Molecular Biology of Lung Cancer—A Review

Dinesh Pendharkar · B. V. Ausekar · Sumant Gupta

Received: 15 November 2012 / Accepted: 7 January 2013 / Published online: 15 January 2013
© Indian Association of Surgical Oncology 2013

Abstract Lung cancer is leading site of cancer in Indian males today. The incidence is on the rise. It is generally diagnosed in late stages. The progression free survival and overall survival still remains low. Majority of oncology research today is driven towards molecular profiling of lung cancer. Receptor tyrosine kinases have emerged as major molecular markers and targets. These are the results of gene mutations or amplifications. There are many developed targets where therapeutic option is available like EGFR, ALK, MET, KRAS and so on. This review features on few important molecular profiles of lung cancer, especially with more therapeutic implications.

Keywords Lung cancer · NSCLC · EGFR · ALK

There are about 1 million new cases of cancer diagnosed every year in India. Lung cancer has emerged as number one cancer in Indian metros [1]. As a single disease it is most common cancer in males. Annually about 11 % of all cancer in males constitute lung cancer. Various regions of the country have large differences. The age adjusted rate in males in Delhi is about 13.5, whereas same in Aizwal is 42.5. The treatment is based upon multiple factors mainly histology and stage. Non-small cell lung cancer forms the major bulk.

Lung cancer is generally diagnosed in locally advanced or metastatic stages. Most patients with advanced non small cell lung cancer (NSCLC) are treated with chemotherapy regimens that provide an overall survival of less than 1 year. Platinum base doublet is the standard of care in non surgical cases which constitute majority. The survival still remains low. Due to these poor clinical outcomes there has been substantial research into the mechanisms of pathogenesis of this disease. These advances in molecular biology and pathogenesis of NSCLC provide insights into potential therapeutic targets. Investigators have identified multiple processors that are responsible to the contribution of tumour progression and responsible for cell growth.

Molecular Profiling and Testing in NSCLC

Through molecular diagnostics tumour cells may be distinguished based on the presence or absence of receptor proteins, driver mutations, or oncogenic fusion rearrangements. Over the last decade growing number of various markers EGFR, ALK, KRAS, HER2, MET, BRAF, PIK3CA, MEK1, NRAS, AKT1, ROS1 have been identified in NSCLC. Multiple mutations may occur in multiple oncogenes leading to transcription of mutated signals. This leads to change in protein structure, copy number. Similarly it may lead to various abnormal combinations of proteins. The basic consequence of such a mutation is induction and sustenance of tumorigenesis. These so called driver mutations are common in kinase genes. They may be seen in all subtypes of non small cell lung cancer. Adenocarcinoma in particular is likely to possess therapeutically amenable mutations. Interestingly they may have some clinical correlations. Like females, non smokers are more likely to harbor EGFR mutations. The more common and most studied mutations include EGFR, ALK, HER2, RET and ROS1.

D. Pendharkar (✉)
Asian Institute of Oncology, S. L. Raheja Hospital,
Mahim, Mumbai 400016, India
e-mail: drpendharkar@gmail.com

B. V. Ausekar
Shree Siddheshwar Cancer Hospital & Research Center, Sholapur,
India

S. Gupta
Cancer Institute Adiyar, Chennai, India

This review will deal with few of these molecular oncogenic abnormalities (Table 1).

Biomarker status is quickly becoming an important tool based on which treatment is decided. There are more and more drugs which are being incorporated into this list. For example positive EGFR mutation status shows greater response to TKI's, low ERCC1 expression is associated with greater response to platinum chemotherapy and RRM1 expression with greater response to gemcitabine.

ERCC1 is repair protein which repairs damaged DNA. NSCLC tumors have been found to harbor mutation in this gene. It may be playing role in carcinogenesis. But in case of carcinoma lung it plays a unique predictor role. It has been observed that high ERCC1 activity is associated with resistance to platinum drugs. That means a standard doublet using a platinum compound may not be effective in patients with high ERCC1 Expression. Nucleoside excision repair is the primary DNA repair mechanism that removes platinum-DN compound from tumor DNA. It possible to do ERCC1 measurement in the lab today and is being clinically utilized.

