


Review Article

Epidermal Growth Factor Receptor Gene in Non-Small-Cell Lung Cancer: The Importance of Promoter Polymorphism Investigation

Vladimir Jurišić ¹, Jasmina Obradovic,² Sonja Pavlović,³ and Nataša Djordjevic¹

¹Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovica 69, 34000 Kragujevac, Serbia

²Institute of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovica 12, 34000 Kragujevac, Serbia

³Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11010 Belgrade, Serbia

Correspondence should be addressed to Vladimir Jurišić; juriscvladimir@gmail.com

Received 23 February 2018; Revised 5 July 2018; Accepted 7 August 2018; Published 14 October 2018

Academic Editor: Fernando Schmitt

Copyright © 2018 Vladimir Jurišić et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Recently, epidermal growth factor receptor (EGFR) was a key molecule in investigation of lung cancer, and it was a target for a new therapeutic strategy, based on molecular analyses. In this review, we have summarized some issues considering the role of EGFR in lung cancer, its coding gene, and its promoter gene polymorphisms (SNPs) -216G/T and -191C/A in non-small-cell lung cancer (NSCLC). The position of the SNPs indicates their significant role in EGFR regulation. The accumulation of knowledge regarding SNPs lately suggests their significant and important role in the onset of carcinogenesis, the prediction of the onset of metastases, the response to therapy with TKI inhibitors, and the onset of toxic effects of the applied therapy. Based on this, we suggest further studies of the relationship of clinical significance to SNPs in patients with lung tumors.

1. Non-Small-Cell Lung Cancer

Over the years, many scientific reports referred to lung cancer as “the leading cause of death” worldwide [1–6]. Non-small-cell lung cancer (NSCLC) is the most common form of lung cancer and accounts for about 85% of all cases of cancer [7–10]. Classical chemotherapy has been a major option for this type of tumor for many years, but the mortality remained high. For this incurable disease, the hope seems to lie in preventive medicine, i.e., various education strategies about risk factors, introduction of new programs for early cancer screening and early diagnostics, and providing equal chances for proper treatment to all patients [6, 8].

Carcinogenesis is a multistep process that usually takes many years to develop, as there are several mechanisms that prevent it, including the immune system, antioxidative system, and DNA repair mechanisms [11, 12]. The recent development of new techniques and methods has increased the knowledge of molecular mechanisms during carcinogenesis [13–15]. These mechanisms, including increased gene amplification and protein expression, abnormal cell activation,

allelic disbalance, and epigenetic mechanisms [13–20], might be just the top of the iceberg for all undiscovered interactions and signaling networks that are present in cancer cells. Studies in animal transgenic mice have shown that during carcinogenesis, one of the important molecules is epidermal growth factor receptor (EGFR) [13, 20].

2. Epidermal Growth Factor Receptor

Epidermal growth factor receptor (EGFR), usually being overexpressed in many cancers, such as non-small-cell lung cancer and colorectal and breast cancers [21], has drawn scientists’ attention early. It is a transmembrane protein with the N-terminal extracellular-ligand binding domain, transmembrane lipophilic domain, and C-terminal intracellular tyrosine kinase (TK) domain. The binding of ligand to the extracellular domain leads to formation of homo- or heterodimers within the EGFR family and a subsequent activation of the TK domain. In normal cells, it is a trigger molecule for many important processes, including growth, development, and differentiation. In altered cells, it conducts many abnormal messages through a signaling network cascade,

leading to carcinogenesis [22]. Binding of the adaptor proteins such as Grb2 and Shc induces activation of three main signaling pathways Ras/MAPK, PI3K/Akt, and JAK/STAT, which in altered cells lead to uncontrolled proliferation, angiogenesis, inhibition of apoptosis, invasion, metastasis, and immortalization [13, 23, 24]. These key molecules of signaling cascades might also be affected by gene mutations, altering the process of carcinogenesis [13, 20, 25, 26].

In nontransformed cells, EGFR activation triggers inhibitory mechanisms including dephosphorylation and inactivation with inducible feedback inhibitors, acting as tumor suppressors [27]. There are three main mechanisms that lead to EGFR activation in malignant cells: increased EGFR expression, increased ligand production, and the presence of EGFR-activating mutations [21]. In NSCLC with overexpressed EGFR, the inhibition of the receptor signaling has been introduced as a targeted treatment, with tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, rendered optimal in carriers of *EGFR*-activating mutations [21, 28, 29].

3. *EGFR* Gene Regulation

EGFR is located at the short arm of the chromosome 7 (7p11.2), spans about 200 kb, contains 28 exons, and encodes a protein of 1210 amino acids [30]. Currently, the regulation of *EGFR* expression is not completely understood, and different factors have been proposed to have a role in the process. Namely, most of the eukaryotes have regulatory elements for binding transcription factors (so-called “TATA” and “CAAT” sequences), located about 30–80 bp upstream of the start transcription site [31, 32]. *EGFRs*' 5' region differs from the 5' region of the most of eukaryotes, as it has less regulatory elements and high GC content in the promoter region, providing multiple start sites for the initiation of RNA transcription [31, 32].

EGFR promoter activation requires transcription factor Sp1, for which multiple binding sites were discovered [31, 33–37]. *EGFR* transcription is upregulated by at least three enhancers that act cooperatively: two of them localized upstream, i.e., near the start transcription site, and the third one in introne [38–40]. In the context of *EGFR* regulation, different *cis* and *trans* elements are reviewed, including TP53 (so-called “guardian of the genome”), p63, epidermal growth factor (EGF) responsive DNA-binding protein 1 (ERDBP-1), early growth response factor 1 (Egr-1), *EGFR*-specific transcription factor (ETF) (ETR–*EGFR*), *cis*-acting EGF receptor transcriptional repressor, repressor regulatory element in the first introne of *EGFR*, transforming growth factor β (TGF- β), GC-binding factor (GCF), microsatellite CA sequence, AP1, and AP2 [33, 34, 41–50].

