhancing the antitumour activities. Solving the problem of side-effects and cytotoxicity, the novel preparation of curcumin is now used with oral bioavailability and reduced dosage against NSCLC. This curcumin treatment specially targeted the expression of EGFR and had the cancer inhibitory property. Recent studies indicated that the diminished EGFR activity is mediated through the intrinsic pathway of cell apoptosis accompanied by rapid phosphorylation of p38 and Bax activation in intestinal epithelial cells. In addition, curcumin has been discovered as a p38 modulator that prevented the loss of integrity of the intestine villi and reduced apoptosis in the intestine, *in vivo*. This is the first report to show that curcumin can overcome the gefitinib inefficiency and inhibited EGFR protein kinase activity through down-regulating endogenous EGFR level via accelerating proteasome-ubiquitin activity and could significantly reduce not only pEGFR, but also EGFR protein expression in a concentration-dependent manner. Although previous studies have indicated that curcumin inhibited EGFR kinase activity and blocked ligand-induced EGFR activation in the different types of cells, these reports showed the amount of EGFR did not alter in the presence of curcumin (Lee et al., 2011). The roles of these natural products or secondary metabolites played in plants have only recently come to be appreciated in an analytical context.

Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for anti-bacterial, antineoplastic, and other pharmaceutical functions. Phenolic compounds are widely distributed in the plant kingdom. Flavonoids are the most abundant, commonly known for their anti-oxidant and anti-tumour efficacy and for their use in human diet, due to their widespread distribution, and their relatively low toxicity, compared to other active plant compounds (i.e. alkaloids). Strong experimental evidences also show their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities. A growing number of epidemiological studies suggest that high flavonoid intake may be correlated with a decreased risk of cancer. Flavonoids have been demonstrated to inhibit carcinogenesis *in vitro* and substantial evidences indicate that they can also do so *in vivo*. As example, when given intraperitoneally, quercetin and apigenin inhibited melanoma cell (B16eBL6) growth and metastatic potential in syngenic mice, and interestingly, they significantly decreased the invasion of B16-BL6 cells *in vitro*. Important flavones, specifically genistein, apigenin, luteolin, quercetin, and some other anti-cancer polyphenols are found in tea, in particular catechins. In green tea, epigallocatechin gallate (EGCG), epigallocatechin, epicatechin-3-gallate and epicatechin are the major compounds (Lentini et al., 2010). Traditional Chinese medicine herbal treatment (TCM) can improve the prognosis of advanced pulmonary adenocarcinoma patients, especially targeting EGFR (Guo et al., 2011).

Recent study on the ethanolic extract of *Thuja occidentalis* against A549-NSCLC and ethanolic extract of *Marsdenia condurango* against A549, H522-NSCLC also reported anticancer potential, *in vitro* (Mukherjee et al., 2012; Sikdar et al., 2013). Although the anti-EGFR activity had not been studied earlier, these reports on anti-lung cancer potential encouraged us to conduct further research on some of these traditional medicines.

DISCUSSION

Promising characteristics of the EGFR as a molecular target for cancer therapy have prompted an extensive drug development effort to design pharmacologic inhibitors of EGFR signaling. The agents developed can be crudely classified into two major categories, namely, large molecule anti-EGFR antibodies and small molecule EGFR-TKIs. We have previously described that recent therapeutic advances include the use of EGFR-TKIs including gefitinib and erlotinib. It is thought that these drugs would be most effective with patient selection on the basis of target expression. In NSCLC patient populations, EGFR mutation prevalence is 10% - 23% (in Caucasians) and 30% - 40% (in East Asian populations) (Weiss et al., 2008).

Activated EGFR has been reported to promote cell survival, proliferation, invasion, and metastasis through activation of JAK/STAT, PI3K/Akt and MAPK pathways previously mentioned. These observations have established EGFR as a target for lung cancer therapy, especially NSCLC and have led to the development of the EGFR-TKI gefitinib and erlotinib. Recent research has indicated that patients with NSCLC with EGFR-activating mutations exhibit a dramatic clinical response to EGFR-TKIs especially in Asians, females, non-smokers, and patients with adenocarcinoma (Chang et al., 2010).