RRM1 gene is gene encoding ribonucleoside-diphosphate reductase large subunit. This enzyme is extremely important for the production of deoxyribonucleotides prior to DNA synthesis by a cell. The location of this gene is on 11p15.5, close to tumor-suppressor genes. Changes of this region have been recorded in various tumors including NSCLC. RRM1 is a molecular target for the drug gemcitabine [2]. So changes in the levels of RRM1 interfere with the efficacy of

gemcitabine, higher levels are associated with low response rate. This particular expression can well be studied in individual patients. This warrants analysis of these genes ERCC1 and RRM1 in patients where usage of gemcitabine and platinum is being considered. There are no exact guidelines on this as of now but there is lot of data accumulation going keeping these molecules in the hypothesis.

Signal Transduction

Cell growth is initiated with a signal from outside the cell. Signal transduction is one of such mechanisms. This signal acts on the receptors which are situated outside the cell membrane and they then transmit the signal inside the cell through the intracellular domains. This leads to the downstream activation of multiple pathways which ultimately leads to cell growth, differentiation, migration and proliferation.

Receptors have to receive activation signal before they start signaling below and downstream. The signaling is increased when there is overexpression of the receptors that means there are multiple receptors on the cell surface to get the growth signal. Sometimes there is mutation in the receptors. These mutations also lead to over signaling downstream thereby promoting growth and other associated processes. If we are able to lock the receptors or the downstream pathway then it is possible to halt the process of growth. In other words this may lead to apoptosis. There is a special group of targeted molecules which act either on the external receptors or on the pathway and causes inhibition. Tyrosine kinase inhibitor are such a group.

Table 1 Various molecular profiles and efficacy of treatment in NSCLC

Molecular profile	Type	Management relevance
EGFR	Wild type	Reduced efficacy of EGFR TKIs
	Mutated	Increased sensitivity to EGFR TKIs
EML4-ALK fusion	Positive	Increased sensitivity to Crizotinib Decreased sensitivity to EGFR TKIs
	Negative	Decreased sensitivity to Anti ALK TKIs
KRAS	Mutation positive	Reduced sensitivity to EGFR TKIs
MET	Amplification positive	Increased sensitivity to MET TTKIs
		Decreased sensitivity to EGFR TKIs
ROS1	Mutation positive	Increased sensitivity to Crizotinib
		Decreased sensitivity to EGFR TKIs

EGFR

The ERB family is a group of receptors on cell surface in non-small cell lung cancer. This family of receptor TKI is that control major intracellular signaling pathways. These pathways are responsible for ultimate survival of the cancer cells. There are major receptors like-EGFR or ERBB1, B2, B3 and B4 also termed as HER1/HER2/HER3/HER4. All of them play an important role in cell signaling. Generally in response to extracellular growth factor, which binds to 2 ligands which in turn form a dimer activating downstream signaling. Once activated the EGFR then phosphorylates its substrates resulting in downward chain reaction. The two major downstream signaling pathways are RAS-RAF-MEK-ERK pathway responsible for cell proliferation and PI3K-AKT-mTOR pathway determining cell survival. These are multi chain reactions, meaning every partner in the chain may play an important role.

EGFR mutations occur in approximately 10 % to 15 % of NSCLC tumours and other oncogenic drivers in these tumours. More than 20 to 30 % of adenocarcinoma have

EGFR mutations. In squamous cell carcinomas the incidence of mutation is less. The most commonly acquired mutations are in the tyrosine kinase domain of EGFR gene such as deletions in EGFR EXON 18–21 and point mutations resulting in an oncogenic stick. Approximately 90 % of these mutations are exon 19 deletions or 21 L858R point mutations. These mutations increase the tyrosine kinase activity. In addition EGFR overexpression and gene amplification have been observed in patients with non-small cell lung cancer.

