# Review on EGFR-ERK1/2 signaling cascade: implications on cell proliferation in health and disease

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Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that is often increased in malignancies such as non–small cell lung cancer, metastatic colorectal cancer, head and neck cancer, pancreatic cancer, and breast cancer. EGFR activity may be enhanced by different ways. These include typical mutations and truncations in the extracellular domain, and in the kinase domain. Overactivation of downstream ERK1/2 signaling pathway occurs as a result of these EGFR abnormalities. Cancer cell proliferation is aided by the chronic start and advancement of the cell cycle, which is triggered once these pathways are activated. This article discusses the ligand-binding and dimerization molecular processes that control EGFR signal transmission and its relationship to the ERK1/2 signaling axis that forces cells toward the G1 phase of the cell cycle. Furthermore, it illustrates how EGFR signaling pathways promote cyclin D expression via ERK1/2 activation.

**Keywords:**
cell cycle, cell proliferation, epidermal growth factor receptor, extracellular signal-regulated kinase (ERK), signal transduction

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## Introduction

Epidermal growth factor receptor (EGFR) is the progenitor of the EGFR family, which also includes ErbB2/HER2/Neu, ErbB3/HER3, and ErbB4/HER4. The EGFR has been branded as a proto-oncogene due to its ability to stimulate cell growth while counteracting apoptosis [1]. Ongoing research on the intricate signaling cascade mediated by the receptor tyrosine kinase has led to the discovery of novel insights. However, a comprehensive understanding of the EGFR is still necessary to optimize the efficacy of EGFR-targeted therapeutics. This essay aims to provide a comprehensive overview of the current knowledge on the mitogen EGF and its impact on the ERK1/2 axis. Furthermore, the final effect on the cell cycle progression will be also explored in health and disease. Finally, the rationale of EGFR inhibitors will be mentioned in certain disorders.

*Stanley Cohen's* Nobel Prize-winning research pioneered EGFR. *Cohen* and *Rita Levi-Montalcini* studied the effects of the recently discovered nerve growth factor (NGF) on neonatal mice in 1959 [2]. Humans have four different members of the ErbB gene family, designated EGFR/ERBB1/HER1, NEU/ERBB2/HER2, ERBB3/HER3, and ERBB4/HER4. In this family, there are a remarkable 28 possible configurations due to the members' propensity to form homo- and heterodimers [3]. Despite the absence of a ligand-binding domain and the lack of a known direct ligand, ERBB2 seems to be the preferred binding partner to its family members, and its dimerization arm is constitutively exposed [4,5]. There is modest autophosphorylation activity in ERBB3 homodimers, although it is low since ERBB3 lacks a kinase domain [6]. It is still possible to phosphorylate ERBB3 and trigger strong downstream signaling [4]. By upregulation of ETF (EGFR-specific transcription factor) synthesis, EGF can regulate its own receptor's function. Other proteins, including E1A, Sp-1, and AP2, may also have an impact on the EGFR promoter's activity [7].

With the exception of hematopoietic cells, almost every cell type contains members of the ErbB family [8]. Members of EGFR family play an essential role in vertebrate embryo development [9]. ErbB gene deletions in mice result in death before or shortly after birth [10]. The particular phenotype is influenced not just by the mouse's genetic background, but also by which member of the ErbB family is deleted. Mice without the EGFR gene do not make it very far before they die, and this has been related to several problems with organ development

and stem cell renewal, including issues in the brain, skin, lungs, and intestines [11,12]. Mice lacking ERBB2 die from cardiac dysfunctions as well as issues with motor neurons and sensory ganglia [13]. Cardiovascular development defects and severe neuropathies were seen in ERBB3-deficient animals [14]. Like ERBB2 knockout mice, ERRB4 knockout animals die due to abnormal functions in the heart and have abnormal migration of hindbrain-derived cranial neural crest cells [15]. Development of the epidermis, hair follicles, hair cycle, and cornea in embryonic rats requires EGFR [16]. Throughout puberty, the mammary ducts can only develop properly if certain genes from the EGFR family are active. Mutations like T743G, which diminish tyrosine kinase activity, impair mammary gland development in female mice [17]. This is because the mice are unable to produce enough milk for their young. In the majority of the adult CNS, EGFR activity is still rather strong. While EGFR is widely expressed in developing mouse and rat astrocytes, it is lost in mature astrocytes [18]. Reactive astrogliosis is a reaction in which astrocytes re-upregulate EGFR in response to insults to the CNS such as ischemia, tumor growth, or neurodegenerative illnesses [19,20].

