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MINI-REVIEW

Molecular target therapeutics of EGF-TKI and downstream signaling pathways in non-small cell lung cancers

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Abstract: Lung carcinoma (LC) is the third most common cancer diagnosis and accounted for the most cancer-related mortality worldwide in 2018. Based on the type of cells from which it originates, LC is commonly classified into non-small cell lung cancers (NSCLC) and small cell lung cancers (SCLC). NSCLC account for the majority of LC and can be further categories into adenocarcinoma, large cell carcinoma, and squamous cell carcinoma. Accurate classification of LC is critical for its adequate treatment and therapeutic outcome. Since NSCLC express more epidermal growth factor receptor (EGFR) with activation mutations, targeted therapy EGFR-tyrosine kinase inhibitors (TKIs) have been considered as primary option of NSCLC patients with activation EGFR mutation. In this review, we present the genetic alterations, reported mutations in EGFR, and TKIs treatment in NSCLC patients with an emphasis on the downstream signaling pathways in NSCLC progression. Among the signaling pathways identified, mitogen activation protein kinase (MAPK), known also as extracellular signal-regulated protein kinase (Erk) pathway, is the most investigated among the related pathways. EGFR activation leads to the autophosphorylation of its kinase domain and subsequent activation of Ras, phosphorylation of Raf and MEK1/2, and the activation of ERK1/2. Phosphatidylinositol 3-kinase (PI3K)/Akt is another signal pathway that regulates cell cycle and has been linked to NSCLC progression. Currently, three generations of EGFR TKIs have been developed as a first-line treatment of NSCLC patients with EGFR activation and mutation in which these treatment options will be further discussed in this review. The Supplementary Appendix for this article is available at http://links.lww.com/JCMA/A138.

Keywords: Epidermal growth factor receptor; Lung carcinoma; Mitogen activation protein kinase pathway; Non-small cell lung cancers; Tyrosine kinase inhibitors.

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1. INTRODUCTION

Lung carcinoma (LC) is the third most common cancer diagnosis by gender, behind prostate cancer for men and breast cancer for women.[1] LC make up about 13% of all cancer cases. However, LC accounted for the most cancer-related mortality, around two million dead worldwide in 2018 (WHO, 2018). LC is more common in older patients with around 60% LC patients are above 70 years of age.[2] The highest risk factor of LC is cigarette smoking, which contributed approximately 86% of total LC cases.[3-5] Followed by radon gas which is a naturally occurring gas emitted by rocks, the concentration of radon gas differs based on geographical areas and types of rocks, where higher concentration of radon gas has a significant relationship in the increased risk of LC.[6,7]

LC classification is generally based on the origin of cells. Generally, LC can be categories into non-small cell lung cancers (NSCLCs) and small cell lung cancers (SCLCs).[8] NSCLCs

account for about 85% of LC and can be further subdivided into adenocarcinoma, large cell carcinoma, and squamous cell carcinoma,[9] while SCLCs contributed to the remaining 15%.[9] Accurate classification of LC is important because it can determine the therapeutic treatment plan of an LC patient. For instance, NSCLC patients with activation epidermal growth factor receptor (EGFR) mutation can benefit more from targeted therapy EGFR-tyrosine kinase inhibitor (TKI) (such as gefitinib) than SCLC because NSCLCs express more EGFR with active mutations than SCLC.[10,11]

In summary, LC is classified into two groups, NSCLC and SCLC. In addition, their genetic composition and mutations also have noticeable influence on the types of treatment. Notably, this review will focus on NSCLC which accounts for the majority of LC cases.