T790 M is a point mutation in the ATP binding pocket of EGFR that can render NSCLC tumours unresponsive to first-generation EGFR TKIs although the T790 and mutation has been reported in 2 months prior to therapy with an EGFR TKI patients may develop resistance to first-generation TKIs. This mutation which may be either intrinsic or acquired renders these tumours unresponsive to first-generation EGFR tyrosine kinase inhibitors. The T790M mutation can be found in approximately 50 % of the patients who become resistant the first condition of EGFR tea caddies. Thus the tumour may ultimately harbor both an activating mutation EGFR and T790M.

In majority of tumors the EGFR may be wild type. This confers reduced efficacy to TKIs. Despite this the drugs may still be effective. For example erlotinib is approved in second–third line irrespective of EGFR status. Mutations in the EGFR domain are in majority of the cases—non overlapping with other mutations in NSCLC like KRAS mutations, ALK gene rearrangements.

AKT 1

This is serine-threonine protein kinase, which is one of the members of the family AKT2 and AKT3 [3]. It also plays role in many cell processes, like other kinases namely growth, survival, proliferation, angiogenesis. The major downstream pathway for this domain is PI3K pathway.

The incidence of mutation in this domain is about 1 % of NSCLC [3]. The drugs to target this mutation are in very early stages of development. This mutation is generally seen in tumors with wild type EGFR, ALK mutations.

ALK

ALK is a receptor tyrosine kinase, otherwise called as anaplastic lymphoma kinase, is seen in multiple cancers. ALK fusions are commonly found in anaplastic lymphomas, colorectal cancer, ovarian cancer. These fusions have been found in NSCLC [4]. Nearly 3–7 % of lung tumors have ALK fusions [5, 6]. They have been more commonly observed in never smokers or light smokers, in patients with younger age and adenocarcinomas particularly with acinar

histology [7] or signet ring cell histology [8]. There are many ALK rearrangements in NSCLC. The commonest is EML4-ALK fusion genes. Like other markers these fusions are generally non-overlapping with other mutations.

This receptor has already gained clinical relevance and importance. Thanks to the positive trial with drug-crizotinib it was possible to get good progression free survival of 72 % in patients with ALK fusion positivity [8]. Crizotinib being dual kinase inhibitor inhibiting ALK, MET and ROS 1 kinases The test has already become part of routine armamentarium for NSCLC.

The other trials with HSP90 are on. It is believed that patients harboring this fusion show less responsiveness to EGFR TKI's [9]. Availability of this and other molecules markers is bringing us closer to achieving the goal of personalized treatment.

BRAF

It belongs to the group of serine-threonine kinases. It plays vital role in MAP kinase pathway through activation of MEK. Mutation of BRAF has been implicated in tumorigenesis of many cancers mainly melanoma, NSCLC. In melanoma this has already achieved a role of important therapeutic target. Thanks to this there is increased interest to further study it in many cancers, but mainly in NSCLC. Lung cancer do harbor this mutation in 1–3 % of patients, majority of whom are adenocarcinoma more often found in former/current smokers [10]. This mutation also is generally non-overlapping with other mutations in NSCLC. Multiple trials in this situation are already on going. Mutations giving rise to BRAF proteins are associated with decreased sensitivity to gefitinib [11].

KRAS

KRAS is one of the genes from RAS family. Proteins produced by these genes are central mediators of growth factor receptor signaling and hence responsible for cell properties. RAS can activate both PI3K and RAS-RAF pathway. Many tumors have found to have role of this genes, mainly colon cancer [12]. Incidence of KRAS mutation in lung adenocarcinoma is about 15–25 %. Like many other mutations they are not common in squamous cell lung cancers. Similarly with other genes explained above this is generally a non-overlapping mutation, that mean in tumors who do not EGFR or ALK mutation there is more chance of having KRAS mutation. This information is being further investigated in clinical trials. There are more than 25 trials presently underway trying to target information from KRAS gene. Generally patients with KRAS mutation have low response gefitinib and erlotinib [13].

