The biology of Epidermal Growth Factor Receptor (EGFR) from regulating cell cycle to promoting carcinogenesis: the state of art including treatment options

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Abstract

The current definition of cancer is the creation of atypical cells capable to rapidly grow beyond the normal boundaries and spread to distant organs. To do so tumour cells have to acquire the ability to proliferate continuously and avoid apoptosis. An important role in this process is played by growth factors and their receptors. Amongst many, one of the most important interaction is between the Epidermal growth factor (EGF) and its receptor (EGFR) which are frequently mutated or upregulated in human cancers particularly in non-small cell carcinoma of the lung (NSCLC). The binding leads to protein activation, cell proliferation and decreased apoptosis. However, in this subset of tumours, the blockage of this interaction by EGFR-targeting drugs has shown an overall outcome improvement leading to the era of target therapy. The result is that patients are now routinely screened for a series of actionable mutations to be given the best possible therapy available for their specific type of tumour limiting the side effect of broad-spectrum chemotherapy. This paper will review the biology of EGFR receptor, the type and role of its mutation and the clinical implication for patients with NSCLC harbouring these mutations.

Review of the Literature

Structure and function of egfr

Receptor Tyrosine Kinases (RTKs) are cell surface receptors for many ligands such as polypeptide growth factors, cytokines, and hormones for which they have a high affinity [1]. So far 58 RTKs have been identified which are distributed in 20 families and amongst those, the Epidermal Growth Factor Receptor (EGFR) and its other family members (erbB2/ HER2, erbB3/HER3, erbB4/HER4) have been discovered to play an important role in signalling and carcinogenesis [1]. These receptors can initiate intracellular signalling regulating cell proliferation and survival [2]. The mechanisms through which this happens and can be targeted by specific drugs is related to the structure and biology of the receptor itself [3]. The EGFR gene is located on the short arm of chromosome 7 (7p11.2) and encodes a 170-kDa type I transmembrane growth factor receptor with tyrosine kinase activity. All these trans-membrane proteins are composed of an extracellular ligand-binding domain, a transmembrane lipophilic domain, and an intracellular tyrosine kinase domain and all bind to receptor-specific ligands except HER2 [4].

After binding with its specific ligand, the receptor undergoes dimerization (homo or heterodimerisation according to the type of the other receptor involved in the process). The subsequent phosphorylation of the key tyrosine residues within the COOH-
The Role of egfr mutations in cancerogenesis

All these effects are initiated by EGFR activation which can happen in three ways: ligand–dependent activation, ligand–independent activation and overexpression. Six EGFR ligands have been identified which causes the dimerization of the receptor and its autophosphorylation [2,16]. On the contrary, ligand–independent activation is caused by stress such as radiation and finally overexpression of the EGFR which is mediated with interaction with integrins [2].

EGFR has long been studied with important discoveries regarding its role in cell signalling and potential oncogenic role dated back in the 1980s [17,18].

Through the years and with a better understanding of those pathways [19], it has been recognised that EGFR plays an important role in tumorigenesis with at least three different mechanisms: overexpression of EGFR ligands, receptor amplification or activating mutation of EGFR [20]. The overall effect is the acquired ability of cells to proliferate continuously and avoid apoptosis which is one of the hallmarks of cancer described by Hanahan and Weinberg [21,22]. This involves bypassing a series of check–point that control the main phases of the replication cycle such as cell size control, DNA damage responses or monitoring DNA replication [23]. Besides playing an important role in initiating tumorigenesis in many solid tumours, genetic alteration of growth factor receptors has also clinical relevance in particularly in a subtype of carcinoma of the lung were is considered a predictive marker [24].

Types, frequency and effect of egfr mutations

In their comprehensive metanalysis, Pao, et al. [20], have summarised all type of mutations affecting EGFR tyrosine kinase domain known to date.

According to their review, 85.9% of the mutations reported happening in two “hot spots” in the EGFR gene between exon 19 and exon 21.