Mutations in the kinase domain of EGFR are very common in non-small cell lung cancers (NSCLCs), whereas ectodomain anomalies are more common in glioblastomas [1,21]. Mutations in the EGFR gene are seen in colorectal tumors much less often [22]. Instead, an increase in the copy number of the EGFR gene by three to five-fold has been documented in around half of colorectal malignancies [23]. Overexpression of the EGFR is also seen in 40-80% of NSCLC [24]. Constitutive activation of EGFR is promoted by most EGFR mutations and truncations because they enhance ligand-independent dimerization with ERBB family receptors [25]. Endocytosis is normally responsible for downregulating receptor activity [26]. Mutations in the transmembrane domain of EGFR are quite rare. The oncogenic alterations in EGFR ectodomain frequently result in the elimination of inhibitory regulatory domains that are responsible for dimerization. v-ERBB, which is the viral counterpart of EGFR, is typically observed to exist in dimeric form due to its absence of an ectodomain. This characteristic renders it the most widely recognized mutant of the EGFR ectodomain [27]. Furthermore, around 20% of glioblastomas have the EGFR variation EGFRvIII [21,26]. EGFRvIII exhibits a distinctive characteristic of being capable of signaling in the absence of a ligand, while simultaneously displaying low constitutive activity. Despite exhibiting low constitutive activity, cancer cells derive advantages from heightened signaling owing to the fact that these receptors evade downregulation via endocytosis [26]. L858R is the most prevalent EGFR point mutation and accounts for around 45% of all mutations in the tyrosine kinase domain [28,29]. The L858R mutation of the activation loop is considered a 'classical' activating mutation since it increases kinase activity by 50-fold [30]. Activating mutations in the kinase domain, such as multiple EGFR exon deletions, are often seen in NSCLC [31]. T790M is a mutation in the kinase domain that is known to raise EGFR phosphorylation levels and provide resistance to pharmacological EGFR tyrosine kinase inhibitors (TKIs) [32]. The mechanism behind T790M resistance remains elusive as its precise nature has not yet been ascertained. However, it is possible that the extended side chain of methionine could create a hindrance for the binding of TKI, as suggested by previous research [33]. The T790M insertion is among several identified in the EGFR exon that have been linked to resistance towards tyrosine kinase inhibitors, as reported in reference [31].