4. *EGFR* Gene Amplification and Overexpression in Tumors

Expression of *EGFR* is a complex process, and it differs in normal and cancerous cells. Although the genetic mechanism of *EGFR* protein overproduction is not completely elucidated, it represents a very common event in different

tumors [21] and is usually associated with a more progressive stage of disease, worse prognosis, and higher mortality [51, 52]. In the literature, there is a certain controversy concerning the correlation among *EGFR* gene amplification, *EGFR* overexpression, and the efficacy of the TKI treatment. Namely, while earlier investigations did not observe clear relationship between *EGFR* expression and clinical outcomes for the NSCLC patients treated with TKI [53, 54], succeeding studies reported significant association of both high *EGFR* gene copy number (due to gene amplification or chromosome polysomy) and high protein expression with better response to gefitinib or erlotinib [55–57].

Some studies showed no correlation between *EGFR* gene amplification and protein expression [58, 59], while others reported the association [60–62]. It was observed that the amplification of *EGFR*, as a result of gene rearrangement in chromosome 7, leads to formation of aberrant RNA [60]. Several studies showed that *EGFR* amplification, as well as *EGFR*-activating mutations, are associated with the increased iRNA expression and in turn with a better therapy outcome [55, 63, 64]. Described inconsistency in reports suggests that *EGFR* genetic variations might play a role in both NSCLC carcinogenesis and TKI therapy success.

5. *EGFR* Variations

The most common *EGFR* somatic mutations are positioned in the TK domain, i.e., within exons 18 to 24 [30, 65]. These mutations are clustered around the *EGFR* ATP-binding pocket, affecting ATP affinity and altering sensitivity to TKIs [65]. Most of them, including E746_A750del and L858R, are classified as activating or “gain-of-function” mutations and could be found in NSCLC patients that respond well to gefitinib or erlotinib [66]. Others, such as T790M, usually emerge later during the treatment, causing secondary resistance to TKI therapy [67]. Currently, both are considered pharmacogenetic biomarkers in oncology, which could help in predicting the outcome of the treatment [68–70]. Yet, even with the *EGFR* somatic mutation data, a part of the observed interindividual difference in clinical response to gefitinib and erlotinib remains unexplained.

There are numerous germline single nucleotide polymorphisms (SNPs) found within *EGFR* [71], some already associated with increased risk of certain tumors [72–74] or with altered response to drug therapy [15, 19, 75–78]. Among the best studied *EGFR* SNPs are -216G/T and -191C/A, whose location within the *EGFR* promoter region indicates their potential role in *EGFR* regulation. Namely, -216G/T (rs712829) is placed within the transcription factor Sp1 binding site of the *EGFR* promoter, and -191C/A (rs712830) 4bp upstream from one of the start transcription binding site [32–34, 40, 75] (Figure 1). A low level of linkage disequilibrium (LD) that was observed between -216G/T and other important *EGFR* SNPs suggests its independent role in gene regulation, with G to T substitution resulting in significant increase of both promoter activity and mRNA expression [40, 79]. On the other hand, tight LD with other variations and lower effect on *EGFR* activity have been described for -191C/A [40, 70].

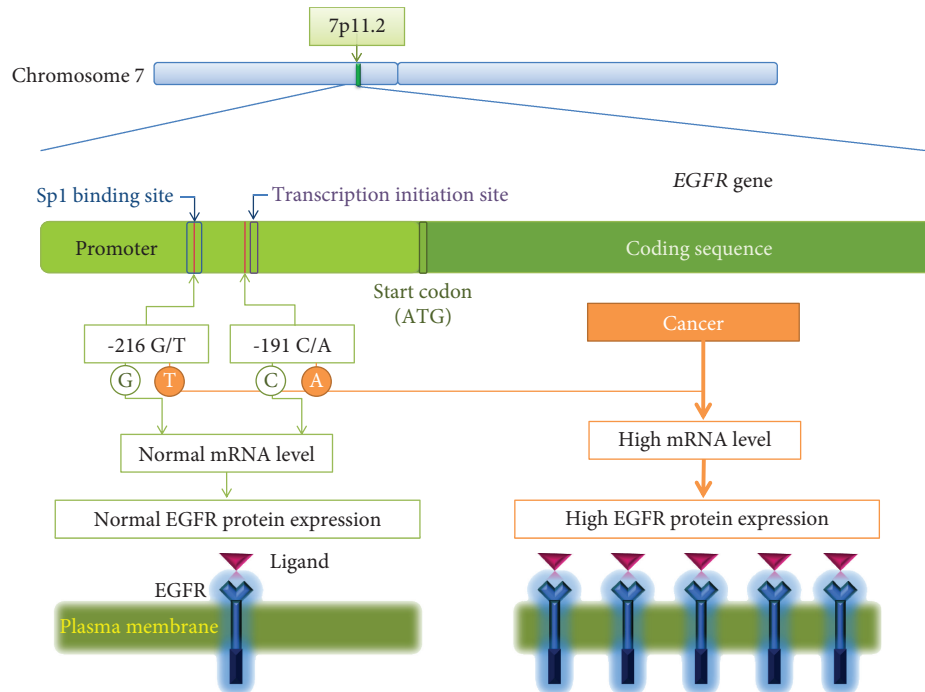


FIGURE 1: *EGFR* gene location on chromosome 7 and functional characteristics of two SNPs -191C/A and -216G/T placed in the *EGFR* promoter region.

6. Ethnicity and Variants of EGFR

Ever since the significance of EGFR variations for the clinical response to therapy of lung cancer has been recognized [63, 64, 80], they have been the subject of intense research around the world. Based on the obtained results, modern classification and diagnostics of the lung cancer are nowadays performed based on molecular analysis [81].