Exon 19 encodes highly conserved amino acids (LREA) which in the majority of those mutations (55.8%) are eliminated by multi–nucleotide in–frame deletions. This occurs in the activation loop, adjacent to a highly conserved DFG motif [25]. The remaining 44.2% of mutation are point mutations on exon 21 causing substitution between leucine and arginine at position 858 (L858R). The rest of the mutations amounting to 14.1% includes non–synonymous mutations between exons 18–21, a multi–nucleotide in–frame deletion in exon 19 downstream LREA and ultimately in–frame duplications/insertions in exon 20 [20]. It has been postulated that all these mutations lead to conformational change (for example affecting the alfa C-helix or phosphate–binding loop) resulting in increased activity of the receptor and some cases to sensitivity to TK inhibitors [26–28]. In their study Shan et al. have gone into the depth of how mutations affect the receptor structure and function [29]. They started from the observation that the extracellular domain has a very low tendency for dimerisation in the absence of interaction with its ligand [30]. Then they described the conformational changes of the receptor, adding new data to those previously reported [31–33].

Amongst those, Zhang et al. described how asymmetric dimers are created upon stabilisation of one EGFR kinase (the receiver) by another EGFR kinase (the activator) through the placement of the alpha–C helix in a way that the catalytic KE salt bridge between Lys721 and Glu738 is maintained (alphaC–inactive) [33]. However, EGFR can adopt also alphaC–out active conformation where the above–mentioned bridge is broken and the receptor is inactive [31,32]. Shan et al. have shown a third conformation, in which EGFR kinase exhibits a partial unfolding at the alphaC helix or local intrinsic disorder [29]. Intrinsic disorder has been shown to have an important role for the function of proteins [34] and the experiment conducted by Shan et al. not only enforced this concept but showed a possible mechanism through which EGFR mutations can act as a cancer promoter. The authors focused on how L858R mutation may cause aberrant activity by suppressing the local intrinsic disorder, increasing the dimerization affinity of the receptor and lowering the threshold for its activation [29]. This is
supported by the higher dimerisation affinity that the mutant receptor as compared to the wild-type counterpart. Eventually, they also discussed the possibility of lateral signal propagation involving the cytoplasmic portion of the receptor which happens during the dimerisation through the phosphorylation of a tyrosine residue (tyr845) in the activation loop which suppresses the intrinsic disorder [29].

Targeting egfr mutations: the advent of tyrosine kinase Inhibitors

The investigation of the structure of EGFR during the early 1990s, initially led to the identification of a new molecule: gefitinib, an anilinoquinazoline with antineoplastic activity targeting the EGFR-TK activity and other similar receptors [35]. Gefitinib inhibits the catalytic activity of other tyrosine kinases, which may result in inhibition of tyrosine kinase-dependent tumour growth. It competes with the binding of ATP to the tyrosine kinase domain of EGFR and inhibits receptor autophosphorylation. The final result is inhibition of signal transduction, cell cycle arrest and inhibition of angiogenesis [35,36]. The growth inhibition was particularly seen in different solid tumours including lung, prostate, breast, colon and ovarian cancer in preclinical studies of cell lines and human tumour xenografts [37]. The results seen in the treatment of NSCLC led to two clinical trials (IDEAL 1 and 2) and in 2003 the drug received US Food and Drug Administration approval in patients with advanced NSCLC as monotherapy treatment after failure of standard chemotherapy regimen [20]. At this time the role of a second molecule, a quinazoline derivative (erlotinib), was also investigated. Similarly, to the previous inhibitor described, erlotinib is an orally ATP–competitive inhibitor capable to block EGFR phosphorylation and delay tumour growth. It has also been shown to block the cell cycle in G1, to reduce RB phosphorylation and to induce apoptosis [20]. It gained FDA approval a year later (2004) for the treatment of patients with disease progression at least after one cycle of chemotherapy [38].

All EGFR TKIs competitively block the binding of ATP to the catalytic site in the tyrosine kinase domain of EGFR, subsequently inhibiting autophosphorylation. The binding is reversible for gefitinib and erlotinib whilst afatinib, a second-generation TKI has an irreversible binding [39].

