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Oncogenic mutant forms of EGFR: lessons in signal transduction and targets for cancer therapy

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Abstract

The EGF receptor is frequently mutated in a large variety of tumors. Here we review the most frequent mutations and conclude that they commonly enhance the intrinsic tyrosine kinase activity, or they represent loss-of-function of suppressive regulatory domains. Interestingly, the constitutive activity of mutant receptors translates to downstream pathways, which are subtly different from those stimulated by the wild type receptor. Cancer drugs intercepting EGFR signaling have already entered clinical application. Both kinase inhibitors specific to EGFR, and monoclonal antibodies to the receptor are described, along with experimental approaches targeting the HSP90 chaperone. Deeper understanding of signaling pathways downstream to mutant receptors will likely improve the outcome of current EGFR-targeted therapies, as well as help develop new drugs and combinations.

Introduction

The laboratory of Stanley Cohen discovered in 1980 that addition of EGF to the culture medium of human epidermoid carcinoma (A431) cells yields massive tyrosine phosphorylation, similarly to cells infected with oncogenic viruses [1]. Although not known at the time, it was speculated by Stanley Cohen and colleagues, that the EGF-receptor (EGFR) and the observed kinase activity are present in the same molecule [2]. In the past thirty years we have learned a great deal about the structure of growth factor receptors, their intrinsic ligand recognition and kinase functions, and the cellular outcomes of their activation. Upon their ligand-induced dimerization, growth factor receptors initiate a vast array of cell signaling pathways (Fig. 1), with profound effects on cell fate decisions such as proliferation, cell lineage determination and differentiation, migration and even cell death. Due to their importance, these mechanisms evolved to be tightly regulated and robust. One of the best-studied examples for such mechanisms is the EGFR family, also called the ErbB family, which belongs to the super-family of receptor tyrosine kinases (RTKs). These plasma membrane bound receptors are mainly composed of an extracellular ligand-binding, and a single trans-membrane domain, followed by an intracellular tyrosine kinase domain and a non-catalytic carboxyl terminal tail. This configuration allows extracellular signals to be relayed into the cell, and to be interpreted in order to evoke a proper response: Activation is achieved following a series of processes starting with the interaction between the ligand and the ligand-binding domain of the receptor. This binding induces a structural change which exposes an otherwise tethered dimerization arm [3], leading to coupling of two receptor molecules. Now, closer together in the correct

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orientation, the two kinase domains interact asymmetrically, while one kinase acts as an activator and the second being activated [4]. The activated kinase phosphorylates tyrosine residues located at the receptor's tail, which then act as docking sites for adaptor molecules linking the receptor to its downstream signaling pathways (Fig. 1).

During evolution, the single nematode ErbB receptor, Let-23, underwent two duplication events and subsequent incorporation of further mutations, resulting in a family of four members in mammals [5], which seem to be similar at the first glance, but were discovered to be profoundly different [6,7]. While ligand binding activates the kinase domain of EGFR/ErbB-1, as well as ErbB4, ErbB-2 has no compatible ligand and is kept in a constitutively active state [8]. On the other hand, ErbB-3 maintained its ligand-binding capacity, but its kinase domain was mutated in a way that it can only serve as a catalytically inactive activator of kinases in the context of ErbB receptor heterodimers [9]. Owing to their significant similarities, these receptors may form homo- as well as hetero-dimers, upon ligand binding, thus assembling a spectrum of activation options [10]. To further increase this system's complexity, ligands have evolved as well and are composed of a family of eleven molecules, distinct with respect to their expression patterns, binding specificities and affinities to the ErbB receptors [11–16]. Remarkably, each ligand is unique in multiple aspects, which determine physiological roles, as well as oncogenicity. These include cleavage of the precursor form, retention in the extracellular environment, preference for certain receptor heterodimers and intracellular trafficking.