It has been observed that *EGFR* variants occur more frequently in Asia, unlike *KRAS* mutations, which are more common in Caucasians [82–84]. This suggests that there are significant interethnic differences in the molecular basis of carcinogenesis of the lung cancer. In addition, it was shown that the frequency of EGFR mutations is also higher in women, nonsmokers, and patients with adenocarcinoma, as compared to other types of lung cancer [85]. In our study from 2016, for the first time in our knowledge, the white people in the Balkans have described the frequency -216G/T and -191C/A, we found that the distribution of these SNPs coincides with their distribution in the whites from other areas [85]. Another investigation, which was carried out in a Caucasian population from the Balkan country, also showed the correlation of EGFR polymorphisms with the histological type of cancer, with the variant alleles being the most frequent in adenocarcinoma [86].

On the other hand, the interethnic differences in incidence, mortality, prognosis, and survival of NSCLC are already known [87–89]. In most cases, these differences can be associated with a different frequency of *EGFR* variations [40, 88–94]. Although many polymorphisms and mutations of *EGFR* have been described, the two polymorphisms of the promoter region, namely, -216G/T and -191C/A, were

shown to be especially important [40], as they convey ethnicity-dependent genetic susceptibility for lung cancer [40, 90–94]. The frequencies of EGFR variations in different ethnic populations are summarized in Table 1.

Based on the previous reports, there are interethnic differences in frequency distribution of *EGFR* promoter SNPs. Namely, in Caucasians and Afro-Americans, -216G/T is much more frequent than in Asians [40, 94] while -191C/A was detected almost only in Caucasians [40] and with extremely low frequency in East Asians [94–99].

Furthermore, these polymorphisms have been associated with the localization of tumor metastases. Namely, as the process of cell proliferation and differentiation is strictly related to EGFR, tumor metastasis should be affected by variations in *EGFR*. In line with the expectations, significant differences in genotype and allele frequencies of the -216G/T polymorphism between the patient group with the pleural metastasis in comparison with the nonmetastasis group have been observed [100]. Based on these findings, the authors have concluded that the polymorphism in exon 13 of the *EGFR* gene might be one of the molecular mechanisms of pleural metastasis of lung cancer.

Having in mind the importance of these polymorphisms for the NSCLC therapy outcome, both ethnic background and the cancer stage should be considered in making a decision on a proper treatment approach.

7. TKI and EGFR Variants

Tyrosine kinase inhibitors (TKIs) specifically bind to the intracellular tyrosine kinase (TK) domain of the EGFR receptor and thereby prevent the transmission of a signal directed

TABLE 1: EGFR -191C/A and -216G/T minor allele frequencies in lung cancer patients of different ethnicities.

SNP	Minor allele frequency			Publication
	Caucasians	Asians	African Americans	
rs712830 (-191C/A)	0.136 (6/44)	0.000 (0/46)	0.000 (0/48)	Liu et al. [40]
	0.114 (37/324)	NA	NA	Cusatis et al. [95]
	NA	0.000 (0/54)	NA	Choi et al. [90]
	0.071 (13/184*)	NA	NA	Liu et al. [15]
	0.099 (19/192)	NA	NA	Giovannetti et al. [17]
	0.128 (85/662)	NA	NA	Winther Larsen et al. [78]
	0.226 (19/84)	NA	NA	Obradović et al. [85]
rs712829 (-216G/T)	NA	0.035 (9/260)	NA	Bashir et al. [96]
	0.318 (14/44)	0.071 (3/46)	0.292 (14/48)	Liu et al. [40]
	0.444 (144/324)	NA	NA	Cusatis et al. [95]
	NA	0.040 (2/54)	NA	Choi et al. [90]
	0.400 (73/184*)	NA	NA	Liu et al. [15]
	0.440 (144/328)	NA	NA	Gregorc et al. [109]
	0.401 (77/192)	NA	NA	Giovannetti et al. [17]
	NA	0.020 (23/1128)	NA	Dong et al. [97]
	NA	0.050 (14/282)	NA	Liu et al. [98]
	NA	0.056 (8/142)	NA	Jung et al. [19]
	NA	0.283 (361/1276)	NA	Guo et al. [99]
	0.326 (216/662)	NA	NA	Winther Larsen et al. [78]
	NA	0.130 (60/460)	NA	Zhang et al. [110]
0.310 (26/84)	NA	NA	Obradović et al. [85]	
NA	0.287 (491/856)	NA	Guo et al. [100]	
NA	0.596 (155/260)	NA	Bashir et al. [96]	

NA: not available; * population mainly Caucasian.

to the development of malignancy. In the NSCLC treatment, first-generation TKIs include gefitinib and erlotinib, the second generation TKI involves afatinib and dacomitinib, and the third generation involves recently approved osimertinib [101–106].

There are many reasons for obtaining resistance to drugs used in targeted therapy. In regard to TKIs, one of the possibilities includes EGFR wild-type allele amplification, highlighting the importance of EGFR genotype for the treatment efficacy [107].

Regarding safety, according to earlier *in vitro* data, neither -216G/T nor -191C/A seems to be associated with cytotoxicity of different TKIs, including erlotinib [79]. These findings have been supported by few *in vivo* reports, where correlation between any of the two EGFR promoter polymorphisms and the occurrence of skin rash or diarrhea with gefitinib treatment was not detected [108, 109]. However, numerous other studies involving advanced NSCLC patients on gefitinib therapy demonstrated the opposite. Namely, a higher response rate and prolonged progression-free and overall survivals but also significantly higher risk of treatment-related rash and diarrhea were observed in carriers of at least one -216T allele [15, 110]. In similar studies on the role of -216G/T and -191C/A polymorphisms in patients with advanced NSCLC treated with gefitinib or erlotinib, variant haplotypes were associated with the clinical benefit, time to progression, and the overall survival [19, 109]

but also with the gastrointestinal and skin drug toxicities [17, 111]. The similar association found between -216G/T variant allele and the successful clinical response to anti-EGFR monoclonal antibodies such as cetuximab or panitumumab further supports the proposed role of EGFR promoter polymorphism in therapy targeting NSCLC patients [112].