Previous study examined the effect of drugs on NSCLC and elucidates the molecular mechanism(s) by which it exhibits anticancer activity. Plumbagin could down-regulate cyclin B1

and Cdc25B protein expressions, which might have resulted in the induction of G2/M arrest in H460 cells and also inhibit the EGFR expression. These results are supported by previous studies which suggest that inhibition of cyclin B1 and EGFR by different agents such as ionizing radiation, adriamycin, or sulforaphane caused significant G2/M arrest in NSCLC. The results of this study support the effect of plumbagin on EGFR and its downstream signaling (Janmaat et al., 2003).

Clinical responses to gefitinib (ZD1839) in NSCLC have varied among populations, with higher rates of response seen in females, non-smokers, and those patients with adenocarcinoma or bronchio-alveolar carcinoma (Tracy et al., 2004). Cisplatin causes monoadducts and intrastrand or interstrand cross-links in DNA (Ceppi et al., 2006).

Another study noted increased response rate and prolonged progression-free but not overall survival in patients treated with gefitinib (Dziadziuszko et al., 2006). Recently, two consecutive studies reported that patients with EGFR exon 19 deletions had a longer survival than patients with EGFR L858R point mutations. Current study demonstrated that FISH analysis for EGFR gene copy number in combination with immunohistochemistry assessment for EGFR protein is a useful paradigm for selection of advanced NSCLC patients to gefitinib (Hirsch et al., 2007). Several experimental and clinical studies were recently initiated to explore the role of targeting EGFR in cancer therapy. Lev-Ari et al. (2006) showed that combining COX-2 and EGFR inhibitors was more effective than using either agent alone for the suppression of the development of lung carcinoma *in vivo*. Furthermore, following establishment of cetuximab-resistant cell lines, it was confirmed that gefitinib and erlotinib retain the capacity to inhibit cellular growth and downstream EGFR signaling in the cetuximab-resistant cells. These results suggest that combining distinct classes of EGFR inhibitors may not only potentiate cellular toxicity caused by non-overlapping mechanisms of action but also may assist to overcome inherent or acquired resistance to one class of EGFR inhibitor.

All the previous data suggest that combining distinct classes of EGFR inhibitors can augment the antitumour response over that realized with a single EGFR inhibitor. The data further suggest that acquired resistance to one class of EGFR inhibitor may be partially overcome by challenge with another class of EGFR inhibitor. These preclinical data from the *in vitro* and *in vivo* setting warrant validation across other laboratories and may provide a scientific platform for the future design of clinical trials, which further explore this dual EGFR inhibitor strategy (Huang et al., 2004).

But the involvement of side-effects in EGFR-targated therapy forced the recent researchers to use the natural agents. Curcumin has long been known as a potential therapeutic or preventive agent for several major human cancers, by regulating multiple targets on tumour signaling pathways, such as protein kinases, transcriptional factors and apoptosis-related proteins. Also, since curcumin displayed synergistic effects of several chemotherapeutic drugs, it has been suggested as an adjuvant for anti-cancer therapy. However, the potential anti-tumour effect of curcumin combined with EGFR-TKIs on NSCLC has not yet been investigated. Lee et al. (2011) recently found that curcumin has dual beneficial effects on gefitinib therapy in NSCLC (Lee et al., 2011). In that study, it was found that curcumin inhibited cell survival in lung adenocarcinoma cell lines, an effect that was associated with the down regulation of EGFR and inhibition of Erk1/2 activity for the first time. In conclusion, EGFR is down-regulated and the Erk1/2 activity is decreased by curcumin. This inhibition was associated with decreased survival and enhanced induction of apoptosis in lung and pancreatic adenocarcinoma cells (Lev-Ari et al., 2006).

Our recent unpublished works on benzo[a]pyrene-induced NSCLC and post cancerous treatment with glycoside-rich components of ethanolic extract of Condurango and potentized form of it, also gave a positive signal towards EGFR-inhibitory activity of plant-derived components and homeopathic drugs.

CONCLUSION

All the previous studies have indicated a novel approach for the treatment of lung cancer patients, specially the patients bearing NSCLC with the EGFR-targated therapy by using some traditional alternative medicines alone, or in combination with EGFR-TKIs. These therapies might lead to an efficient and prolonged control of lung tumour growth directed against EGFR and ligands with no or little side-effects on normal cells and/or other organs of the patient's body. So, in future it is an urgent need to test some traditionally used plant extracts and homeopathic remedies in clinical trials to prove their efficacy in EGFR-targeted lung cancer (especially NSCLC) therapy over conventional synthetic chemotherapeutic drugs.