Overcoming egfr tki resistance

Despite promising results, the response to these inhibitors was different amongst the patients therefore additional studies were conducted focusing on how to improve patient selection for these treatments [20]. A milestone, of course, was the discovery of somatic driver mutations in EGFR which led to a drastic shift in the clinical management of NSCLC from standard chemotherapy to precision medicine [38]. Additional studies and clinical trials (most relevant was IPASS study) were carried out and the most important finding was that presence of EGFR mutation is the best rationale to select patients to treat with TKI rather than clinic-pathological parameters [40]. Nevertheless, a higher frequency of those mutations was reported in Asiatic female, never smoker and with adenocarcinoma as histological type [40]. Even though most NSCLC patients harbouring TKI-sensitizing EGFR mutations show an initial pronounced response to EGFR–TKI treatment, they acquire resistance to these drugs after ~9 to 14 months of such therapy [38]. Many studies have been conducted to investigate the underlying mechanisms of acquired resistance to EGFR–TKIs: Amongst these are mutation in exon 20 of EGFR, MET amplification, overexpression of hepatocyte growth factor (HGF), and activation of the Insulin–like Growth Factor 1 Receptor (IGF1R) [41].

The T790M mutation of EGFR is the most common mechanism of such an acquired resistance, having been detected in up to 50% of patients treated with either the first-generation EGFR–TKIs (erlotinib or gefitinib) or in patients receiving first-line treatment with the second–generation EGFR–TKI (afatinib) [42]. This point mutation which involves a threonine–to–methionine substitution in exon 20, increases the affinity of EGFR for ATP and consequently reduces the binding efficacy of EGFR–TKIs: the clinical implication is poor clinical outcomes in patients treated with EGFR–TKIs [43]. In 2009 Zhou et al. studied three closely related pyrimidines (WZ3146, WZ4002, and WZ8040) to specifically target the T790M mutation and spare the WT–EGFR [44]. Despite one of the three compounds being very effective (WZ4002), it was never developed into a commercial drug (38). Osimertinib was recently approved for patients with T790M mutation resistance to a first– or second–generation EGFR TKI and is the only commercially available third–generation EGFR TKI. It has been approved by the FDA in 2017 for the treatment of metastatic EGFR T790M mutation–positive NSCLC, as detected by the FDA–approved test, and disease progression during or after EGFR TKI therapy [38]. In a randomized phase III trial (AURA 3), osimertinib was compared with standard chemotherapy (platinum–based doublet chemotherapy) in patients with T790M–positive lung cancer [45]. The results reported by Mok et al. showed better progression–free survival and less toxicity of osimertinib compared to chemotherapy. Therefore patients experiencing failure after a first–line EGFR TKI should be tested for the presence of a T790M mutation and considered for third–line TKI. Following these results, osimertinib was compared to a first–generation EGFR TKI as a first–line treatment for EGFR mutation–positive NSCLC in the FLAURA trial. This study demonstrated a significant improvement in Progression–Free Survival (PFS) with osimertinib [46] and it obtained from FDA an additional indication for the first–line treatment of patients with metastatic NSCLC positive for exon–19 deletions or the L858R point mutation of EGFR.

However, management of this subset of patients is still controversial and ongoing research is investigating also the role of immunotherapy in this population.

Egfr and beyond: Is there a rationale for combined therapies?

Many authors have been investigating the relationship between EGFR mutation and the presence of other molecular alterations or immunohistochemical expression of other proteins. The most important are Anaplastic Lymphoma...

Citation: Viola P (2020) The biology of Epidermal Growth Factor Receptor (EGFR) from regulating cell cycle to promoting carcinogenesis: the state of art including treatment options. Ann Cytol Pathol 5(1): 048-053. DOI: https://dx.doi.org/10.17352/acp.000015
Kinase (ALK) and ROS-1 which are often rearranged in NSCLC: in 2007 the first reports of the expression of oncogenic EML4-ALK and SLC34A2/CD74-ROS fusion proteins in lung cancer came out [47,48]. Although oncogenic ALK and ROS1 gene rearrangements in NSCLC are only found in respectively 4% and 2%, there are three drugs (crizotinib, ceritinib, and alectinib) available for the treatment of ALK-rearranged lung tumours, and one drug (crizotinib) is for ROS1-rearranged tumours [49]. Crizotinib became the first ALK inhibitor to receive FDA approval for the treatment of patients with ALK-positive metastatic NSCLC in November 2013 [49]. Other relevant markers which have been investigated are BRAF, MET, RET, ERBB2 (HER2), and KRAS, ERCC1 but currently, no therapy is formally approved [50,51]. EGFR, KRAS, and ALK mutations in NSCLC are mutually exclusive and the presence of one or another can influence response to targeted therapy [52,53]. More recently the focus has been shifted towards the relationship between EGFR and immunotherapy.