MET

The MET gene is located on 7th chromosome. This gene is site for encoding receptor tyrosine kinase. Like other tyrosine kinase receptors this receptor after phosphorylation also regulates downstream signaling. Both the pathways PI3K-AKT-mTOR and RAS-RAF-MEK-ERK pathways get activated. This receptor is activated in many tumors. This molecular marker is activated in 100 % of papillary renal cell cancers and cancers like head and neck, HCC, NSCLC and few small cell lung cancer tumors also possess this mutation [14]. Along with mutation amplification is also noticed in many tumor tissues. In NSCLC mutations and amplifications have been reported. About 2–4 % of tumors of NSCLC show MET amplification. Interestingly 5–20 % of patients with EGFR mutations or with acquired resistance to EGFR TKIs have been found to have MET amplification. The mutations are generally germline mutations. There are assays available to test both mutations and overexpression. MET protein has also been seen to be elevated in tumor tissue from lung cancer. It is associated with poor prognosis [15].

There are multiple TKIs, as well monoclonal antibody available with activity against MET. These trials are on in NSCLC.

ROS1

This is a tyrosine kinase receptor from insulin family. Role of ROS 1 fusions in NSCLC has been evident for last few years [16]. Nearly 2 % of patients have these fusions. The incidence is higher in light and never smokers, in younger population. They are as well non-overlapping mutations. There is ROS inhibitor already available for trial. In fact ALK inhibitor crizotinib has also shown some activity. These patients show lesser sensitivity to EGFR TKIs.

Summary

Lung cancer is one of the greatest challenges in oncology. The survival is still very low. Newer insights in to molecular biology are helping us in locating various subtypes. It has become obvious that non small lung cancer is a group of diseases, which behave differently and respond to treatment differently. As of now there are few important targets which have been defined—EGFR, ALK, MET, KRAS, BRAF. A lung cancer specimen should be studied for EGFR and EML4ALK mutations. ERCC1 and RRM1 gene analysis should also be done in select cases. These investigations as well drugs targeting these anomalies are already available in the country. There are many such markers. Extensive research and trials are on to find there clinical relevance and therapeutic

potential. There availability in particular tumor is crucial for selecting patients. They may either be mutated and or amplified. There relative positivity to one another is also important. Present day management has to base the decisions on availability of this information in particular patient. Personalised treatment is becoming the theme of the day.