8. Conclusion

EGFR is usually overexpressed in many epithelial cancers; thus, the inhibition of its signaling pathway has been introduced as a potential very successful treatment of NSCLC. Yet, there are pronounced interindividual differences in response to TKIs, with EGFR being among the most important determinants. Although EGFR gene amplification, gene mutation, and chromosome polysomy have all been associated with TKI therapy success, there is a part of the observed interindividual difference in clinical response to therapy that remained unexplained. EGFR SNPs -216G/T and -191C/A discussed here are located in the promoter region of the gene, which indicates their potential role in EGFR regulation. The data from *in vivo* studies involving NSCLC patients demonstrate that both these SNPs but especially -216G/T affect efficacy and safety of the TKI treatment, suggesting their importance in making a decision on a proper therapy approach.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

The study was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, 175056.

References

- [1] H. Witschi, "A short history of lung cancer," *Toxicological Sciences*, vol. 64, no. 1, pp. 4–6, 2001.
- [2] D. S. Ettinger, W. Akerley, H. Borghaei et al., "Non-small cell lung cancer," *Journal of the National Comprehensive Cancer Network*, vol. 10, no. 10, pp. 1236–1271, 2012.
- [3] L. Esposito, D. Conti, R. Ailavajhala, N. Khalil, and A. Giordano, "Lung cancer: are we up to the challenge?," *Current Genomics*, vol. 11, no. 7, pp. 513–518, 2010.
- [4] L. Nichols, R. Saunders, and F. D. Knollmann, "Causes of death of patients with lung cancer," *Archives of Pathology & Laboratory Medicine*, vol. 136, no. 12, pp. 1552–1557, 2012.
- [5] X. Mu, Y. Zhang, X. Qu et al., "Ubiquitin ligase Cbl-b is involved in Icotinib (BPI-2009H)-induced apoptosis and G1 phase arrest of EGFR mutation-positive non-small-cell lung cancer," *BioMed Research International*, vol. 2013, Article ID 726375, 8 pages, 2013.
- [6] C. Printz, "Lung cancer new leading cause of death for women in developed countries: data reflects increased rates of smoking," *Cancer*, vol. 121, no. 12, pp. 1911–1912, 2015.
- [7] J. Ferlay, I. Soerjomataram, R. Dikshit et al., "Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012," *International Journal of Cancer*, vol. 136, no. 5, pp. E359–E386, 2015.
- [8] M. Malvezzi, P. Bertuccio, T. Rosso et al., "European cancer mortality predictions for the year 2015: does lung cancer have the highest death rate in EU women?," *Annals of Oncology*, vol. 26, no. 4, pp. 779–786, 2015.
- [9] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2015," *CA: A Cancer Journal for Clinicians*, vol. 65, no. 1, pp. 5–29, 2015.
- [10] A. Spira and D. S. Ettinger, "Multidisciplinary management of lung cancer," *The New England Journal of Medicine*, vol. 350, no. 4, pp. 379–392, 2004.
- [11] G. Konjevic, V. Jurisic, V. Jovic et al., "Investigation of NK cell function and their modulation in different malignancies," *Immunologic Research*, vol. 52, no. 1–2, pp. 139–156, 2012.
- [12] Y. A. Mebratu and Y. Tesfaigzi, "Does the BCL-2 family member BIK control lung carcinogenesis?," *Molecular & Cellular Oncology*, vol. 5, no. 4, article e1435182, 2018.
- [13] Y. Yarden, "The EGFR family and its ligands in human cancer. Signalling mechanisms and therapeutic opportunities," *European Journal of Cancer*, vol. 37, Supplement 4, pp. 3–8, 2001.
- [14] R. Jaenisch and A. Bird, "Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals," *Nature Genetics*, vol. 33, no. 3s, pp. 245–254, 2003.
- [15] G. Liu, S. Gurubhagavatula, W. Zhou et al., "Epidermal growth factor receptor polymorphisms and clinical outcomes in non-small-cell lung cancer patients treated with gefitinib," *The Pharmacogenomics Journal*, vol. 8, no. 2, pp. 129–138, 2008.
- [16] E. Brambilla and A. Gazdar, "Pathogenesis of lung cancer signalling pathways: roadmap for therapies," *The European Respiratory Journal*, vol. 33, no. 6, pp. 1485–1497, 2009.
- [17] E. Giovannetti, P. A. Zucali, G. J. Peters et al., "Association of polymorphisms in *AKT1* and *EGFR* with clinical outcome and toxicity in non-small cell lung cancer patients treated with gefitinib," *Molecular Cancer Therapeutics*, vol. 9, no. 3, pp. 581–593, 2010.
- [18] O. Rho, D. J. Kim, K. Kiguchi, and J. DiGiovanni, "Growth factor signaling pathways as targets for prevention of epithelial carcinogenesis," *Molecular Carcinogenesis*, vol. 50, no. 4, pp. 264–279, 2011.
- [19] M. Jung, B. C. Cho, C. H. Lee et al., "EGFR polymorphism as a predictor of clinical outcome in advanced lung cancer patients treated with EGFR-TKI," *Yonsei Medical Journal*, vol. 53, no. 6, pp. 1128–1135, 2012.
- [20] L. Kim, G. Liu, and M. Tsao, "Predictive tumor biomarkers for EGFR inhibitors," in *Lung Cancer*, J. A. Roth, W. K. Hong, and R. U. Komaki, Eds., pp. 435–453, John Wiley & Sons, 2014.
- [21] B. A. Chan and B. G. Hughes, "Targeted therapy for non-small cell lung cancer: current standards and the promise of the future," *Translational Lung Cancer Research*, vol. 4, no. 1, pp. 36–54, 2015.
- [22] P. A. Jänne, J. A. Engelman, and B. E. Johnson, "Epidermal growth factor receptor mutations in non-small-cell lung cancer: implications for treatment and tumor biology," *Journal of Clinical Oncology*, vol. 23, no. 14, pp. 3227–3234, 2005.
- [23] R. N. Jorissen, F. Walker, N. Pouliot, T. P. Garrett, C. W. Ward, and A. W. Burgess, "Epidermal growth factor receptor: mechanisms of activation and signalling," *Experimental Cell Research*, vol. 284, no. 1, pp. 31–53, 2003.
- [24] N. Normanno, A. de Luca, C. Bianco et al., "Epidermal growth factor receptor (EGFR) signaling in cancer," *Gene*, vol. 366, no. 1, pp. 2–16, 2006.
- [25] S. V. Sharma, D. W. Bell, J. Settleman, and D. A. Haber, "Epidermal growth factor receptor mutations in lung cancer," *Nature Reviews. Cancer*, vol. 7, no. 3, pp. 169–181, 2007.
- [26] X. Cai, J. Sheng, C. Tang et al., "Frequent mutations in EGFR, KRAS and TP53 genes in human lung cancer tumors detected by ion torrent DNA sequencing," *PLoS One*, vol. 9, no. 4, article e95228, 2014.
- [27] O. Segatto, S. Anastasi, and S. Alema, "Regulation of epidermal growth factor receptor signalling by inducible feedback inhibitors," *Journal of Cell Science*, vol. 124, no. 11, pp. 1785–1793, 2011.
- [28] B. Brandt, S. Meyer-Staeckling, H. Schmidt, K. Agelopoulos, and H. Buerger, "Mechanisms of EGFR gene transcription modulation: relationship to cancer risk and therapy response," *Clinical Cancer Research*, vol. 12, no. 24, pp. 7252–7260, 2006.
- [29] J. Greenhalgh, K. Dwan, A. Boland et al., "First-line treatment of advanced epidermal growth factor receptor (EGFR) mutation positive non-squamous non-small cell lung cancer," *The*