ACKNOWLEDGEMENTS

This study was supported by the grant of the Boiron Laboratories, Lyon, France.

CONFLICT OF INTEREST

None to declare.

REFERENCES

Aaujo A, Ribeiro R, Azevedo I, Coelho A, Soares M, Sousa B, Pinto D, Lopes C, Medeiros R, Scagliotti GV. Genetic polymorphisms of the epidermal growth factor and related receptor in non-small cell lung cancer—a review of the literature. Oncologist. 2007;12:201-210.

Ceppi P, Volante M, Novello S, Rapa I, Danenberg KD, Danenberg PV. ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine. Ann Oncol. 2006;17:1818-1825.

Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, Haney J, Witta S, Danenberg K, Domenichini I, Ludovini V, Magrini E, Gregorc V, Doglioni C, Sidoni A, Tonato M, Franklin WA, Crino L, Bunn PA Jr, Varella-Garcia M. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst. 2005;97:643-655.

Chang TH, Tsai MF, Su KY, Wu SG, Huang CP, Yu SL, Yu YL, Lan CC, Yang CH, Lin SB, Wu CP, Shih JY, Yang PC. Slug confers resistance to the epidermal growth factor receptor tyrosine kinase inhibitor. Am J Respir Crit Care Med. 2011; 183:1071-1079.

Dougherty U, Sehdev A, Cerda S, Mustafi R, Little N, Yuan W, Jagadeeswaran S, Chumsangsri A, Delgado J, Tretiakova M, Joseph L, Hart J, Cohen EE, Aluri L, Fichera A, Bissonnette M.

Epidermal growth factor receptor controls flat dysplastic aberrant crypt foci development and colon cancer progression in the rat azoxymethane model. Clin Cancer Res. 2008;14:2253-2262.

Dziadziuszko R, Witta SE, Cappuzzo F, Park S, Tanaka K, Danenberg PV, Barón AE, Crino L, Franklin WA, Bunn PA Jr, Varella-Garcia M, Danenberg KD, Hirsch FR. Epidermal growth factor receptor messenger RNA expression, gene dosage, and gefitinib sensitivity in non small cell lung cancer. Clin Cancer Res. 2006;12:3078-3084.

Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, Ince WL, Jänne PA, Januario T, Johnson DH, Klein P, Miller VA, Ostland MA, Ramies DA, Sebisanovic D, Stinson JA, Zhang YR, Seshagiri S, Hillan KJ. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol. 2005;23:5900-5909.

Efferth T, Li PCH, Konkimalla VSB, Kaina B. From traditional Chinese medicine to rational cancer therapy. Trends Mol Med. 2007;13:353-361.

Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Feyereislova A, Dong RP, Baselga J. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. J Clin Oncol. 2003;21:2237-2246.

Gomathinayagam R, Sowmyalakshmi S, Mardhatillah F, Kumar R, Akbarsha MA, Damodaran C. Anticancer mechanism of plumbagin, a natural compound, on non-small cell lung cancer cells. Anticancer Res. 2008;28:785-792.

Guo H, Liu JX, Xu L, Madebo T, Baak JP. Traditional Chinese medicine herbal treatment may have a relevant impact on the prognosis of patients with stage IV adenocarcinoma of the lung treated with platinum-based chemotherapy or combined targeted therapy and chemotherapy. Integr Cancer Ther. 2011;10:127-137.

Herbst RS, Maddox AM, Rothenberg ML, Small EJ, Rubin EH, Baselga J, Rojo F, Hong WK, Swaisland H, Averbuch SD, Ochs J, LoRusso PM. Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in non-small-cell lung cancer and other solid tumors: results of a phase I trial. J Clin Oncol. 2002; 20:3815-3825.

Herbst RS, Prager D, Hermann R, Fehrenbacher L, Johnson BE, Sandler A, Kris MG, Tran HT, Klein P, Li X, Ramies D, Johnson DH, Miller VA. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol. 2005;23:5892-5899.