#### Mechanistic pathway

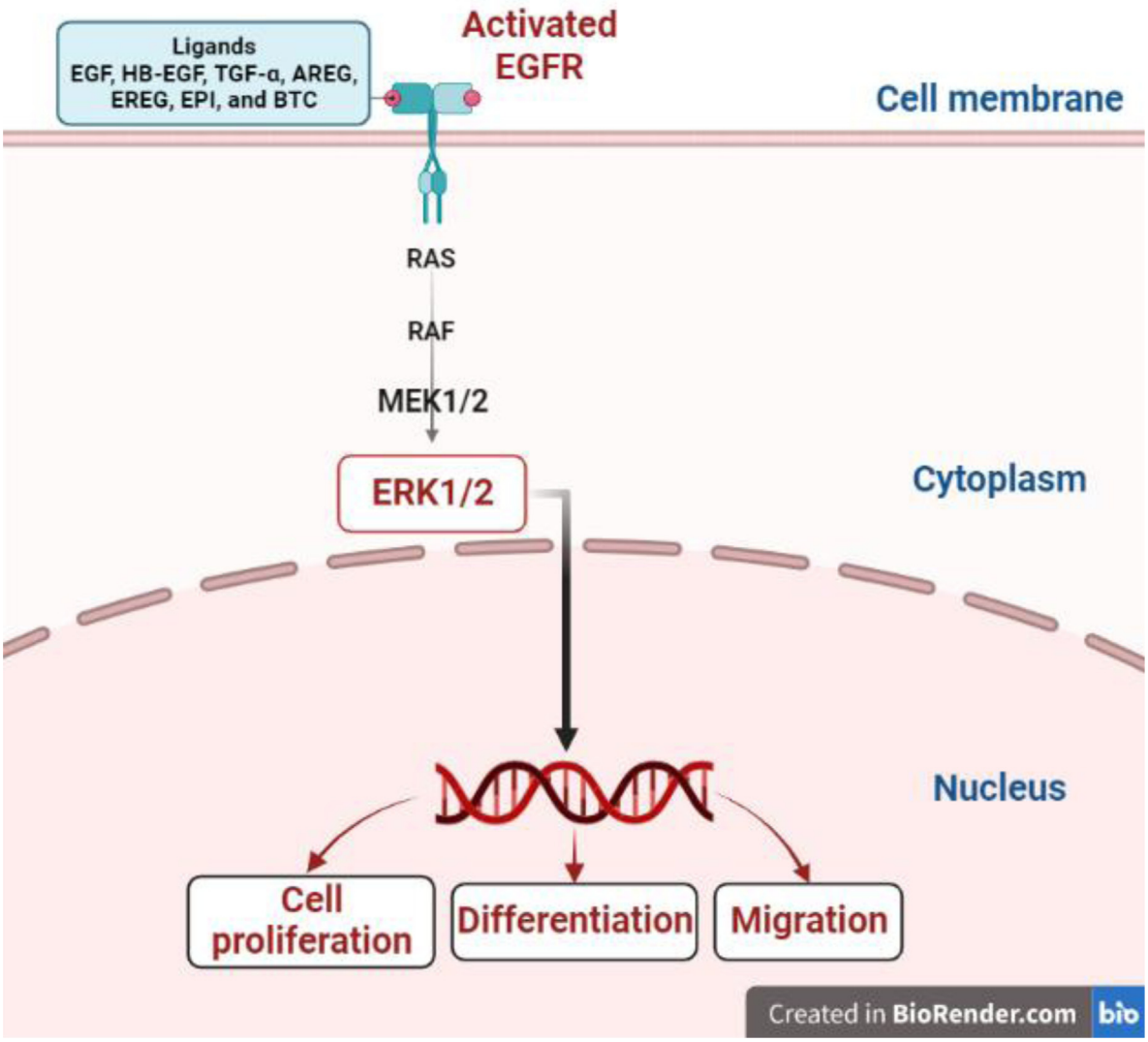
EGF initially increased epidermal proliferation and keratinization in animals [34]. EGF stimulates cell proliferation, differentiation, growth, and migration, and inhibits apoptosis. EGF stimulates the EGFR in HeLa cells to phosphorylate 2244 proteins [35]. Human mammary epithelial cells are also susceptible to changes in 3172 genes and 596 proteins when stimulated with EGF [36]. Several studies have shown that the ERBB signaling network should include 122 proteins and 211 interactions in total [37]. EGF's effects on the cell are significant even without post-translational changes. EGFR signal transduction is complicated. EGFR activation by a ligand causes receptor dimerization, C-terminal tail transphosphorylation, and signal propagation via complex signaling pathways to trigger gene expression. EGFR mutations and truncations cause ligand-independent signaling, which upregulates pro-oncogenic pathways such as persistent cell cycle proliferation.

Evidence on where the human body gets its EGF has been collected [38]. EGF has been hypothesized to have a role in a wide variety of morphogenetic processes, including those involving the teeth, the brain, the genitourinary system, the skin, the intestines, the heart, the blood vessels, the epithelium that lines the cornea [39]. Nevertheless,

owing to the availability of additional EGFR ligands, no diseases caused by EGF deficiency have been discovered [40]. As shown in Fig. 1, other EGFR ligands to EGF include six more. Among them are transforming growth factor alpha (TGF-α), amphiregulin (AREG), epiregulin (EREG), betacellulin (BTC), heparin-binding EGF-like growth factor (HB-EGF), and epigene (EPI). For the EGFR, the only known ligands are EGF, TGF-α, and amphiregulin [41]. TGF-α has 35% to 40% sequence identity with EGF [8]. BTC, HB-EGF, and EREG have all been shown to have dual selectivity for EGFR and ERBB4 [42].

