EGFR in Cancer

Subjects: Oncology
Contributor: Mary Luz Uribe

EGFR is one of the most successful pharmacological targets of anti-cancer drugs . Both monoclonal antibodies (mAbs) and TKIs demonstrated efficacy and acceptable toxicity in large phase III clinical trials , hence were approved for treatment of lung, colorectal and head/neck cancer (see a list of anti-EGFR and anti-HER2 approved drugs in Table 1). However, despite major therapeutic advances, both primary and acquired resistance to these drugs occur and result in disease recurrence.

Keywords: anti-cancer drug ; drug resistance ; growth factor ; signal transduction ; signaling pathway ; transcription network ; tyrosine kinase

1. Introduction

Although numerous growth factors have been isolated and we are still debating the full spectrum of their physiological and pathological functions, there has been no question about the origin of growth factor research. Because fewer embryonic nerve cells were observed when the limb buds had been removed, he concluded that nerve cells failed to grow because the limb was not producing an organizing factor on which they depended. Studying the nerve cells at more frequent intervals, she noted that these cells did proliferate initially, but then died because the limb bud was not producing the putative growth factors needed for their survival. Although subsequent studies found that EGFR and some ligand growth factors were overexpressed in human tumors and the EGFR gene is amplified or rearranged in brain tumors, frequent oncogenic point mutants of EGFR have been first identified in 2004, in human non-small cell lung cancer (NSCLC) from patients who were sensitive to EGFR-specific tyrosine kinase inhibitors (TKIs; e.g., gefitinib and erlotinib) [1][2][3].

This review will focus on EGFR (also called ERBB1 and HER1) and its seven growth factor ligands (EGF; transforming growth factor alpha, TGFa; heparin-binding EGF, HB-EGF; betacellulin; amphiregulin; epiregulin and epigen). Notably, the EGFR family of receptor tyrosine kinases (RTKs) includes a catalytically defective member, HER3/ERBB3. This receptor acts as an amplifier of growth factor signals, in the context of a layered signaling network [4]. The reader is referred to similar reviews covering additional aspects of EGFR and the three other type I RTKs [5][6].

2. Physiological and Mutational Activation of EGFR

Activation of the receptor depends on the formation of an asymmetric dimer of kinase domains, in which one kinase domain allosterically activates the other [7]. Together, these signaling effectors and adaptor proteins link activated receptors directly or indirectly to canonical intracellular pathways, as well as to the endocytic machinery, which desensitizes active receptors. In addition, receptor connectivity is achieved by phosphorylation of cytoplasmic EGFR residues by intracellular kinases, such as SRC, which phosphorylates tyrosine 845, a residue that serves as an extra docking site. Altogether, this configuration permits the ligand-activated EGFR to simultaneously stimulate multiple intracellular signaling routes and gain robustness.

Oncogenic mutant forms of EGFR mimic the ligand-activated wild type form. Nevertheless, although the mutated EGFRs of tumors are enzymatically active and transforming, their tyrosine phosphorylation status is significantly lower compared to ligand-activated wild type receptors [8]. Apparently, this relatively low but persistent activity generates intracellular signals that differ from the canonical biochemical machinery. In addition, EGFR mutants frequently evade negative regulation (desensitization), such as receptor endocytosis and degradation [9], thus allowing the mutated receptors to act “under the radar” of receptor attenuating mechanisms.

Likewise, several relatively rare point mutations in the EGFR’s extracellular and intracellular domains have been documented. EGFRvIII (also called EGFRΔIII) represents the most frequent genetic aberration in brain tumors. Importantly, this and other EGFR mutations occur on background of EGFR amplification. Furthermore, cells ectopically

expressing EGFRvIII displayed reduced adhesion due to decreased focal adhesion size and number, as well as displayed enhanced migration [10].

A fraction of NSCLC patients presents activating mutations in the EGFR gene [1][2][3][11][12]. In similarity to brain tumors, lung tumors displaying EGFR mutations frequently associate with EGFR gene amplification [13]. In addition, the prevalence of the mutations varies in different human populations: approximately 10–12% of Caucasian patients with lung cancer versus up to 40% among East Asians. In fact, the discovery of these mutations accompanied efforts to understand why Asians, more than Caucasians, were highly sensitive to EGFR tyrosine kinase inhibitors (TKIs).