Hirsch FR, Varella-Garcia M, Cappuzzo F, McCoy J, Bemis L, Xavier AC, Dziadziuszko R, Gumerlock P, Chansky K, West H, Gazdar AF, Crino L, Gandara DR, Franklin WA, Bunn PA Jr. Combination of EGFR gene copy number and protein expression predicts outcome for advanced non-small-cell lung cancer patients treated with gefitinib. Ann Oncol. 2007;18:752-760.

Hirsch FR, Herbst RS, Olsen C, Chansky K, Crowley J, Kelly K, Franklin WA, Bunn PA Jr, Varella-Garcia M, Gandara DR Increased EGFR gene copy number detected by fluorescent in situ hybridization predicts outcome in non-small-cell lung cancer patients treated with cetuximab and chemotherapy. J Clin Oncol. 2008;26:3351-3357.

Huang S, Armstrong EA, Benavente S, Chinnaiyan P, Harari PM. Dual-agent molecular targeting of the epidermal growth factor receptor (EGFR): combining anti-EGFR antibody with tyrosine kinase inhibitor. Cancer Res. 2004;64:5355-5362.

Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer. 2005;5:341-354.

Janmaat ML, Kruyt FA, Rodriguez JA, Giaccone G. Response to epidermal growth factor receptor inhibitors in non-small cell lung cancer cells: limited antiproliferative effects and absence of apoptosis associated with persistent activity of extracellular signal-regulated kinase or Akt kinase pathways. Clin Cancer Res. 2003;9:2316-2326.

Kelly K, Huang C. Biological agents in non-small cell lung cancer: a review of recent advances and clinical results with a focus on epidermal growth factor receptor and vascular endothelial growth factor. J Thorac Oncol. 2008;3:664-673.

Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG, Halmos B. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med. 2005;352:786-792.

Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res. 2004;64:8919-8923.

Kwak EL, Sordella R, Bell DW, Godin-Heymann N, Okimoto RA, Brannigan BW, Harris PL, Driscoll DR, Fidias P, Lynch TJ, Rabindran SK, McGinnis JP, Wissner A, Sharma SV, Isselbacher KJ, Settleman J, Haber DA. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. Proc Natl Acad Sci USA. 2005;102:7665-7670.

Larco JEde and Todaro GJ. Growth factors from murine sarcoma virus-transformed cells. Proc Natl Acad Sci USA. 1978;75:4001-4005.

Lee JY, Lee YM, Chang GC, Yu SL, Hsieh WY, Chen JJ, Chen HW, Yang PC. Curcumin induces EGFR degradation in lung adenocarcinoma and modulates p38 activation in intestine: the versatile adjuvant for gefitinib therapy. PLoS One. 2011;6:e23756.

Lentini A, Tabolacci C, Provenzano B, Rossi S, Beninati S. Phytochemicals and proteinepolyamine conjugates by transglutaminase as chemopreventive and chemotherapeutic tools in cancer. Plant Physiol Biochem. 2010;48:627-633.

Lev-Ari S, Starr A, Vexler A, Karaush V, Loew V, Greif J, Fenig E, Aderka D, Ben-Yosef R. Inhibition of pancreatic and lung adenocarcinoma cell survival by curcumin is associated with increased apoptosis, down-regulation of COX-2 and EGFR and inhibition of Erk1/2 activity. Anticancer Res. 2006;26:4423-4430.

Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saijo Y, Hagiwara K, Morita S, Nukiwa T; North-East Japan Study Group. Gefitinib or chemotherapy for non-small cell lung cancer with mutated EGFR. N Engl J Med. 2010;362:2380-2388.

Marchetti A, Martella C, Felicioni L, Barassi F, Salvatore S, Chella A, Camplese PP, Iarussi T, Mucilli F, Mezzetti A, Cuccurullo F, Sacco R, Buttitta F. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. J Clin Oncol. 2005;23:857-865.

Misra R, Acharya S, Sahoo SK. Cancer nanotechnology: application of nanotechnology in cancer therapy. Drug Discov Today. 2010;15:842-850.

Mitsudomi T, Kosaka T, Endoh H, Horio Y, Hida T, Mori S, Hatooka S, Shinoda M, Takahashi T, Yatabe Y. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. J Clin Oncol. 2005;23:2513-2520.