- Cochrane Database of Systematic Reviews*, vol. 5, article CD010383, 2016.
- [30] U. Hodoglugil, M. W. Carrillo, J. M. Hebert et al., "PharmGKB summary: very important pharmacogenetic information for the epidermal growth factor receptor," *Pharmacogenetics and Genomics*, vol. 23, no. 11, pp. 636–642, 2013.
- [31] S. Ishii, Y. H. Xu, R. H. Stratton, B. A. Roe, G. T. Merlino, and I. Pastan, "Characterization and sequence of the promoter region of the human epidermal growth factor receptor gene," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 82, no. 15, pp. 4920–4924, 1985.
- [32] A. C. Johnson, S. Ishii, Y. Jinno, I. Pastan, and G. T. Merlino, "Epidermal growth factor receptor gene promoter. Deletion analysis and identification of nuclear protein binding sites," *The Journal of Biological Chemistry*, vol. 263, no. 12, pp. 5693–5699, 1988.
- [33] R. Kageyama, G. T. Merlino, and I. Pastan, "Epidermal growth factor (EGF) receptor gene transcription: requirement for Sp1 and an EGF receptor-specific factor," *The Journal of Biological Chemistry*, vol. 263, pp. 6329–6336, 1988.
- [34] R. Kageyama, G. T. Merlino, and I. Pastan, "A transcription factor active on the epidermal growth factor receptor gene," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 14, pp. 5016–5020, 1988.
- [35] J. Xu, K. L. Thompson, L. B. Shephard, L. G. Hudson, and G. N. Gill, "T3 receptor suppression of Sp1-dependent transcription from the epidermal growth factor receptor promoter via overlapping DNA-binding sites," *The Journal of Biological Chemistry*, vol. 268, no. 21, pp. 16065–16073, 1993.
- [36] E. Grinstein, F. Jundt, I. Weinert, P. Wernet, and H. D. Royer, "Sp1 as G1 cell cycle phase specific transcription factor in epithelial cells," *Oncogene*, vol. 21, no. 10, pp. 1485–1492, 2002.
- [37] J. Obradovic, V. Jurisic, N. Tomic et al., "Optimization of PCR conditions for amplification of GC-rich EGFR promoter sequence," *Journal of Clinical Laboratory Analysis*, vol. 27, no. 6, pp. 487–493, 2013.
- [38] T. Maekawa, F. Imamoto, G. T. Merlino, I. Pastan, and S. Ishii, "Cooperative function of two separate enhancers of the human epidermal growth factor receptor proto-oncogene," *The Journal of Biological Chemistry*, vol. 264, no. 10, pp. 5488–5494, 1989.
- [39] J. M. McInerney, M. A. Wilson, K. J. Strand, and S. A. Chrysogelos, "A strong intronic enhancer element of the EGFR gene is preferentially active in high EGFR expressing breast cancer cells," *Journal of Cellular Biochemistry*, vol. 80, no. 4, pp. 538–549, 2001.
- [40] W. Liu, F. Innocenti, M. H. Wu et al., "A functional common polymorphism in a Sp1 recognition site of the epidermal growth factor receptor gene promoter," *Cancer Research*, vol. 65, no. 1, pp. 46–53, 2005.
- [41] R. Kageyama, G. T. Merlino, and I. Pastan, "Nuclear factor ETF specifically stimulates transcription from promoters without a TATA box," *The Journal of Biological Chemistry*, vol. 264, no. 26, pp. 15508–15514, 1989.
- [42] L. L. Chen, M. L. Clawson, S. Bilgrami, and G. Carmichael, "A sequence-specific single-stranded DNA-binding protein that is responsive to epidermal growth factor recognizes an S1 nuclease-sensitive region in the epidermal growth factor receptor promoter," *Cell Growth & Differentiation*, vol. 4, no. 12, pp. 975–983, 1993.
- [43] X. Hou, A. C. Johnson, and M. R. Rosner, "Induction of epidermal growth factor receptor gene transcription by transforming growth factor beta 1: association with loss of protein binding to a negative regulatory element," *Cell Growth & Differentiation*, vol. 5, no. 8, pp. 801–809, 1994.
- [44] X. Hou, A. C. Johnson, and M. R. Rosner, "Identification of an epidermal growth factor receptor transcriptional repressor," *The Journal of Biological Chemistry*, vol. 269, no. 6, pp. 4307–4312, 1994.
- [45] A. L. Reed, H. Yamazaki, J. D. Kaufman, Y. Rubinstein, B. Murphy, and A. C. Johnson, "Molecular cloning and characterization of a transcription regulator with homology to GC-binding factor," *The Journal of Biological Chemistry*, vol. 273, no. 34, pp. 21594–21602, 1998.
- [46] F. Gebhardt, K. S. Zanker, and B. Brandt, "Modulation of epidermal growth factor receptor gene transcription by a polymorphic dinucleotide repeat in intron 1," *The Journal of Biological Chemistry*, vol. 274, no. 19, pp. 13176–13180, 1999.
- [47] H. Buerger, F. Gebhardt, H. Schmidt et al., "Length and loss of heterozygosity of an intron 1 polymorphic sequence of egfr is related to cytogenetic alterations and epithelial growth factor receptor expression," *Cancer Research*, vol. 60, no. 4, pp. 854–857, 2000.
- [48] H. Nishi, M. Senoo, K. H. Nishi et al., "p53 homologue p63 represses epidermal growth factor receptor expression," *The Journal of Biological Chemistry*, vol. 276, no. 45, pp. 41717–41724, 2001.
- [49] H. Nishi, K. H. Nishi, and A. C. Johnson, "Early growth response-1 gene mediates up-regulation of epidermal growth factor receptor expression during hypoxia," *Cancer Research*, vol. 62, no. 3, pp. 827–834, 2002.
- [50] M. A. Wilson and S. A. Chrysogelos, "Identification and characterization of a negative regulatory element within the epidermal growth factor receptor gene first intron in hormone-dependent breast cancer cells," *Journal of Cellular Biochemistry*, vol. 85, no. 3, pp. 601–614, 2002.
- [51] R. I. Nicholson, J. M. W. Gee, and M. E. Harper, "EGFR and cancer prognosis," *European Journal of Cancer*, vol. 37, pp. 9–15, 2001.
- [52] G. Selvaggi, S. Novello, V. Torri et al., "Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small-cell lung cancer," *Annals of Oncology*, vol. 15, no. 1, pp. 28–32, 2004.
- [53] M. G. Kris, R. B. Natale, R. S. Herbst et al., "Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial," *JAMA*, vol. 290, no. 16, pp. 2149–2158, 2003.
- [54] M. Fukuoka, S. Yano, G. Giaccone et al., "Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer," *Journal of Clinical Oncology*, vol. 21, no. 12, pp. 2237–2246, 2003.
- [55] F. Cappuzzo, F. R. Hirsch, E. Rossi et al., "Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer," *Journal of the National Cancer Institute*, vol. 97, no. 9, pp. 643–655, 2005.
- [56] M. S. Tsao, A. Sakurada, J. C. Cutz et al., "Erlotinib in lung cancer—molecular and clinical predictors of outcome," *The*