3. Roles for Non-Coding RNAs in EGFR Signaling

Epigenetic deregulation of gene expression is involved in the initiation and progression of multiple cancers. ncRNAs (lncRNAs) and circular RNAs (circRNAs). Importantly, the surprising discovery that up to 90% of the human genome is subjected to pervasive transcription, although only less than 2% of the total genome encodes protein-coding genes, has placed ncRNAs in the limelight of the signal transduction and many other fields. As a result, we now understand that analyses of the approximately 7000 small RNAs, around 16,000 lncRNAs, and a slightly smaller number of pseudogenes [14] significantly changes our view of EGFR signaling.

For example, it has been reported that the levels of NEAT1, a lncRNA, were regulated by EGFR pathway activity, and this was critical for glioma cell growth and invasion [15]. Through binding to EZH2 and controlling the trimethylation of H3K27 in specific promoters, the EGFR/NEAT1/EZH2 axis contributes to glial cell tumorigenesis. Interestingly, LIMT downregulation required an active ERK pathway, and low expression of LIMT correlated with poor prognosis of patients with breast cancer. Such downregulation allows mammary cells to cross the extracellular matrix in vitro and enhance tumor metastasis in vivo.

In similarity to lncRNAs, microRNAs-mediated regulation has been shown to be involved in a wide range of biological processes, such as cell-cycle control, apoptosis, and several developmental and physiological mechanisms. Reciprocally, these two miRNAs associated with long overall survival time of patients with glioma. Likewise, another signaling axis, EGFR/miR-338-3p/EYA2, has been linked by a recent study to tumor growth and lung metastasis [16]. Remarkably, through the miR-338-3p/EYA2 pathway, EGFR increased breast cancer cell growth, EMT, migration, invasion, and metastasis in an allograft tumor mouse model [16].

Circular RNAs (circRNAs) are widespread circles of non-coding RNAs, which sequester microRNAs and RNA binding proteins, and, in some cases, contain short open reading frames [17]. However, although stimulation of epithelial cells with EGF leads to dynamic changes in the abundance of coding and non-coding RNA molecules, circRNAs display no similarly dynamic alterations [18]. Interestingly, it has recently been reported that a secretory E-cadherin protein variant (C-E-Cad), encoded by a circular E-cadherin RNA (circ-E-Cad), can directly activate EGFR [19]. In summary, recent reports are revealing intricate relations between EGFR signaling and several types of ncRNAs, both short (microRNAs) and long RNA molecules, such as lncRNAs and circRNAs.

4. Patient Resistance to Anti-Cancer Drugs Targeting EGFR

EGFR is one of the most successful pharmacological targets of anti-cancer drugs [20]. Both monoclonal antibodies (mAbs) and TKIs demonstrated efficacy and acceptable toxicity in large phase III clinical trials [5][21][22][23], hence were approved for treatment of lung, colorectal and head/neck cancer (see a list of anti-EGFR and anti-HER2 approved drugs in Table 1). Notably, while drug resistance arises from evolutionary pressures that select specific clones, resistance to TKIs often associates with appearance of new on-target mutations, but this mechanism rarely confers resistance to mAbs [24]. In addition, although the sequence of events preceding establishment of resistant clones is poorly understood, one commonality, which is shared by antibiotic-treated bacteria [25], entails an epigenetic transitory state, called drug tolerant persister

Both pre-existing and newly appearing on-target mutations drive the majority of resistance to first-generation EGFR TKIs [26]. Other mechanisms of resistance to the first-generation TKIs include amplification of MET [27] or HER2 [28], overexpression of AXL [29] or the hepatocyte growth factor (HGF) [30][31]. In addition, emergence of mutant forms of RAS [32] and BRAF [33], as well as phenotypic alterations [34], bestow resistance to the first-generation drugs. Recent data showed that the most common mechanisms of resistance to osimertinib in first-line settings are MET amplification, C797X mutations, which prevent covalent binding of the drug, amplification of wild-type EGFR or HER2 and mutations in downstream signaling proteins [35].