2. CHARACTERISTIC GENETIC ALTERATIONS IN NSCLC

Copy number alteration is one of the major ways through which NSCLC disrupts its gene to suite the mechanism supporting its progression.[12] Weir et al[13] identified 57 significant gene amplifications in lung adenocarcinoma (LAC), which is a type of NSCLC. In addition, these genetic amplifications were mostly identified on chromosome 14q13.3, accounting for 12% of all the tumor samples.[13] Examples of genes located at this chromosome locus (14q13.3) is NKX2-1. However, Nkx2-1 protein and genomic expression in NSCLC have opposing roles in NSCLC prognosis and may occur preferentially in different subsets of NSCLC patients with distinct oncogenic mutations.[14]

In East Asian patients' tumor samples, copy number gain on 16p13.13 and 16p13.11 were reported. In contrast to gain of copy numbers, higher rates of genomic loss on 19p13.3 and 19p13.11 occurred in white patients.[15]

In addition to the finding of an increase in the copy number of the MYC gene in nonsmokers, an oncogene FUS was also associated with gains in the copy number on 16p. In tumors harboring activating mutations of both EGFR and KRAS genes, higher copy number gains are observed.[16]

In addition to copy number alteration, many genetic mutations have been identified in LC, including KRAS, ROS1, BRAF, RET, NTRK1, and ERBB2, and most of them encode tyrosine kinase domain.[17] The receptors regulate cell survival and proliferation by activating downstream MAP Kinase, PI3K, and JAK-STAT pathways.

3. REPORTED MUTATION IN EGFR

The TK domain mutation of EGFR results in destabilized of domain conformation and EGFR become inactive, resulting the tyrosine kinase domain structure to become autoinhibition of its activity,[18] then kinase activity is constitutively activated, and its downstream signaling pathways are activated.[19] Since the first reported mutation in EGFR short deletions in exon 19 and point mutations (G719S, L858R, and L861Q) in exons 19 and 21, many mutations in EGFR have been discovered.[20] Based on nucleotide changes, the mutations have been classified into three categories.[21] Class I mutations is an in-frame deletions of up to six amino acids loss (E746 to S752) that was encoded by exon 19. Class II mutations are substitutions of single nucleotides from either point of exons 18 to 21. Class III mutations are in-frame duplications and/or insertions that frequently observed at exon 20. In TK domain mutations, class I deletions and exon 21 L858R mutations account for the majority of the cases of approximately 85% to 90%.[20] In the beginning, studies and reports have suggested exon 19 deletions and L858R mutations hold an equal occurrent rate. However, recent clinical trials data suggest that deletions occurs at a higher frequency compared to point mutations. It has been observed that a rare mutation of exon 22 (E884K) can lead to different EGFR small-molecule inhibitor sensitivity.[22]

4. THE DOWNSTREAM SIGNALING PATHWAYS OF EGFR IN NSCLC

4.1. Ras/Raf-MEK-MAPK signaling pathway

The implication of EGFR and its downstream signaling pathways in NSCLC progression and even in therapeutic targeting have been well established by various studies.[10,23,24] One of the key EGFR downstream pathways is mitogen activation protein kinase (MAPK) also known as extracellular signal-regulated protein kinase (Erk) pathway.[25] Out of all ERK family proteins, ERK1/2 activation will be introduced here because of its implication in NSCLC progression.[26] EGFR activation will lead to autophosphorylation of its kinase domain which recruits and activates SOS and GRB2 subsequently activates RAS.[27] RAS phosphorylates RAF, particularly important b-RAF, RAF then phosphorylates MEK1/2 which will activate Erk1/2 by phosphorylation.[27] Upon Erk1/2 phosphorylation, phospho-Erk1/2 (p-Erk1/2) dimerization occurs,[25] and then the p-Erk1/2 dimer either translocate to the nucleus where it will activate several genes and transcription factors such as c-MYC, ETS, c-Jun, and c-Fos.[25,28] These nuclear targets are very important because of their implication in cell proliferation, survival, and metastasis.[25] Interestingly, the RAF/RAS-MEK-Erk pathway can lead to the transcription and production of more TGF-β which can sustain the activation of this pathway.[29] On another note, regulations of this signaling pathway depends predominantly on the activity of the phosphatases targeting the proteins. For instance, von Kriegsheim et al[30] reported that protein phosphatase 5 controls Raf-MEK-Erk pathway by dephosphorylating Raf-'s ser 338 which inactivates Raf-1 and its downstream MEK and Erk proteins. Likewise, DUSP family proteins have been identified as a group of phosphatases targeting MAPK/Erk for dephosphorylation and subsequent inactivation.[31,32] More so, various small-molecule inhibitors were established to block the activation (phosphorylation) of the proteins in this pathway. For example, PD98059 is an allosteric inhibitor of MEK1/2, which inhibits MEK activation and its downstream Erk1/2 activation.[33] The Raf-MEK-Erk pathway is well known to promote cell proliferation, migration, and metastasis in several types of malignancies including NSCLC.[34,35]