Immunotherapy has become another important treatment for NSCLC. In particular, complete activation of T-cells is regulated by direct recognition of antigen through major histocompatibility complex or can be mediated by the antigen-presenting cells and their costimulatory molecules. T-cells can also be negatively regulated as in the cytotoxic T-lymphocyte-associated antigen-4–B7 (CTLA4-B7) pathway and the programmed cell death protein–1 (PD-1)/programmed cell death protein–ligand 1 (PD-L1) pathway. PD-1 is an important immunological checkpoint that can inhibit the activation of T cells and the production of cytokines by binding to its two ligands, PD-L1 and PD-L2. Tumour cells can escape the immune system by upregulating PD-L1 expression and binding to PD-1 on the surface of tumour-specific CD8+ T cells to limit the host's immune response, therefore blockade of the PD-1/PD-L1 signalling pathway using monoclonal antibodies has been investigated in many solid tumours with promising results [54,55]. To date, the FDA has approved multiple anti-PD-1/PD-L1 antibodies for first- and second-line treatment of patients with advanced NSCLC and clinical prognosis has significantly improved [56,57]. Pembrolizumab, the first one approved, is used in combination with carboplatin and pemetrexed for first-line combination therapy as well as for first-line treatment of metastatic non–squamous cell NSCLC [58]. However, immunotherapy is only effective for a small number of patients and despite initial response, some of them show a subsequent rapid disease progression. Therefore, clinical trials and further studies on biomarkers are ongoing to understand how to better identify patients more likely to respond. But the very big question is whether combined immunotherapy and treatment with TKI can be an option.

Liang and colleagues in their review have given an excellent insight on preclinical studies and clinical trials related to this topic. It has been demonstrated that activation of the EGFR pathway can upregulate the expression of PD-1, PD-L1, and CTLA-4 through p–ERK1/2p–c–Jun, leading to the apoptosis of T cells in the tumour-associated microenvironment (TEM). This brings a state of immunosuppression and the escape of malignant cells from the host immune response [59]. EGFR TKIs besides killing tumour cells enhances the immune response and inhibit the expression of NF–KB reducing the expression of PD-1/PD-L1 [59]. Hence, these preclinical studies that the authors have summarised suggest the use of EGFR-TKIs in combination with immunotherapy to improve clinical outcomes in patients with EGFR positive NSCLC, accompanied by upregulation of PD–L1 expression. However, when translated in a clinical trial the results are not as good and so sharply demarcated. Some early clinical studies confirmed the efficacy of immunotherapy in patients with EGFR-mutant NSCLC; however, toxicity is the main limiting factor (ILD incidence and alanine aminotransferase/aspartate aminotransferase elevated levels resulted in treatment failure). On the contrary results from large randomized Phase III clinical trials have not demonstrated clinical benefit from this combined treatment. The reasons are difficult to understand given the association with smoking history, lymphocytic infiltrate and tumour mutation burden amongst other variables [59].

**Conclusion**

EGFR and its ligand have a pivotal role in controlling cell proliferation and somatic mutations of the receptor can lead to neoplastic transformation. However, in a subset of lung cancers, the presence of these mutations can be targeted by specific drugs. In clinical practice patients are now routinely tested for predictive biomarkers making precision medicine a reality. Despite many clinical trials showing better efficacy and fewer side effects in patients treated with TKIs compared to standard chemotherapy, many questions are still unanswered. For example, how to better tailor the treatment of WT-EGFR lung cancer or those with T790M germline mutation. Research is still ongoing and immunotherapy seems to be a promising option, however, their role and its association with TKIs in EGFR positive NSCLC has still to be addressed.

**References**


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