The clinical approval of mAbs targeting EGFR for treatment of patients with metastatic colorectal cancer (mCRC) has represented a major step forward, primarily due to high efficacy in terms of progression-free survival and overall patient survival, along with improved quality of life [36][37]. Accordingly, the commonest mechanism of primary resistance of CRC to anti-EGFR antibodies involves genomic alterations affecting downstream effectors, such as KRAS, NRAS, and PIK3CA mutations. For instance, KRAS mutations in exon 2 (codons 12 and 13) were identified by several retrospective analyses as determinants of primary resistance to the antibodies [38][39]. Hence, patients with mutant forms of KRAS or NRAS are ineligible for treatment, since RAS mutations activate downstream pathways and establish a bypass survival route.

Similarly, because EGFR is neutralized by anti-EGFR antibodies, the bypass route permitting resistance likely involves HER3 and its ligands, neuregulins. In analogy, several preclinical studies implicated the hepatocyte growth factor (HGF) and its receptor, MET, in resistance of CRC to anti-EGFR antibodies. However, only extreme amplification of the MET locus has been associated with lack of response, which suggests that resistance is driven by a dosage effect. These observations reinforce the roles played by tumor heterogeneity, pre-existing minor clones of cancer cells, and the adaptive mutability taking place while tumors are under treatment [40][41].

References

1. Lynch, T.J.; Bell, D.W.; Sordella, R.; Gurubhagavatula, S.; Okimoto, R.A.; Brannigan, B.W.; Harris, P.L.; Haserlat, S.M.; Supko, J.G.; Haluska, F.G.; et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N. Engl. J. Med. 2004, 350, 2129–2139.
2. Paez, J.G.; Janne, P.A.; Lee, J.C.; Tracy, S.; Greulich, H.; Gabriel, S.; Herman, P.; Kaye, F.J.; Lindeman, N.; Boggon, T.J.; et al. EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 2004, 304, 1497–1500.
3. Pao, W.; Miller, V.; Zakowski, M.; Doherty, J.; Politi, K.; Sarkaria, I.; Singh, B.; Heelan, R.; Rusch, V.; Fulton, L.; et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc. Natl. Acad. Sci. USA 2004, 101, 13306–13311.
4. Yarden, Y.; Sliwkowski, M.X. Untangling the ErbB signalling network. Nat. Rev. Mol. Cell. Biol. 2001, 2, 127–137.
5. Arteaga, C.L.; Engelman, J.A. ERBB receptors: From oncogene discovery to basic science to mechanism-based cancer therapeutics. Cancer Cell 2014, 25, 282–303.
6. Lemmon, M.A.; Schlessinger, J.; Ferguson, K.M. The EGFR family: Not so prototypical receptor tyrosine kinases. Cold Spring Harb. Perspect. Biol. 2014, 6, a020768.
7. Kovacs, E.; Zorn, J.A.; Huang, Y.; Barros, T.; Kuriyan, J. A Structural Perspective on the Regulation of the Epidermal Growth Factor Receptor. Annu. Rev. Biochem. 2015, 84, 739–764.
8. Shtiegman, K.; Kochupurakkal, B.S.; Zwang, Y.; Pines, G.; Starr, A.; Vexler, A.; Citri, A.; Katz, M.; Lavi, S.; Ben-Basat, Y.; et al. Defective ubiquitinylation of EGFR mutants of lung cancer confers prolonged signaling. Oncogene 2007, 26, 6968–6978.
9. Mosesson, Y.; Mills, G.B.; Yarden, Y. Derailed endocytosis: An emerging feature of cancer. Nat. Rev. Cancer 2008, 8, 835–850.
10. Banisadr, A.; Eick, M.; Beri, P.; Parisian, A.D.; Yeoman, B.; Placone, J.K.; Engler, A.J.; Furnari, F. EGFRvIII uses intrinsic and extrinsic mechanisms to reduce glioma adhesion and increase migration. J. Cell Sci. 2020, 133, jcs247189.
11. Wang, Z.; Longo, P.A.; Tarrant, M.K.; Kim, K.; Head, S.; Leahy, D.J.; Cole, P.A. Mechanistic insights into the activation of oncogenic forms of EGF receptor. Nat. Struct. Mol. Biol. 2011, 18, 1388–1393.
12. Zhang, Y.L.; Yuan, J.Q.; Wang, K.F.; Fu, X.H.; Han, X.R.; Threapleton, D.; Yang, Z.Y.; Mao, C.; Tang, J.L. The prevalence of EGFR mutation in patients with non-small cell lung cancer: A systematic review and meta-analysis. Oncotarget 2016, 7, 78985–78993.
13. Cappuzzo, F.; Hirsch, F.R.; Rossi, E.; Bartolini, S.; Ceresoli, G.L.; Bemis, L.; Haney, J.; Witta, S.; Danenberg, K.; Domenichini, I.; et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J. Natl. Cancer Inst. 2005, 97, 643–655.
14. Harrow, J.; Frankish, A.; Gonzalez, J.M.; Tapanari, E.; Diekhans, M.; Kokocinski, F.; Aken, B.L.; Barrell, D.; Zadissa, A.; Searle, S.; et al. GENCODE: The reference human genome annotation for The ENCODE Project. Genome Res. 2012, 22, 1760–1774.

