Review

Anti-EGFR/BRAF-Tyrosine Kinase Inhibitors in Thyroid Carcinoma

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Abstract. Alterations in significant genes located on chromosome 7 - including epidermal growth factor receptor (EGFR) and also v-Raf murine sarcoma viral oncogene homolog B (BRAF) as a mitogen-activated protein kinase (MAPK) - combined or not with numerical imbalances of the whole chromosome (aneuploidy-polysony) are crucial genetic events involved in the development and progression of malignancies. Identification of EGFR/BRAF-dependent specific somatic mutations and other mechanisms of deregulation (i.e., amplification) is critical for applying targeted therapeutic approaches [tyrosine kinase inhibitors (TKIs) or monoclonal antibodies (mAbs). Thyroid carcinoma is a specific pathological entity characterized by a variety of histological sub-types. Follicular thyroid carcinoma (FTC), papillary thyroid carcinoma (PTC), medullary thyroid carcinoma (MTC), and anaplastic thyroid carcinoma (ATC) represent its main sub-types. In the current review, we explore the role of EGFR/BRAF alterations in thyroid carcinoma in conjunction with the corresponding anti-EGFR/BRAF TKI-based novel therapeutic strategies for patients with specific genetic signatures.

Epithelial cell neoplastic and malignant transformation is a multi-step process. It includes chromosome instability (CI) as gross chromosome numerical and structural alterations such as polysomy/aneuploidy, monosomy, and rearrangements (i.e., translocations) in specific or vast chromosome regions (1, 2). Concerning thyroid carcinoma, the corresponding follicular epithelia demonstrate chromosome gains and losses and also specific gene amplifications, mutations or allelic losses (3). Thyroid carcinoma includes a broad spectrum of different histological sub-types, such as papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), anaplastic thyroid cancer (ATC), and medullary thyroid carcinoma (4). Each pathological entity is characterized by specific cyto-morphological and genetic profiles. Epidermal growth factor
receptor (EGFR) and also v-Raf murine sarcoma viral oncogene homolog B (BRAF) genes are localized on chromosome 7 and their numerical or structural abnormalities lead to specific molecular signatures in the corresponding patients (5, 6). In the current review, we described EGFR-dependent signaling transduction pathways and their impact on thyroid carcinoma biological behavior in conjunction with modern oncological therapeutic approaches.

EGFR-mediated Pathways: Molecules and Mechanisms

Among genes that are localized on chromosome 7, EGFR and BRAF represent major genetic markers implicated in the development, progression, and prognosis of thyroid carcinoma (7). Additionally, another important gene is the ret proto-oncogene (RET) (8). These genes are involved in a variety of signaling transduction pathways altered in carcinogenic process. Interestingly, the BRAF protein-encoded by the corresponding gene (cytogenetic band: 7q34) is a target for a critical driver mutation (BRAF V600G/E). Based on this mutation, a series of inhibitors has been developed and applied in sub-groups of patients (trametinib, dabrafenib, vemurafenib) (9, 10). Concerning the EGFR (ERBB, ERBB1, HER1) gene, it is located on the short (p) arm of chromosome 7 (cytogenetic band: 7p12.1). The gene encodes the EGFR protein acting as a transmembrane glycoprotein receptor (11). Referring to its composition, a large extra cellular ligand-binding region, a single hydrophobic transmembrane bridge adjusting to an intracellular juxtamembrane (JM) region, a tyrosine kinase domain and a C terminal tail including multiple tyrosine residues are the main distinct domains. Due to similar domains with HER2 protein, formations of HER2-EGFR heterodimers have been already identified in cancer cell lines (12).