The process of EGFR activation through a particular ligand appears to entail a sequence of occurrences, comprising ligand attachment, receptor dimerization, receptor trans-autophosphorylation, and the enlistment of signaling adaptors, as illustrated in Fig. 1. In addition, it has been observed that all ligands of the epidermal growth factor receptor (EGFR) induce the internalization of EGFR, which is subsequently conveyed to early endosomes [43]. Notwithstanding these common characteristics, research has demonstrated that the stiumlation of the EGFR leads to a range of distinct biological effects at the intended location [40]. It is uncertain how exactly each ligand produces its unique biological effects, however, there are several hypotheses. One possible explanation is that the various members of the EGFR family each form different dimer pairs in response to certain ligands [44]. An alternative explanation considers how the receptor's ligand interacts with it to determine its final destination. In general, receptors bound with EGF are destined for lysosomal degradation or recycling, receptors bound with HB-EGF and BTC are destined for lysosomal degradation, receptors bound with TGF-α and EPI are

**Figure 1**




Schematic diagram illustrates the mechanistic pathway of EGFR-ERK1/2.

destined for recycling, and receptors bound with AREG are destined for fast and slow EGFR recycling [43]. The pH sensitivity of each ligand-receptor pair may be at play in the aforementioned differential sorting. Upon reducing the pH to 6, it was observed that TGF-α dissociated from the EGFR, thereby facilitating the recycling of the receptor to the plasma membrane. However, the EGF-EGFR complex remained stable and consequently underwent trafficking to breakdown components, as reported in reference [45]. Studies have demonstrated that the endosomal sorting of the EGFR can be influenced by recombinant EGF mutants possessing varying affinities for the EGFR. Further research has shown that long-lasting EGFR signaling can be achieved by the recycling of TGF-α-bound EGFR back to the plasma membrane [46]. As the ligand influences where the EGFR is localized inside the cell, it follows that different ligands may generate unique signaling patterns, allowing fine-tuning of the biological response. Moreover, it has been postulated that the conformations of dimerized receptors are influenced by the ligands, resulting in varying substrate accessibility to the C-terminal tail [47]. Researchers have unable to pin down the precise mechanism of differential signaling. The vast majority of growth factors function in a paracrine manner, meaning that a growth factor secreted by one cell affects another cell in close proximity. Yet, there are known instances of autocrine systems in action. Initially elucidated comprehensively by Sporn and Roberts, this perilous circumstance confers autonomy upon the cell with respect to its own cellular division [48]. Autocrine signaling is used by several viruses to activate the cell's DNA replication mechanism and so replicate their own genome. Mouse fibroblasts that have been transformed by a sarcoma virus, for example, encode EGF-like molecules to promote their own growth. These molecules interact to the EGFR [49].

EGFR activation is not complete without ligand-binding and dimerization. It was debated, however, as to whether event came first. It is now generally known that EGF-binding to each EGFR monomer is required for EGFR activation before EGFR dimerization can occur. The maintenance and durability of the EGFR dimer is based on the interplay between the intracellular juxtamembrane region and the kinase domain [50].

When EGF binds to the EGFR, a number of tyrosine residues in the receptor's intracellular C-terminal tail are phosphorylated. When EGF is introduced into cells, a number of tyrosine residues are phosphorylated. These tyrosine residues are located at positions 703, 920, 992, 1045, 1068, 1086, 1148, and 1173. It is noteworthy that downstream residues of auto-phosphorylated sites may undergo phosphorylation by other kinases that appear to be post-situated to EGFR activation. For instance, c-SRC phosphorylates at Y845, whereas PKC phosphorylates at Thr654 [51]. No matter the case, the newly phosphorylated tyrosine residues act as docking sites for proteins containing phosphor-tyrosine-binding residues, such as those with Src Homology 2 (SH2) and phosphor-tyrosine binding (PTB) domains [20,52].