Despite multiple regulatory mechanisms acting at the levels of receptors and their downstream signaling pathways, which increase the robustness of this system, the ErbB family represents a major player in many types of malignancies. Robust as it is, every complex system has its weaknesses, and the ErbB system is not different: cellular transformation can arise from receptor gene amplification or overexpression, which is thought to promote dimerization by their sheer numbers [17], or, conversely, by self-production of ligands, which initiate signaling cascades in an autocrine manner [18]. A third option of short-circuiting this highly regulated system is to bypass some of its inherent control. In this review, we will focus on the regulatory elements imposed by the molecular structure of EGFR, along with the question how cancer mutated its way to overcome these regulatory elements. In addition, we will highlight therapeutic approaches aiming to overcome aberrant modes of EGFR activation.

EGFR mutations are not random

Mutant forms of EGFR have been found in several human tumor types. The aberrant forms characterize a limited set of tumors, but their causes and reasons for cell lineage specificity remain unknown. In addition, these alterations are not evenly dispersed along the EGFR gene, but rather cluster in specific areas, leading to the assumption that mutational “hot spots” entail elements of special functional or regulatory importance (Fig. 2).

The ectodomain hotspot

A primary safeguard mechanism, which prevents premature receptor dimerization and subsequent activation, is the ligand-binding domain. Formation of stable receptor dimers can only be achieved upon ligand binding, followed by a constructive dimer formation. The viral EGFR homologue, v-ErbB, which induces erythroblastosis in birds and contributed its name to the ErbB family, lacks the whole extracellular domain. With the dimerization constraints removed, this viral protein was found to exist primarily in dimers, leading to constitutive kinase activation and subsequent oncogenicity [19]. Interestingly, similar deletion mutants have been discovered in human gliomas (Table 1 and Fig. 2). One of these mutant forms, EGFR variant III (EGFRvIII), was found to lack a large part of the extracellular portion, including components of the ligand-binding domain [20]. Indeed, although ligand insensitive, this mutated form was

found to be constitutively dimerized and highly tumorigenic [21], resembling the activity of its viral counterpart. Additionally, apart from deletions, this ectodomain's hot spot attracts point mutations, most of which were reported to be oncogenic [22], presumably by promoting receptor dimerization.

The kinase hotspot

Unlike other RTKs, which must undergo phosphorylation within the activation loop of the kinase domain in order to achieve the catalytically active conformation, EGFR does not require this modification [23]. Here, the activity of the kinase domain is controlled by conformational changes induced by dimer formation and possibly by the distal carboxyl terminus. Following the rationale of regulation-bypassing, Non-Small Cell Lung Cancer (NSCLC) and other tumors harbor point mutations, small deletions or insertions of the EGFR kinase domain [24–26], rendering this domain constitutively active [27] even in the absence of upstream events such as ligand binding or dimerization.

The C-tail hotspot

In addition to activation by means of kinase mutations, a deletion mutant found in gliomas, named EGFRvIV, lacks either two or three exons in its carboxyl tail, downstream to the kinase domain [20,28], while keeping most of the auto-phosphorylation tyrosine residues intact. According to structural [29,30] and computational analyses, the deleted segment likely plays an essential role in kinase auto-regulation by folding over the kinase domain, implying that its removal will result in constitutive kinase activity [31]. Additionally, the notion of a regulatory role of the carboxyl tail may explain the constitutive activation of a kinase-duplication mutant, in which only one kinase is predicted to be inhibited by the tail, while the other may represent an active form [32].

Pathologic aspects of the common oncogenic mutants of EGFR (see Table 1) EGFRvIII in brain and other tumors

Since its discovery in 1990 [33], EGFRvIII has been a subject of thorough investigation, which is justified by its relative abundance in brain tumors, and frequent detection in other malignancies, including lung, breast and ovarian cancers [20,34–36]. As mentioned above, by deletion of a segment of the ligand-binding domain, EGFRvIII bypasses the need of ligand. This deletion spans exons 2–7, resulting in the introduction of a novel glycine residue at the fusion junction. Interestingly, EGFRvIII is commonly diagnosed in tumors that overexpress the wild type form of EGFR, suggesting that receptor overexpression precedes deletion of exons 2–7 [28,33]. While this mutant cannot bind ligands, it resides at the cell membrane [37], often within receptor dimers [38] that display constitutive basal activity. EGFRvIII has been shown to be transforming both *in vitro* and *in vivo*, as it confers anchorage-independent growth of cultured cells and promotes tumor formation in athymic mice [39]. Curiously, the oncogenic capacity of EGFRvIII may propagate into non-expressing cells by means of cell-to-cell transfer of membrane-derived microvesicles [40].