- New England Journal of Medicine*, vol. 353, no. 2, pp. 133–144, 2005.
- [57] I. J. Dahabreh, H. Linardou, P. Kosmidis, D. Bafaloukos, and S. Murray, “EGFR gene copy number as a predictive biomarker for patients receiving tyrosine kinase inhibitor treatment: a systematic review and meta-analysis in non-small-cell lung cancer,” *Annals of Oncology*, vol. 22, no. 3, pp. 545–552, 2011.
- [58] Y. H. Xu, N. Richert, S. Ito, G. T. Merlino, and I. Pastan, “Characterization of epidermal growth factor receptor gene expression in malignant and normal human cell lines,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 81, no. 23, pp. 7308–7312, 1984.
- [59] C. R. King, M. H. Kraus, L. T. Williams, G. T. Merlino, I. H. Pastan, and S. A. Aaronson, “Human tumor cell lines with EGF receptor gene amplification in the absence of aberrant sized mRNAs,” *Nucleic Acids Research*, vol. 13, no. 23, pp. 8477–8486, 1985.
- [60] G. T. Merlino, S. Ishii, J. Whang-Peng et al., “Structure and localization of genes encoding aberrant and normal epidermal growth factor receptor RNAs from A431 human carcinoma cells,” *Molecular and Cellular Biology*, vol. 5, no. 7, pp. 1722–1734, 1985.
- [61] G. T. Merlino, Y. H. Xu, N. Richert et al., “Elevated epidermal growth factor receptor gene copy number and expression in a squamous carcinoma cell line,” *The Journal of Clinical Investigation*, vol. 75, no. 3, pp. 1077–1079, 1985.
- [62] F. R. Hirsch, M. Varella-Garcia, P. A. Bunn Jr et al., “Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis,” *Journal of Clinical Oncology*, vol. 21, no. 20, pp. 3798–3807, 2003.
- [63] T. J. Lynch, D. W. Bell, R. Sordella et al., “Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib,” *The New England Journal of Medicine*, vol. 350, no. 21, pp. 2129–2139, 2004.
- [64] J. G. Paez, P. A. Jänne, J. C. Lee et al., “EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy,” *Science*, vol. 304, no. 5676, pp. 1497–1500, 2004.
- [65] S. A. Forbes, N. Bindal, S. Bamford et al., “COSMIC: mining complete cancer genomes in the catalogue of somatic mutations in cancer,” *Nucleic Acids Research*, vol. 39, Supplement 1, pp. D945–D950, 2011.
- [66] C. Zhou, Y. L. Wu, G. Chen 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,” *The Lancet Oncology*, vol. 12, no. 8, pp. 735–742, 2011.
- [67] W. Pao, V. A. Miller, K. A. Politi et al., “Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain,” *PLoS Medicine*, vol. 2, no. 3, article e73, 2005.
- [68] W. Brugger, N. Triller, M. Blasinska-Morawiec et al., “Prospective molecular marker analyses of EGFR and KRAS from a randomized, placebo-controlled study of erlotinib maintenance therapy in advanced non-small-cell lung cancer,” *Journal of Clinical Oncology*, vol. 29, no. 31, pp. 4113–4120, 2011.
- [69] M. Fukuoka, Y. L. Wu, S. Thongprasert et al., “Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS),” *Journal of Clinical Oncology*, vol. 29, no. 21, pp. 2866–2874, 2011.
- [70] US Food and Drug Administration, *Table of Pharmacogenomic Biomarkers in Drug Labeling*, U.S. Food and Drug Administration, Silver Spring (MD), 2018, <http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm>.
- [71] National Center for Biotechnology Information, *Database of Single Nucleotide Polymorphisms (dbSNP)*, National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda (MD), 2018, <http://www.ncbi.nlm.nih.gov/snp>.
- [72] Y. S. Jou, Y. L. Lo, C. F. Hsiao et al., “Association of an EGFR intron 1 SNP with never-smoking female lung adenocarcinoma patients,” *Lung Cancer*, vol. 64, no. 3, pp. 251–256, 2009.
- [73] M. Sanson, F. J. Hosking, S. Shete et al., “Chromosome 7p11.2 (EGFR) variation influences glioma risk,” *Human Molecular Genetics*, vol. 20, no. 14, pp. 2897–2904, 2011.
- [74] P. Rajaraman, B. S. Melin, Z. Wang et al., “Genome-wide association study of glioma and meta-analysis,” *Human Genetics*, vol. 131, no. 12, pp. 1877–1888, 2012.
- [75] C. M. Rudin, W. Liu, A. Desai et al., “Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity,” *Journal of Clinical Oncology*, vol. 26, no. 7, pp. 1119–1127, 2008.
- [76] F. Ma, T. Sun, Y. Shi et al., “Polymorphisms of EGFR predict clinical outcome in advanced non-small-cell lung cancer patients treated with gefitinib,” *Lung Cancer*, vol. 66, no. 1, pp. 114–119, 2009.
- [77] M. Tiseo, G. Rossi, M. Capelletti et al., “Predictors of gefitinib outcomes in advanced non-small cell lung cancer (NSCLC): study of a comprehensive panel of molecular markers,” *Lung Cancer*, vol. 67, no. 3, pp. 355–360, 2010.
- [78] A. Winther Larsen, P. H. Nissen, P. Meldgaard, B. Weber, and B. S. Sorensen, “EGFR CA repeat polymorphism predict clinical outcome in EGFR mutation positive NSCLC patients treated with erlotinib,” *Lung Cancer*, vol. 85, no. 3, pp. 435–441, 2014.
- [79] W. Liu, X. Wu, W. Zhang et al., “Relationship of EGFR mutations, expression, amplification, and polymorphisms to epidermal growth factor receptor inhibitors in the NCI60 cell lines,” *Clinical Cancer Research*, vol. 13, no. 22, pp. 6788–6795, 2007.
- [80] W. Pao, V. Miller, M. Zakowski 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,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 36, pp. 13306–13311, 2004.
- [81] W. D. Travis, E. Brambilla, M. Noguchi et al., “International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society international multidisciplinary classification of lung adenocarcinoma,” *Journal of Thoracic Oncology*, vol. 6, no. 2, pp. 244–285, 2011.
- [82] K. Suda, K. Tomizawa, and T. Mitsudomi, “Biological and clinical significance of KRAS mutations in lung cancer: an