References

1. Behera D (2012) Lung cancer in India. *JAPI Med Updat* 22:401–407
2. Rosell R, Danenberg KD, Alberola V et al (2004) Ribonucleotide reductase messenger RNA expression and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. *Clin Cancer Res* 10:1318–1325
3. Bleeker FE, Felicioni L, Buttitta F, Lamba S, Cardone L, Rodolfo M, Scarpa A, Leenstra S, Frattini M, Barbareschi M, Grammasro MD, Sciarrotta MG, Zanon C, Marchetti A, Bardelli A (2008) AKT1(E17K) in human solid tumours. *Oncogene* 27(42):5648–5650, Epub 2008 May 26
4. Choi YL, Takeuchi K, Soda M, Inamura K, Togashi Y, Hatano S, Enomoto M, Hamada T, Haruta H, Watanabe H, Kurashina K, Hatanaka H, Ueno T, Takada S, Yamashita Y, Sugiyama Y, Ishikawa Y, Mano H (2008) Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer. *Cancer Res* 68(13):4971–4976
5. Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, Choi HG, Kim J, Chiang D, Thomas R, Lee J, Richards WG, Sugarbaker DJ, Ducko C, Lindeman N, Marcoux JP, Engelman JA, Gray NS, Lee C, Meyerson M (2008) EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clin Cancer Res* 14(13):4275–4283
6. Wong DW, Leung EL, So KK, Tam IY, Sihoe AD, Cheng LC, Ho KK, Au JS, Chung LP, Pik Wong M, Jänne PA, University of Hong Kong Lung Cancer Study Group (2009) The EML4-ALK fusion gene is involved in various histologic types of lung cancers from non-smokers with wild-type EGFR and KRAS. *Cancer* 115(8):1723–1733
7. Inamura K, Takeuchi K, Togashi Y, Hatano S, Ninomiya H, Motoi N, Mun MY, Sakao Y, Okumura S, Nakagawa K, Soda M, Choi YL, Mano H, Ishikawa Y (2009) EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. *Mod Pathol* 22(4):508–515, Epub 2009 Feb 20
8. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Jänne PA, Costa DB, Varela-Garcia M, Kim WH, Lynch TJ, Fidias P, Stubbs H, Engelman JA, Sequist LV, Tan W, Gandhi L, Mino-Kenudson M, Wei GC, Shreeve SM, Ratain MJ, Settleman J, Christensen JG, Haber DA, Wilner K, Salgia R, Shapiro GI, Clark JW, Iafrate AJ (2010) Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 363(18):1693–1703
9. Sequist LV, Gettinger S, Senzer NN, Martins RG, Jänne PA, Lilenbaum R, Gray JE, Iafrate AJ, Katayama R, Hafeez N, Sweeney J, Walker JR, Fritz C, Ross RW, Grayzel D, Engelman JA, Borger DR, Paez G, Natale R (2010) Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. *J Clin Oncol* 28(33):4953–4960, Epub 2010 Oct 12
10. Paik PK, Arcila ME, Fara M, Sima CS, Miller VA, Kris MG, Ladanyi M, Riely GJ (2011) Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. *J Clin Oncol* 29(15):2046–2051, Epub 2011 Apr 11

11. Gandhi J, Zhang J, Xie Y, Soh J, Shigematsu H, Zhang W, Yamamoto H, Peyton M, Girard L, Lockwood WW, Lam WL, Varella-Garcia M, Minna JD, Gazdar AF (2009) Alterations in genes of the EGFR signaling pathway and their relationship to EGFR tyrosine kinase inhibitor sensitivity in lung cancer cell lines. *PLoS One* 4(2):e4576, Epub 2009 Feb 24
12. Schubbert S, Shannon K, Bollag G (2007) Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer* 7(4):295–308
13. Riely GJ, Marks J, Pao W (2009) KRAS mutations in non-small cell lung cancer. *Proc Am Thorac Soc* 6(2):201–205
14. Kong-Beltran M, Seshagiri S, Zha J, Zhu W, Bhawe K, Mendoza N, Holcomb T, Pujara K, Stinson J, Fu L, Severin C, Rangell L, Schwall R, Amler L, Wickramasinghe D, Yauch R (2006) Somatic mutations lead to an oncogenic deletion of met in lung cancer. *Cancer Res* 66(1):283–289
15. Xu L, Nilsson MB, Saintigny P, Cascone T, Herynk MH, Du Z, Nikolinakos PG, Yang Y, Prudkin L, Liu D, Lee JJ, Johnson FM, Wong KK, Girard L, Gazdar AF, Minna JD, Kurie JM, Wistuba II, Heymach JV (2010) Epidermal growth factor receptor regulates MET levels and invasiveness through hypoxia-inducible factor-1alpha in non-small cell lung cancer cells. *Oncogene* 29(18):2616–2627, Epub 2010 Feb 15
16. Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, Nardone J, Lee K, Reeves C, Li Y, Hu Y, Tan Z, Stokes M, Sullivan L, Mitchell J, Wetzel R, Macneill J, Ren JM, Yuan J, Bakalarski CE, Villen J, Kornhauser JM, Smith B, Li D, Zhou X, Gygi SP, Gu TL, Polakiewicz RD, Rush J, Comb MJ (2007) Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* 131(6):1190–1203