4.2. PI3K-Akt signaling pathway

PI3K-Akt works as an intracellular signaling pathway known for its regulation of the cell cycle and therefore is linked to cell proliferation implicated in cancer progression.[36] In NSCLC, PI3K-Akt activation was found to be regulated by growth receptors such as EGFR and its implication has been studied in various disease including cancer, insulin resistance type 2 diabetes, cardiovascular diseases, and autoimmune diseases.[37] Precisely, when a growth receptor is activated, it leads to the recruitment of a PI3K, then PI3K can increase PIP3 levels to the recruitment of PDK1. Subsequently, PDK1 can either directly phosphorylates Akt or it can indirectly activate mTOR complex 2, which itself can phosphorylate Akt.[38] When Akt is activated in the cell, it inhibits AS160 through phosphorylation, and since AS160 is a negative regulator of GLUT4 translocation,[39] GLUT4 containing vesicles undergo translocation leading to GLUT4 plasma membrane localization, facilitating glucose entering into the cell to drive glycolysis.[40] Akt also inhibits TSC1/2 through phosphorylation activates mTOR complex 1, which leads to the

activation of p70S6K and S6, resulting in an increase in protein synthesis.[41] Another major protein that AKT regulates through phosphorylation is FOXO, which inhibits cell survival and proliferation.[42] AKT also inhibits GSK3 through phosphorylation. Glycogen synthase is critical or needed for glycogen synthesis. This means that because Akt inhibits an inhibitor of glycogen synthesis it activates glycogen synthesis. Akt activates ATP citrate lyase and ATP citrate lyases are important for fatty acid synthesis.[43] Because of the various functions of the Akt signaling pathway, it is commonly implicated in diseases such as insulin resistance and diabetes as well as cancer. There are several ways that the Akt pathway can be turned off. The first way is at the beginning of the pathway, in which PTEN could play the role of downregulating PIP3 by converting PIP3 into PIP2, and by shutting off this step, PDK1 is not recruited and activated.[44] The second way is through PHLPP, as this phosphatase can dephosphorylate Akt.[45] The third is to turn off Akt signaling by another phosphatase PP2A, which dephosphorylates Akt, subsequently turning off the Akt signaling pathway.[46]

5. TKIS TREATMENT IN LAC

Three generations of EGFR TKI are available for the first-line treatment of EGFR activation and mutation positive NSCLC. The first-generation treatment is known as reversible EGFR-TKIs such as gefitinib and erlotinib. Whereas the second-generation is ErbB family blockers such as afartinib and dacomitinib, and finally, the third-generation treatment is related with the irreversible wild-type sparing TKIs Osimertinib.[47]