#### EGFR-ERK1/2 signaling cascade

The SH2 domain of GRB2 forms a direct bond with Y1068 and Y1086, which are phosphorylated during EGFR transphosphorylation [53]. Activated Y1148 and Y1173 residues may additionally attract SHC (Src homology and collagen), predominantly via its PTB domains but also through its SH2 domain [54]. These two adaptors connect ligand-activated EGFR to complicated intracellular biochemical processes. SHC is phosphorylated at Y317 by the EGFR, creating a GRB2 binding site [55]. SRC phosphorylates SHC at Y239/240 for GRB2 association [56]. SOS1 (son of sevenless 1) binds to two flanking SH3 domains of GRB2 [57,58]. When SOS is present, RAS is stimulated and is prompted to convert GDP to GTP. After that, Ras may potentially attach to the RAF-1/Ras-GTPase interaction domain (RBD) [59]. MEK1/2 interacts to phosphorylated Ser338 and Tyr341 residues in RAF-1 [60]. RAF-1 phosphorylates Ser217 and Ser221 residues to activate MEK1/2 [61]. MEK1/2 are rare dual-specificity tyrosine/threonine/serine kinases that activate ERK1/2. MEK1/2 activates ERK1/2 by phosphorylating Thr-Glu-Tyr at T202 and Y204 [62]. By phosphorylating a wide variety of targets, ERK1/2 may trigger a wide range of cellular responses (Fig. 1).

Receptor transphosphorylation is bound by the adaptor proteins SHC and GRB2. Complexes including SHC are superior to direct GRB2-EGFR binding in activating RAS [63]. During two minutes of EGF treatment, SHC or EGFR recruits GRB2 to the plasma membrane [64]. It is possible that GRB2 binds to other proteins in the plasma membrane. Phospholipase D (PLD2), which converts phosphatidylcholine into phosphatidic acid and choline, is bound to GRB2 [65]. Importantly, SOS membrane recruitment requires a binding site created by PLD2-catalyzed phosphatidic acid [66].

In order for SOS to successfully recruit, GRB2, PIP2, and PA are required. SOS's critical GTP exchange activity for RAS may be amplified by a factor of 500 if it is recruited to the plasma membrane, where non-substrate RAS may first establish a binding site [67]. By converting GDP to GTP, SOS stimulates RAS. RAS's intrinsic GTPase activity, which hydrolyzes GTP to GDP, may be stimulated by GTPase activating proteins (GAPs) such NF1 (Neurofibromin 1) [68]. Recent studies have indicated that phosphorylation mediated by Y32-SRC leads to a decrease in the binding of RAS to RAF-1 and an increase in the activity of RAS GTPase [69]. The activation of RAS results in the activation of three significant downstream effectors. The components comprise RAF-1 (also known as c-RAF), PI3K, and RalGDS (Ras-like guanine nucleotide-dissociation stimulator). The prevalence of mutations in the RAS pathways in 30% of human cancers can be attributed to the tendency of RAS to activate these significant pathways [70]. Despite 30 years of research, RAS remains untreatable. Kinases are inhibited by pharmacologically blocking the ATP-binding pocket. Nevertheless, RAS needs a GTP-catalyzing pocket to turn off, prohibiting pharmacological suppression. Thus, RAS targeting is hard. 5% RAS activation in the cell is predicted to activate ERK1/2 completely, requiring a very potent inhibitor to entirely decrease RAS activity [69].

The RAF is partly activated by RAS. RAS facilitates the translocation of RAF to the plasma membrane, thereby enabling the occurrence of activation events that are localized to the membrane. The activation of RAF-1 (CRD) requires the presence of both the RBD and the RAS cysteine-rich domain [71]. Growth factor induces RAF S338 phosphorylation, however the mechanism is unknown. RAF S338 is auto-phosphorylated [60]. RAF-1 activity is also stimulated by phosphorylation of S471 in the catalytic loop [72]. RAF-1 membrane interaction requires PP2A-dephosphorylated pS259 [73].