Mukherjee A, Sikdar S, Bishayee K, Paul A, Ghosh S, Boujedaini N, Khuda-Bukhsh AR. Ethanolic extract of Thuja occidentalis blocks proliferation of A549 cells and induces apoptosis in vitro. Zhong Xi Yi Jie He Xue Bao. 2012;10:1451-1459.

Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, Carotenuto A, De Feo G, Caponigro F, Salomon DS. Epidermal growth factor receptor (EGFR) signaling in cancer. Gene. 2006;366:2-16.

Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, Varmus H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS One. 2005;2:e73.

Pollack VA, Savage DM, Baker DA, Tsaparikos KE, Sloan DE, Moyer JD, Barbacci EG, Pustilnik LR, Smolarek TA, Davis JA, Vaidya MP, Arnold LD, Doty JL, Iwata KK, Morin MJ. Inhibition of epidermal growth factor receptor-associated tyrosine phosphorylation in human carcinomas with CP-358,774: dynamics of receptor inhibition in situ and antitumor effects in athymic mice. J Pharmacol Exp Ther. 1999;291:739-748.

Ramalingam S, Belani C. Systemic chemotherapy for advanced non-small cell lung cancer: recent advances and future directions. Oncologist. 2008;13:5-13.

Rusnak DW, Lackey K, Affleck K, Wood ER, Alligood KJ, Rhodes N, Keith BR, Murray DM, Knight WB, Mullin RJ, Gilmer TM. The effects of the novel, reversible epidermal growth factor receptor/ErbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines in vitro and in vivo. Mol Cancer Ther. 2001;1:85-94.

Schlessinger J. Common and distinct elements in cellular signaling via EGF and FGF receptors. Science. 2004;306:1506-1507.

Shan JZ, Xuan YY, Zheng S, Dong Q, Zhang SZ. Ursolic acid inhibits proliferation and induces apoptosis of HT-29 colon cancer cells by inhibiting the EGFR/MAPK pathway. J Zhejiang Univ Sci B. 2009;10:668-674.

Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer. 2007;7:169-181.

Sikdar S, Mukherjee A, Boujedaini N, Khuda-Bukhsh AR. Ethanolic extract of Condurango (Marsdenia condurango) used in traditional systems of medicine including homeopathy against cancer can induce DNA damage and apoptosis in non small lung cancer cells, A549 and H522, in vitro. TANG. 2013;3:e9.

Tracy S, Mukohara T, Hansen M, Meyerson M, Johnson BE, Jänne PA. Gefitinib induces apoptosis in the EGFRL858R non-small-cell lung cancer cell line H3255. Cancer Res. 2004;64:7241-7244.

Traxler P, Allegrini PR, Brandt R, Brueggen J, Cozens R, Fabbro D, Grosios K, Lane HA, McSheehy P, Mestan J, Meyer T, Tang C, Wartmann M, Wood J, Caravatti G. AEE788: a dual family epidermal growth factor receptor/ErbB2 and vascular endothelial growth factor receptor tyrosine kinase inhibitor with antitumor and anti-angiogenic activity. Cancer Res. 2004;64:4931-4941.

Tsou HR, Overbeek-Klumpers EG, Hallett WA, Reich MF, Floyd MB, Johnson BD, Michalak RS, Nilakantan R, Discafani C, Golas J, Rabindran SK, Shen R, Shi X, Wang YF, Upeslacis J, Wissner A. Optimization of 6,7-disubstituted -4- (arylamino) quinoline-3-carbonitriles as orally active, irreversible inhibitors of human epidermal growth factor receptor-2 kinase activity. J Med Chem. 2005;48:1107-1131.

Wakeling AE, Guy SP, Woodburn JR, Ashton SE, Curry BJ, Barker AJ, Gibson KH. ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. Cancer Res. 2002;62:5749-5754.

Weiss GJ, Bemis LT, Nakajima E, Sugita M, Birks DK, Robinson WA, Varella-Garcia M, Bunn PA Jr, Haney J, Helfrich BA, Kato H, Hirsch FR, Franklin WA. EGFR regulation by microRNA in lung cancer: correlation with clinical response and survival to gefitinib and EGFR expression in cell lines. Ann Oncol. 2008;19:1053-1059.

Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol. 2001;2:127-137.

Yun CH, Boggon TJ, Li Y, Woo MS, Greulich H, Meyerson M, Eck MJ. Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity. Cancer Cell. 2007;11:217-227.