15. Chen, Q.; Cai, J.; Wang, Q.; Wang, Y.; Liu, M.; Yang, J.; Zhou, J.; Kang, C.; Li, M.; Jiang, C. Long Noncoding RNA NEAT1, Regulated by the EGFR Pathway, Contributes to Glioblastoma Progression Through the WNT/beta-Catenin Pathway by Scaffolding EZH2. Clin. Cancer Res. 2018, 24, 684–695.
16. Liang, Y.; Xu, X.; Wang, T.; Li, Y.; You, W.; Fu, J.; Liu, Y.; Jin, S.; Ji, Q.; Zhao, W.; et al. The EGFR/miR-338-3p/EYA2 axis controls breast tumor growth and lung metastasis. Cell Death Dis. 2017, 8, e2928.
17. Verduci, L.; Strano, S.; Yarden, Y.; Blandino, G. The circRNA-microRNA code: Emerging implications for cancer diagnosis and treatment. Mol. Oncol. 2019, 13, 669–680.
18. Enuka, Y.; Lauriola, M.; Feldman, M.E.; Sas-Chen, A.; Ulitsky, I.; Yarden, Y. Circular RNAs are long-lived and display only minimal early alterations in response to a growth factor. Nucleic Acids Res. 2016, 44, 1370–1383.
19. Gao, X.; Xia, X.; Li, F.; Zhang, M.; Zhou, H.; Wu, X.; Zhong, J.; Zhao, Z.; Zhao, K.; Liu, D.; et al. Circular RNA-encoded oncogenic E-cadherin variant promotes glioblastoma tumorigenicity through activation of EGFR-STAT3 signalling. Nat. Cell Biol. 2021, 23, 278–291.
20. Yarden, Y.; Pines, G. The ERBB network: At last, cancer therapy meets systems biology. Nat. Rev. Cancer 2012, 12, 553–563.
21. Mitsudomi, T.; Morita, S.; Yatabe, Y.; Negoro, S.; Okamoto, I.; Tsurutani, J.; Seto, T.; Satouchi, M.; Tada, H.; Hirashima, T.; et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomised phase 3 trial. Lancet Oncol. 2010, 11, 121–128.
22. Rosell, R.; Carcereny, E.; Gervais, R.; Vergnenegre, A.; Massuti, B.; Felip, E.; Palmero, R.; Garcia-Gomez, R.; Pallares, C.; Sanchez, J.M.; et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2012, 13, 239–246.
23. Zhou, C.; Wu, Y.L.; Chen, G.; Feng, J.; Liu, X.Q.; Wang, C.; Zhang, S.; Wang, J.; Zhou, S.; Ren, S.; et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2011, 12, 735–742.
24. Konieczkowski, D.J.; Johannessen, C.M.; Garraway, L.A. A Convergence-Based Framework for Cancer Drug Resistance. Cancer Cell 2018, 33, 801–815.
25. Balaban, N.Q.; Merrin, J.; Chait, R.; Kowalik, L.; Leibler, S. Bacterial persistence as a phenotypic switch. Science 2004, 305, 1622–1625.
26. Hata, A.N.; Niederst, M.J.; Archibald, H.L.; Gomez-Caraballo, M.; Siddiqui, F.M.; Mulvey, H.E.; Maruvka, Y.E.; Ji, F.; Bhang, H.-e.C.; Krishnamurthy Radhakrishna, V.; et al. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. Nat. Med. 2016, 22, 262–269.
27. Engelman, J.A.; Zejnullahu, K.; Mitsudomi, T.; Song, Y.; Hyland, C.; Park, J.O.; Lindeman, N.; Gale, C.-M.; Zhao, X.; Christensen, J.; et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007, 316, 1039–1043.
28. Takezawa, K.; Pirazzoli, V.; Arcila, M.E.; Nebhan, C.A.; Song, X.; de Stanchina, E.; Ohashi, K.; Janjigian, Y.Y.; Spitzler, P.J.; Melnick, M.A.; et al. HER2 amplification: A potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack the second-site EGFRT790M mutation. Cancer Discov. 2012, 2, 922–933.
29. Zhang, Z.; Lee, J.C.; Lin, L.; Olivas, V.; Au, V.; LaFramboise, T.; Abdel-Rahman, M.; Wang, X.; Levine, A.D.; Rho, J.K.; et al. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. Nat. Genet. 2012, 44, 852–860.
30. Mueller, K.L.; Madden, J.M.; Zoratti, G.L.; Kuperwasser, C.; List, K.; Boerner, J.L. Fibroblast-secreted hepatocyte growth factor mediates epidermal growth factor receptor tyrosine kinase inhibitor resistance in triple-negative breast cancers through paracrine activation of Met. Breast Cancer Res. 2012, 14, R104.
31. Yano, S.; Wang, W.; Li, Q.; Matsumoto, K.; Sakurama, H.; Nakamura, T.; Ogino, H.; Kakiuchi, S.; Hanibuchi, M.; Nishioka, Y.; et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. Cancer Res. 2008, 68, 9479–9487.
32. Pao, W.; Wang, T.Y.; Riely, G.J.; Miller, V.A.; Pan, Q.; Ladanyi, M.; Zakowski, M.F.; Heelan, R.T.; Kris, M.G.; Varmus, H.E. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. PLoS Med. 2005, 2, e17.
33. Ohashi, K.; Sequist, L.V.; Arcila, M.E.; Moran, T.; Chmielecki, J.; Lin, Y.L.; Pan, Y.; Wang, L.; de Stanchina, E.; Shien, K.; et al. Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack

mutations in KRAS, NRAS, or MEK1. Proc. Natl. Acad. Sci. USA 2012, 109, E2127–E2133.

34. Jakobsen, K.R.; Demuth, C.; Sorensen, B.S.; Nielsen, A.L. The role of epithelial to mesenchymal transition in resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Transl. Lung Cancer Res. 2016, 5, 172–182.
35. Ramalingam, S.S.; Yang, J.C.; Lee, C.K.; Kurata, T.; Kim, D.W.; John, T.; Nogami, N.; Ohe, Y.; Mann, H.; Rukazenkov, Y.; et al. Osimertinib As First-Line Treatment of EGFR Mutation-Positive Advanced Non-Small-Cell Lung Cancer. J. Clin. Oncol. 2018, 36, 841–849.
36. Sforza, V.; Martinelli, E.; Ciardiello, F.; Gambardella, V.; Napolitano, S.; Martini, G.; Della Corte, C.; Cardone, C.; Ferrara, M.L.; Reginelli, A.; et al. Mechanisms of resistance to anti-epidermal growth factor receptor inhibitors in metastatic colorectal cancer. World J. Gastroenterol. 2016, 22, 6345–6361.
37. Bardelli, A.; Siena, S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J. Clin. Oncol. 2010, 28, 1254–1261.
38. Lievre, A.; Bachet, J.B.; Le Corre, D.; Boige, V.; Landi, B.; Emile, J.F.; Cote, J.F.; Tomasic, G.; Penna, C.; Ducreux, M.; et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006, 66, 3992–3995.
39. Di Fiore, F.; Blanchard, F.; Charbonnier, F.; Le Pessot, F.; Lamy, A.; Galais, M.P.; Bastit, L.; Killian, A.; Sesboue, R.; Tuech, J.J.; et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. Br. J. Cancer 2007, 96, 1166–1169.
40. Russo, M.; Crisafulli, G.; Sogari, A.; Reilly, N.M.; Arena, S.; Lamba, S.; Bartolini, A.; Amodio, V.; Magri, A.; Novara, L.; et al. Adaptive mutability of colorectal cancers in response to targeted therapies. Science 2019, 366, 1473–1480.
41. Gerlinger, M.; McGranahan, N.; Dewhurst, S.M.; Burrell, R.A.; Tomlinson, I.; Swanton, C. Cancer: Evolution within a lifetime. Annu. Rev. Genet. 2014, 48, 215–236.