According to extensive molecular studies, EGFR is responsible for signal transduction to the nucleus in different intra-cellular pathways. The phosphatidyl inositol 3 kinase-v AKT murine thymoma viral oncogene homolog I/phosphatase and TENsin homolog deleted on chromosome ten-mammalian target of rapamycin) (PI3K-AKT/PTEN-mTOR) regulates cellular homeostasis and angiogenesis due to a cell survival/apoptosis balance mechanism (13). Furthermore, the rat sarcoma-mitogen-activated protein kinase (RAS/MAPK) pathway secures normal cell proliferation, tissue differentiation and cell migration processes in the epithelial tissue morphogenesis phase (14). MET proto-oncogene (MET) that encodes for a receptor tyrosine kinase activation is also implicated in these functions (15). Additionally, EGFR-mediated cross talk reactions at the level of JAK gene have been detected proving its involvement in another pathway, the interleukin 6-Janus kinase-signal transducer activator of transcription (IL6-JAK1/2-STAT3) (16).

EGFR Alterations in Thyroid Carcinoma

EGFR overexpression in thyroid carcinoma is a relatively frequent observation. Especially in PTCs, a study group detected a ~20% of all examined tissue microarray-based cases to demonstrate a strong, continuous membrane expression (17). Interestingly, they reported a significant co-over expression of the marker with estrogen (Era/b) and progesterone (PR) receptors in sub-sets of patients. However, statistical significance correlating expression levels with the recurrence rates of the malignancies was not assessed. Another study group performed a meta-analysis focusing on the potential relation between EGFR expression and clinicopathological features in PTCs (18). They detected that EGFR over-expression was strongly associated to TNM stage and extra-thyroid metastases in the examined studies, whereas no correlation was found regarding sex, tumor size, grade of differentiation and age. Concerning the combined EGF/EGFR complex expression in thyroid malignancies, a study group showed not only over expression of the two proteins, but most importantly that EGF over-activation triggers EGFR translocation into the nucleus (19). This biochemical reaction affects indirectly the normal cell cycle regulation leading potentially to increased cell proliferation. This is evidence of EGFR involvement in the corresponding intracellular signaling pathway affecting indirectly nuclear microenvironment. Because this event is detected predominantly in anaplastic carcinomas, it seems to promote dedifferentiation of the neoplasms (aggressive phenotype). Referring to anaplastic thyroid carcinoma, a broad spectrum of point-mutations in critical genes has been already identified (20). Besides EGFR, p53, TERT, BRAF, RAS, PIK3CA, PTEN, AKT1, Mtor, ALK, VEGFR, and CDKN2A demonstrate specific nucleotide substitutions. The encoded proteins are eligible for targeted therapeutic approaches. Similarly, another study co-analyzed the gene expression of EGF/EGFR and another protein, the serpin peptidase inhibitor clade E member 2 (SERPINE2) by implementing a reverse transcription quantitative PCR-based assay (21). SERPINE2 is considered a reliable marker for detecting tissue-to-tissue metastatic potential. They reported a positive feedback loop with the EGF/EGFR system that affects invasion, migration and also proliferation in PTC cells.