Recent clinical trials have demonstrated that afartinib, dacomitinib, and osimertinib far better than the first-generation TKIs as a first-line treatment.[48] However, whichever EGFR-TKIs is chosen, most patients will eventually develop resistance to therapy. The first- and second-generation EGFR-TKIs have similar resistance mechanisms with the gatekeeper T790M mutation in exon 20 of EGFR,[49] as being the most common. Fortunately, a second-line osimertinib is an effective treatment option in this setting. T790M-independent mechanisms of resistance are less well understood. Osimertinib is also indicated as a first-line treatment of EGFR mutation positive NSCLC.[50] However, due to the highly heterogeneous mechanisms of resistance found in this type of cancer, targeted treatment alternatives following osimertinib failure remain uncertain. Ultimately, optimal treatment strategies in individual patients with EGFR mutation positive NSCLC will be facilitated by analysis of how the tumor evolves over time.[51] As tumors have been shown to evolve through their development and during therapy as well as genomic instability, a genetically diverse and heterogenous cell population early oncogenic mutations that drive tumor development such as EGFR mutations, tend to be present in all tumors and affected sites. In contrast. branch mutations, a subsequent subclonal event that appears in a small number of tumor cells and regions creates opportunities for any of these cells that are resistant to therapy to potentially proliferate into a resistant tumor.[52] Therefore, in terms of treatment of EGFR mutation positive tumors, it would be highly advantageous to identify subclonal background mutations and monitor them long eternally over the course of treatment. There are clear indications that EGFR mutation positive tumors are genetically heterogeneous.[53] Recent evidence indicates that in some tumors, small numbers of T790M cells were present before the commencement of treatment.[54] However, treatment with first- or second-generation EGFR TKIs resulted in cells that are subjected to strong selective pressure and these cells developed into resistant tumors that can be subsequently treated with osimertinib. However, not all tumors have pre-existing T790M cells. In some cases, T790M arises over the course of treatment and is consequently only present in some cells of the resistant tumor.[55] Technological developments have identified sources of heterogeneity in EGFR mutation positive tumors. One source of heterogeneity is the existence of uncommon EGFR mutations whereby some tumors were found to have more than one type of EGFR mutation and this phenomenon can exist on the same allele known as compound mutations. Some uncommon mutations, particularly compound mutations, are known to be insensitive to EGFR-TKIs and can therefore drive the development of resistant tumors.[56] Another source of heterogeneity is the existence of genetic aberrations that coexist with EGFR mutations. Recent data have indicated that additional mutations are often observed in the late stage tumors and these include known oncogenic drivers.[57] Additional mutations are often present within T790M positive tumors that are resistant to first- or second-generation EGFR TKIs. These observations have implications for the choice of treatment. First, TKIs should possess a wide range of inhibitory profiles to effectively respond to the heterogeneity of tumors and the potential occurrence of initial expansion among resistant subclones.[58] Emerging data indicate that different TKIs could have a different impact on how tumors evolve.[59] Interestingly, studies have shown that the frequency of T790M alleles was higher in afartinib treated cells when compared to that of erlotinib-treated cells indicating that the T790M clones might be more homogeneous following afartinib treatment.[59] Furthermore, preclinical studies have also indicated that second-generation TKIs appear to have a wider inhibitory effect against uncommon EGFR mutations than those of the first- or third-generation TKIs. Afartinib appears to have broad activity, particularly against compound mutations. While few clinical studies have investigated the activity of EGFR-TKIs against uncommon mutations, afartinib has demonstrated activity against certain uncommon mutations in clinical trials including L861Q, G791X, and S768I.[60] It is indicated for use in this setting. The effectiveness of the second- and third-generation EGFR TKIs appears to correlate well with the clonal evolution perspective. For example, a well-noted study on the clinical benefits with osimertinib in the FLAURA trial had shown strong inhibitory activity against T790M, which resulted in the clonal expansion of pre-existing T790M cells or the establishment of new T790M subpopulations.[61] Furthermore, future emergence of tumor cells commonly seen to resist treatment with the first-generation TKIs is expected to be controlled with the wider range of inhibitory effects generated from the second-generation TKIs against other HerbB family receptors or related mutants. Similarly, clonal expansion and acquired resistance to therapy are possibly being delayed as reflected in the observed improvements in PFS with afartinib and dacomitinib vs the first-generation TKIs to gefitinib in the Lux-lung 3, 6, and 7 studies, respectively.[62] This hypothesis could also explain recent observations of encouraging clinical benefit with sequential second- and third-generation EGFR-TKIs. The recent observational Gio-tag study assessed outcomes in 204 patients who received sequential osimertinib after first-line afartinib.[63] Overall median time on treatment was 27.6 months and was particularly promising in Asian patients with a duration of 46.7 months and patients with an exon 19 deletion mutation showing a duration of 30.3 months. In a post-hoc analysis of Lux-lung 3, 6, and 7, the median OS was not reached in 37 patients who received sequential afartinib and osimertinib.[62] The 3-year survival rate was around 90%.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article an be found at http://links.lww.com/JCMA/A138

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