- oncogenic driver that contrasts with *EGFR* mutation,” *Cancer and Metastasis Reviews*, vol. 29, no. 1, pp. 49–60, 2010.
- [83] H. Shigematsu, L. Lin, T. Takahashi et al., “Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers,” *Journal of the National Cancer Institute*, vol. 97, no. 5, pp. 339–346, 2005.
- [84] J. Soh, S. Toyooka, K. Matsuo et al., “Ethnicity affects *EGFR* and *KRAS* gene alterations of lung adenocarcinoma,” *Oncology Letters*, vol. 10, no. 3, pp. 1775–1782, 2015.
- [85] J. Obradović, N. Djordjević, N. Tošić et al., “Frequencies of *EGFR* single nucleotide polymorphisms in non-small cell lung cancer patients and healthy individuals in the republic of Serbia: a preliminary study,” *Tumor Biology*, vol. 37, no. 8, pp. 10479–10486, 2016.
- [86] N. Elez-Burnjakovic, N. Ugrin, J. Obradovic et al., “Distribution of *EGFR* SNPs -191C/A and 181946G/A in patients with lung cancer depending on smoking status in the Republic of Srpska, Bosnia and Herzegovina,” *Journal of BU ON*, vol. 23, no. 2, pp. 384–390, 2018.
- [87] B. Zaric, V. Stojsic, T. Kovacevic et al., “Clinical characteristics, tumor, node, metastasis status, and mutation rate in domain of epidermal growth factor receptor gene in Serbian patients with lung adenocarcinoma,” *Journal of Thoracic Oncology: Official Publication of the International Association for the Study of Lung Cancer*, vol. 9, no. 9, pp. 1406–1410, 2014.
- [88] M. B. Schabath, W. D. Cress, and T. Muñoz-Antonia, “Racial and ethnic differences in the epidemiology and genomics of lung cancer,” *Cancer Control*, vol. 23, no. 4, pp. 338–346, 2016.
- [89] W. Zhou and D. C. Christiani, “East meets West: ethnic differences in epidemiology and clinical behaviors of lung cancer between East Asians and Caucasians,” *Chinese Journal of Cancer*, vol. 30, no. 5, pp. 287–292, 2011.
- [90] J. E. Choi, S. Ha Park, K. M. Kim et al., “Polymorphisms in the epidermal growth factor receptor gene and the risk of primary lung cancer: a case-control study,” *BMC Cancer*, vol. 7, no. 1, p. 199, 2007.
- [91] K. Shiraishi, H. Kunitoh, Y. Daigo et al., “A genome-wide association study identifies two new susceptibility loci for lung adenocarcinoma in the Japanese population,” *Nature Genetics*, vol. 44, no. 8, pp. 900–903, 2012.
- [92] W. J. Seow, K. Matsuo, C. A. Hsiung et al., “Association between GWAS-identified lung adenocarcinoma susceptibility loci and *EGFR* mutations in never-smoking Asian women, and comparison with findings from Western populations,” *Human Molecular Genetics*, vol. 26, no. 2, pp. 454–465, 2016.
- [93] A. Midha, S. Dearden, and R. McCormack, “*EGFR* mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapII),” *American Journal of Cancer Research*, vol. 5, no. 9, pp. 2892–2911, 2015.
- [94] M. Nomura, H. Shigematsu, L. Li et al., “Polymorphisms, mutations, and amplification of the *EGFR* gene in non-small cell lung cancers,” *PLoS Medicine*, vol. 4, no. 4, article e125, 2007.
- [95] G. Cusatis, V. Gregorc, J. Li et al., “Pharmacogenetics of *ABCG2* and adverse reactions to gefitinib,” *Journal of the National Cancer Institute*, vol. 98, no. 23, pp. 1739–1742, 2006.
- [96] N. Bashir, E. Ragab, O. Khabour, B. Khassawneh, M. Alfaqih, and J. Momani, “The association between epidermal growth factor receptor (*EGFR*) gene polymorphisms and lung cancer risk,” *Biomolecules*, vol. 8, no. 3, 2018.