According to a systematic molecular analysis in a genetically specific population (Kashmiri), harboring EGF mutational landscape includes mainly T790M, L858R, and deletion in exon 19 (22). The study group reported differences between FTCs and PTCs regarding the rates of these point-mutations, but they also concluded that their accumulation is responsible for aggressive phenotypes (increased dedifferentiation). Furthermore, EGFR/BRAF/PIK3CA/mTOR/KRAS pathway is frequently altered in malignancies including thyroid carcinoma, according to an RNA sequencing
profile analysis (23). The study group proposed that over-expression of the markers -due to activating point mutations- leads to aberrant transcriptomic activity in these malignancies. The role of EGFR/RET oncogene activation in thyroid carcinoma is also a very promising field of investigation. A genetic study -based on PTC cell culture transfection protocol and application of real time (RT)-PCR- showed a direct over-activation of EGFR mediated by RET oncogene over expression (24). Besides the elevated proliferation of cancerous cells and the decreased apoptotic rates, RET/EGFR co-activation provided increased epithelial-mesenchymal transition (EMT) as a result of N-cadherin and vimentin over expression. Besides these mechanisms, there is evidence that strong oxidative intracellular stress—which is one of the main causes that induce DNA damage—motivates EGFR mediated signaling pathways such as mitogen-activated protein kinase (MAPK/ERK) and PI3K/Akt (25). A study group co-analyzed EGFR, respectively. Additionally, miR-326 seems to inhibit the base excision repair (BER) pathway initiated by 8-oxoG glycosylase1 (OGG1) and EGFR expression in PTCs. They detected a positive relation between H₂O₂ production and OGG1-BER/EGFR over expression pathways in TPC-1 culture cells. Additionally, novel micro-genetic markers such as micro-RNAs (miRs) and long non-coding RNAs (lncRNA) seem to affect EGFR activation in thyroid carcinoma. Two studies explored the role of specific miRs (miR-30b-5p and miR-7-5p, respectively) in PTC tissues (26, 27). They concluded that miR-30b-5p prevented an aggressive progress of PTCs by inhibiting the EGFR/MAPK pathway, whereas decreased expression of miR-7-5p led to over expression of EGFR, respectively. Additionally, miR-326 seems to inhibit the progression of PTC by suppressing EGFR/MAPK pathway (28). In contrast, miR-133a-3p, which is involved in the axis ZEB1-AS1/miR-133a-3p/LPAR3/EGFR, enhances PI3K/Akt/mTOR signaling transduction pathway activation (29). Concerning lncRNAs, a study group analyzed the role of LncRNA ABHD11-AS1 in a series of PTCs. They detected ABHD11-AS1 high expression levels that led to increased activation EPS15L1/EGFR pathway (30).

**Anti-EGFR/BRAF Tyrosine Kinase Inhibitor (TKI)-based Strategies in Thyroid Carcinoma**

Evolution in targeted therapies significantly affects oncological strategies in malignancies including thyroid carcinoma besides conventional ¹³¹I-based treatment (31). A broad spectrum of mAbs and TKIs that inhibit EGFR-mediated signaling transduction pathways are under evaluation (Table I). Concerning the TKIs, lenvatinib acts as an anti-VEGFR1/2/3 multiple kinase inhibitor, but its function seems to expand to phosphorylated EGFR in thyroid carcinoma. A study group reported increased EGFR phosphorylation in thyroid carcinoma cell lines (32). They also revealed a synergistic anti-EGFR activity mediated by simultaneous lenvatinib/latatinib drug administration. Interestingly, although lenvatinib seem to be a very promising EGFR inhibitor, its impact on the renal function of the corresponding patients is a matter of serious concern for oncologists, as it happens with similar targeted agents. A study group analyzed the data derived from advanced differentiated thyroid carcinoma patients in period of time over 6 months cured by the lenvatinib TKI (33). They reported moderate to high proteinuria levels in subsets of them due to VEGF/VEGFR pathway blocking. They suggested an optimal modification of the lenvatinib in patients with poor renal function. Labatinib, is also a dual anti-EGFR/HER2 TKI inhibitor that perfectly blocks oncogenic signal transduction to nucleus in patients with breast carcinoma by suppressing pyruvate kinase type M2 expression leading to decreased cell proliferation (34). There are relatively limited data regarding its efficacy in thyroid carcinomas. In one study based on a combination of labatinib/vemurafenib – a selective inhibitor of V600E mutated gene (36). Table I. TKIs for direct or indirect anti-EGFR/BRAF-dependent pathways blockade in thyroid carcinoma targeted strategies.