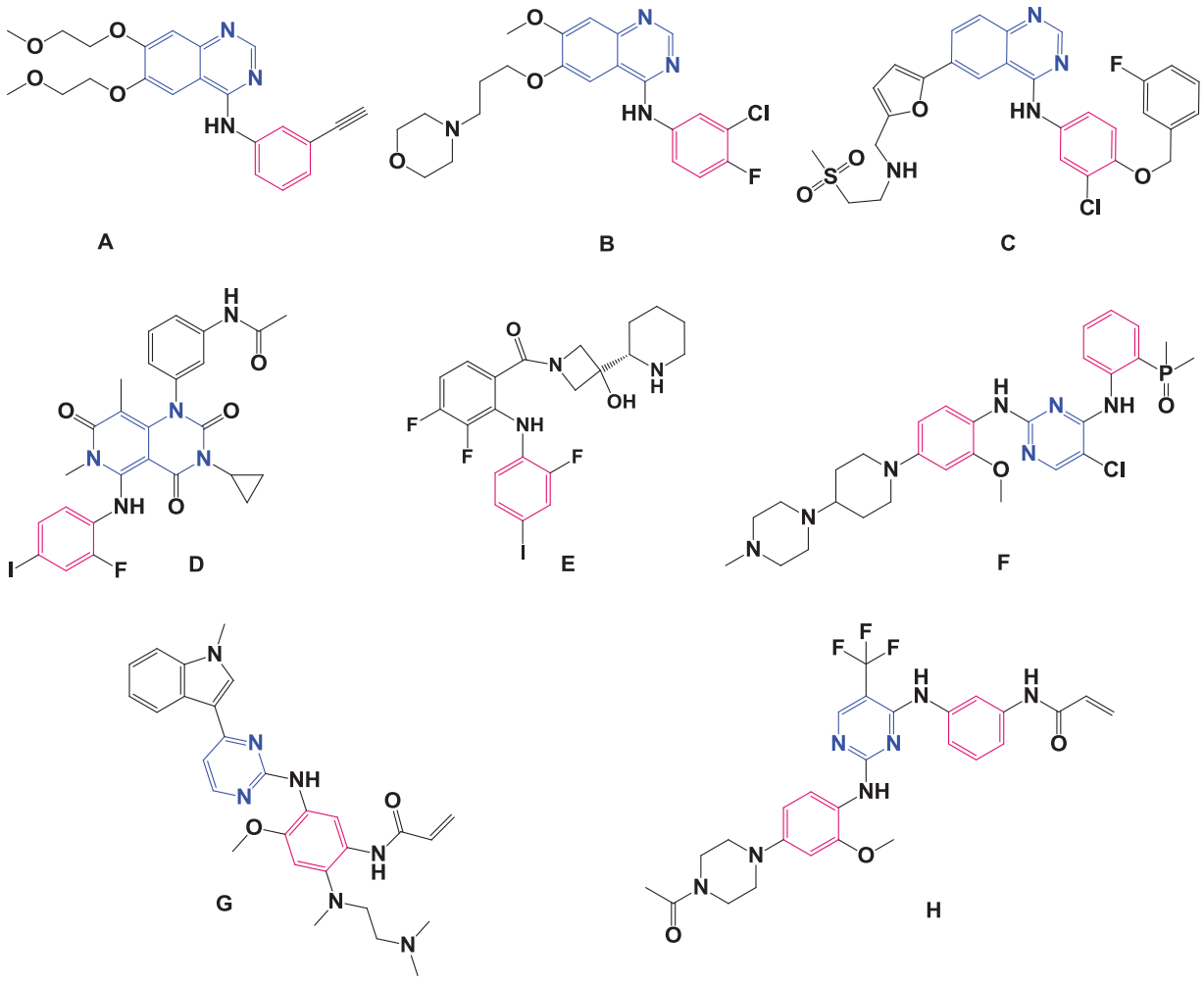
As demonstrated in Fig. 1, RAF-1 activates MEK1 and MEK2. MEK1/2 activates the sole known substrate, ERK1/2. Constitutively activating MEK1 dramatically lowers growth factor requirement for cell proliferation, whereas solid tumors seldom modify MEK1/2 [74]. Serine/threonine kinases ERK1 and ERK2 usually activate together [75]. ERK has approximately 100 downstream cytoplasmic and nuclear substrates, unlike MEK and RAF [76]. After being activated, ERK moves into the nucleus, where it activates TCF transcription factors, which in turn induces early genes Immediate Early Genes (IEGs) [77]. The c-FOS and c-MYC products of the IEG activate late-response genes, leading to an upregulation of activities associated with the extracellular signal-regulated kinase (ERK) pathway [78]. The activation of ERK within the nucleus leads to the activation of ELK-1, ETS, SP-1, and c-JUN [79]. The phosphorylation of S383, S388, and S422 located in the transcription activation domain situated at the C-terminus of ELK-1 is carried out by ERK [80]. The AP-1 complex, comprising of c-FOS and c-JUN, binds to the promoter of CYCLIN D1 to initiate G1 phase [81]. ERK regulates protein synthesis, ribosome biogenesis, and pyrimidine synthesis [82].