- [97] J. Dong, J. Dai, Y. Shu et al., “Polymorphisms in *EGFR* and *VEGF* contribute to non-small-cell lung cancer survival in a Chinese population,” *Carcinogenesis*, vol. 31, no. 6, pp. 1080–1086, 2010.
- [98] W. Liu, L. He, J. Ramirez et al., “Functional *EGFR* germline polymorphisms may confer risk for *EGFR* somatic mutations in non-small cell lung cancer, with a predominant effect on exon 19 microdeletions,” *Cancer Research*, vol. 71, no. 7, pp. 2423–2427, 2011.
- [99] H. Guo, Y. Xing, R. Liu et al., “-216G/T (rs712829), a functional variant of the *EGFR* promoter, is associated with the pleural metastasis of lung adenocarcinoma,” *Oncology Letters*, vol. 6, no. 3, pp. 693–698, 2013.
- [100] H. Guo, Y. Xing, A. Mu et al., “Correlations between *EGFR* gene polymorphisms and pleural metastasis of lung adenocarcinoma,” *OncoTargets and Therapy*, vol. 9, pp. 5257–5270, 2016.
- [101] C.-S. Tan, N. B. Kumarakulasinghe, Y.-Q. Huang et al., “Third generation *EGFR* TKIs: current data and future directions,” *Molecular Cancer*, vol. 17, no. 1, p. 29, 2018.
- [102] V. Hirsh, “Turning *EGFR* mutation-positive non-small-cell lung cancer into a chronic disease: optimal sequential therapy with *EGFR* tyrosine kinase inhibitors,” *Therapeutic Advances in Medical Oncology*, vol. 10, 2018.
- [103] I. Sullivan and D. Planchard, “Next-generation *EGFR* tyrosine kinase inhibitors for treating *EGFR*-mutant lung cancer beyond first line,” *Frontiers in Medicine*, vol. 3, 2017.
- [104] E. A. Barber and K. L. Reckamp, “Best initial treatment strategies for *EGFR*-mutant lung cancer,” *American Journal of Hematology/Oncology*, vol. 12, no. 12, pp. 4–7, 2016.
- [105] C. Bartholomew, L. Eastlake, P. Dunn, and D. Yiannakis, “*EGFR* targeted therapy in lung cancer; an evolving story,” *Respiratory Medicine Case Reports*, vol. 20, pp. 137–140, 2017.
- [106] T. Liu, X. Jin, Y. Wang, and K. Wang, “Role of epidermal growth factor receptor in lung cancer and targeted therapies,” *American Journal of Cancer Research*, vol. 7, no. 2, pp. 187–202, 2017.
- [107] S. Nukaga, H. Yasuda, K. Tsuchihara et al., “Amplification of *EGFR* wild-type alleles in non-small cell lung cancer cells confers acquired resistance to mutation-selective *EGFR* tyrosine kinase inhibitors,” *Cancer Research*, vol. 77, no. 8, pp. 2078–2089, 2017.
- [108] C. L. Huang, C. H. Yang, K. H. Yeh et al., “*EGFR* intron 1 dinucleotide repeat polymorphism is associated with the occurrence of skin rash with gefitinib treatment,” *Lung Cancer*, vol. 64, no. 3, pp. 346–351, 2009.
- [109] V. Gregorc, M. Hidalgo, A. Spreafico et al., “Germline polymorphisms in *EGFR* and survival in patients with lung cancer receiving gefitinib,” *Clinical Pharmacology and Therapeutics*, vol. 83, no. 3, pp. 477–484, 2008.
- [110] X. Zhang, J. Fan, Y. Li et al., “Polymorphisms in epidermal growth factor receptor (*EGFR*) and *AKT1* as possible predictors of clinical outcome in advanced non-small-cell lung cancer patients treated with *EGFR* tyrosine kinase inhibitors,” *Tumor Biology*, vol. 37, no. 1, pp. 1061–1069, 2016.

- [111] T. McKibbin, W. Zhao, M. Tagen et al., "Epidermal growth factor receptor polymorphisms and risk for toxicity in paediatric patients treated with gefitinib," *European Journal of Cancer*, vol. 46, no. 11, pp. 2045–2051, 2010.
- [112] A. Jaka, A. Gutiérrez-Rivera, N. Ormaechea et al., "Association between EGFR gene polymorphisms, skin rash and response to anti-EGFR therapy in metastatic colorectal cancer patients," *Experimental Dermatology*, vol. 23, no. 10, pp. 751–753, 2014.