<table>
<thead>
<tr>
<th>TKI</th>
<th>Direct target gene/s</th>
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<tr>
<td>Erlotinib/Gefitinib</td>
<td>EGFR</td>
</tr>
<tr>
<td>Trametinib</td>
<td>MEK1/2</td>
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<tr>
<td>Dabrafenib</td>
<td>BRAF</td>
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<tr>
<td>Vemurafenib</td>
<td>BRAF</td>
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<tr>
<td>Lenvatinib</td>
<td>VEGFR1/2/3</td>
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<tr>
<td>Lapatinib</td>
<td>EGFR/HER2</td>
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<td>Pazopanib</td>
<td>VEGFR</td>
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<tr>
<td>Cabozantinib</td>
<td>VEGFR2/cMET</td>
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<tr>
<td>Sunitinib/Sorafenib</td>
<td>VEGFR/PGDFR-cKIT/BRAF</td>
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<tr>
<td>Selumetinib</td>
<td>NF-1</td>
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<tr>
<td>Anlotinib</td>
<td>PDGFRCKIT/EGFR/VEGFR</td>
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**Table I.** TKIs: Tyrosine kinase inhibitor. EGFR/BRAF in bold: direct target of TKIs.

In conjunction, another study revealed a positive effect in patients with differentiated thyroid carcinoma patients using a combination of labatinib/vemurafenib/pazopanib/cabozantinib/sunitinib as a salvage treatment when first-line sorafenib-based strategy fails (35). A significantly median progression-free survival period was assessed. Dabrafenib and erlotinib are also significant anti-EGFR TKIs targeting EGFR/RAF/MEK pathway in abroad spectrum of solid malignancies. A clinical study showed that combination of these two agents or using another agent (trametinib) inhibited phosphorylated EGFR even in severe anaplastic carcinoma cases characterized by resistance to dabrafenib-dependent BRAF-mutated gene (36).
Referring to dabrafenib resistance levels, another study focused on an unexplored novel RAC1 (P34R) mutation acquired in BRAF. They reported an excessive EGFR/MET protein over-expression due to gene amplification in PTC cases that led to dedifferentiation enhancing their aggressive genotype/phenotype by increasing their metastatic potential (37). In contrast to successive responses in TKI-based therapeutic approaches, there are sub-groups of patients that lose clinical benefits after discontinuation of lenvatinib-based treatment (38). Mechanisms that lead to absence of response to this agent after a period of time are under investigation. Additionally, increased resistance to selumetinib -another anti-EGFR/BRAF TKI- is a result of insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) over activation (39). Furthermore, a recently published study explored the role of anolitinib, a multi anti-VEGFR, FGFR and PDGFR TKI in anaplastic thyroid carcinoma. They observed that CXCL11-EGF-EGFR signalling interacts with endothelial cells under hypoxia conditions and anolitin blocks EGFR (40). Interestingly, the effect of those TKIs -especially sorafenib, lenvatinib and selumetinib -on technetium-99m uptake in thyroid cancerous cells is under investigation. A study group using cancer cell lines reported elevated rates of the drug in them (41). Finally, another category of inhibitors that target histone deacetylase (HDAC) modify transcription of a variety of genes. A recently published study explored the efficacy of HDAC inhibitors (HDACIs) on anaplastic thyroid carcinoma as a literature meta-analysis. They observed that HDACIs could be a promising mono- or combined with other agents targeted therapeutic approach in sub-groups of patients (42).

In conclusion, EGFR and BRAF over-expression is a frequent event in thyroid carcinoma caused by point mutations or amplification in the corresponding genes. Especially, BRAF mutation is a significant biomarker associated with a more aggressive phenotype in thyroid carcinoma. Elevated EGF/BRADF oncogenic activity triggers a cataract of intracellular reactions leading to RAF and MAPK signaling transduction pathways over-activation. Novel mono- or combined anti-EGFR/BRAF targeted therapies based on TKIs provide benefits (high response rates and expanded life span) in sub-groups of patients characterized by specific genetic signatures.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors’ Contributions

VP, EK, ET: design of the study, ET, AC: manuscript writing, NM, VR, DP, DS: academic advisors: AA, GP, SK, AM, SP, PP; collection and management of references’ data. All Authors read and approved the final manuscript.

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