The expression of cyclin D1 is stimulated by the ERK pathway through the AP-1 complex. ERK-mediated transcriptional activation triggers the expression of IEGs such as c-FOS and c-JUN [78]. The activation of c-FOS through post-translational means by RSK, which is instigated by ERK, has been documented [83]. The activation of c-JUN is directly facilitated by nuclear ERK [79]. The AP-1 complex is formed by the activation of c-FOS and c-JUN, which subsequently activates the promoter of cyclin D1. The transition from G1 to S phase in the cell cycle necessitates sustained ERK activation [84].

#### Inhibitors of EGFR-ERK1/2 signaling axis

Oncogenesis relies heavily on the EGFR and its associated signaling pathways. Two main categories of EGFR-targeted treatments exist at present. Humanized monoclonal antibodies targeting the EGFR extracellular domain have been produced as the first class of treatment, with the goal of inhibiting ligand-binding or mediating downregulation of the receptor [85]. Tyrosine kinase inhibitors (TKIs) make up the second category of drugs. TKIs, which are ATP mimetics, bind to the receptor's kinase pocket, preventing ATP from entering and so blocking signal transmission [86]. Cetuximab (Erbitux) and Panitumumab (Vectibix) are two of the currently available EGFR monoclonal antibodies, whereas TKIs such as Erlotinib (Tarceva) (Fig. 2a), Gefitinib (Iressa) (Fig. 2b), and Lapatinib (Tykerb) (Fig. 2c), a dual EGFR/HER2 inhibitor, have been authorized by the US Food and Drug Administration. Drugs including gefitinib, erlotinib, cetuximab, panitumumab, cetuximab for the treatment of head and neck cancer, erlotinib for the treatment of pancreatic cancer, and lapatinib for the treatment of breast cancer have all been authorized by the FDA [94].

**Figure 2**




The chemical structures of EGFR-ERK1/2 pathway inhibitors : (a) Erlotinib (Tarceva) [87], (b) Gefitinib (Iressa) [88], (c) Lapatinib (Tykerb) [89], (D) Trametinib (Mekinist) [90], (e) Cobimetinib (Cotellic) [91], (F) Brigatinib (Alunbrig) [92], (g) Osimertinib (Tagrisso) [93], and (H) Rociletinib (Xegafri) [93].

Inhibition of the ERK1/2 signaling has been observed to augment the efficacy of EGFR-targeted therapies. Due to its restricted substrate specificity, MEK1/2 represents a viable therapeutic target for the abnormal activation resulting from potent upstream signaling proteins. FDA-approved MEK inhibitors trametinib (Mekinist) (Fig. 2d) and cobimetinib (Cotellic) (Fig. 2e) cure melanoma [95]. It seems that resistance may be overcome by combining MEK and EGFR inhibitors [20,96]. The inhibition of EGFR-ERK at the inhibitory T669 and T677 sites is observed upon MEK inactivation, leading to the adverse regulation of RTKs [97].

### Role of EGFR-ERK1/2 pathway in disease

Several benign and malignant illnesses involve the ERK1/2 pathway. In rats, cycloxygenase-2 over-activates ERK1/2 in benign prostatic hyperplasia (BPH) [98]. ERK1/2 phosphorylation causes cyclosporin-A (CsA) hepatotoxicity and nephrotoxicity [99,100]. Reactive oxygen species (ROS), delta-opioid receptor (DOR), and reticulocalbin-2 (RCN2) stimulate cell proliferation in hepatocellular cancer through the ERK1/2 pathway [101–103]. EGFR/ERK pathway activation of IL-1β drives pancreatic cancer invasion [104]. In epithelial cells, candidalysin toxin induces inflammation through EGFR-ERK1/2 [105].

## Conclusion

Mammalian cell physiology and oncogenesis depend on the EGFR. This review shows that EGFR components have been extensively studied on ERK1/2 signaling axis. This huge diversity of studies is helping us develop more effective EGFR-targeted medicines and medication combinations. EGFR spatial and temporal modulation and cancer signaling pathways are understudied. Understanding EGFR endocytosis and lysosomal degradation may

help lower EGFR levels. EGFR monoclonal antibodies linked to different medicines may also internalize harmful substances to EGFR overexpressing cells through EGFR endocytosis. Besides endocytosis, the EGFR has been well-researched chronologically for its participation in G1, but its role in S, G2, and mitosis has been less investigated. EGFR signaling and endocytosis are differentially regulated during mitosis, however it is seldom explored. EGFR function should be studied throughout the cell cycle.

The inhibition of EGFR-ERK1/2 pathway seems to be effective approach for management of several types of cancerous disorders. Survival rates in EGFR-positive cancer patients have been shown to improve with anti-EGFR treatment [106]. TKIs have been observed to be highly efficacious in cancer cells that exhibit the EGFR L858R mutation and the exon 19 deletion [107]. Over 90% of all EGFR activating mutations are caused by one of these two changes [108]. However, certain obstacles remain in the way of the efficient use of EGFR inhibitors. Various factors, including the type of somatic EGFR mutation, EGFR gene amplification, increased autocrine EGFR ligands, and mutations in proteins involved in the EGFR signaling pathway, may impact a patient's reaction to conventional anti-EGFR therapy [94,109]. Using EGFR inhibitors is difficult since the response is usually short-lived, and many tumors eventually become resistant to the treatment. Despite early success with a TKI, further EGFR mutations may develop over time, rendering the inhibitor ineffective. A prevalent kind of acquired resistance to TKIs is due to mutations like T790M. T790M is being developed as a target for third-generation EGFR TKIs such brigatinib (Alunbrig) (Fig. 2f), osimertinib (Tagrisso) (Fig. 2g), and rociletinib (Xegafri) (Fig. 2h) [110]. The presence of secondary activating mutations in the EGFR signaling pathways, such as those found in K-RAS or PI3K, can lead to the activation of a signaling pathway of EGFR that is independent of the EGFR activation status. This can result in a decrease in the effectiveness of the EGFR inhibitor. Finally, skin toxicity is still very prevalent and is a major reason why patients stop taking the medicine [111]. In addition to this, EGFR inhibitors often cause changes in the cornea and the colon [112].

To boost the effectiveness of EGFR therapy, the possibility of combining EGFR inhibitors with MEK inhibitors has been investigated. Due to its restricted substrate specificity, MEK1/2 is an attractive target for therapeutic intervention because it represents a confluence site of aberrant activation from powerful upstream signaling pathways. Trametinib and cobimetinib are two direct inhibitors of MEK that have been licensed by the FDA for the treatment of melanoma [95]. The concomitant administration of MEK and EGFR inhibitors appears to be efficacious in managing patients who have developed resistance to EGFR inhibitors [96]. It is difficult to block MEK in EGFR-driven malignancies since doing so might increase PI3K/AKT activation. This is due to the fact that EGFR is no longer being phosphorylated at their inhibitory T669 and T677 sites by ERK, which typically contributes to the negative regulation of RTKs [97]. The potential cytotoxicity of cells is a significant consideration, notwithstanding the considerable enthusiasm surrounding the co-administration of a MEK inhibitor with inhibitors targeting the EGFR and PI3K-AKT-mTOR pathways [113].

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#### Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

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