Kinase mutations in NSCLC

Mutations in the EGFR kinase domain are often diagnosed in NSCLC, particularly in tumors occurring in individuals of Eastern Asian origin, females, never smokers, and in adenocarcinomas with bronchioloalveolar histological features [41,42]. These mutations spread along the kinase-coding regions (exons 18–21), and they all seem to catalytically stimulate the basal kinase activity, although by different means. Exons 18 and 19 encode for the phosphate-binding loop, also termed the P-loop. While exon 18 displays point mutations, mainly G719X (X indicates A, C, S or D), which represent about 4% of EGFR mutations in NSCLC, exon 19 is usually affected by deletions, which represent 44% of kinase domain

mutations. Exon 20, which encodes for the α -C helix, harbors relatively rare (4%) insertion mutations, whereas exon 21, encoding the activation loop (A loop), encompasses point mutations that account for 41% of mutations, including the prototypical L858R mutation [43]. All these mutations are believed to destabilize the inactive kinase conformation, hence driving it to a more active state [44]. Notably, in contrast to EGFRvIII, kinase-mutated EGF receptors retain their responsiveness to growth factor ligands [45], which further enhance catalytic activity, relatively to wild type EGFR [24].

Novel signal transduction features of EGFR mutants

Even though the mutated EGF-receptors that are frequently found in tumors were proven to be catalytically active and tumorigenic, their activation profile is essentially different than that of the ligand-activated wild type receptor. Usually, EGFR tyrosine phosphorylation status is significantly lower in mutated receptors compared to ligand-activated wild type receptors, yet clearly exceeds its unstimulated state. Apparently, this basal but chronic activity relays signals in a profoundly different manner than the well-characterized canonical signal-transduction machinery.

The case of EGFRvIII

An example for such discrepancy between wild type and mutant signaling potentials is EGFRvIII activation: while the wild type EGFR activates several signaling pathways upon ligand binding (Fig. 1), the constitutively-dimerized mutant preferentially activates the Phosphatidylinositol 3-Kinase (PI3K) pathway over other pathways such as the Mitogen-Activated Protein Kinase (MAPK) pathway [46–48]. Indeed, EGFRvIII instigates the expression of only a subset of genes induced by the ligand-stimulated wild type EGFR [49].

Mutant EGFRs escape negative regulation

Another important feature of the relatively high basal signaling of EGFR mutants is their ability to evade negative, activation-dependent regulation. The customary EGFR signal attenuator is the ubiquitin-ligase CBL, which is recruited to a specific phosphorylated tyrosine residue located at the receptor's tail (Y1045). When bound, CBL attaches mono- and di-ubiquitin moieties to multiple lysine residues of EGFR, thus tagging the receptor for lysosomal degradation, and thereby defining the temporal window of signaling of activated EGFR. Apparently, in some cases, including the NSCLC single (L858R) and double mutant (L858R/T790M), basal EGFR activation results in differential phosphorylation of tail tyrosine residues [22,50–52], allowing uncontrolled downstream signaling. This aberrant activation pattern is achieved by phosphorylation of tyrosine residues responsible for propagating the signal downstream, while Y1045 is kept at a weakly phosphorylated state, thus allowing the mutated receptors to signal “under the radar” of receptor attenuating mechanisms.

Cancer therapies targeting aberrant forms of EGFR

Due to the frequent involvement of the ErbB family in cancer, intercepting its members became a natural goal of the targeted therapy approach. In contrast to chemotherapy, which is highly toxic and often results in adverse clinical effects, targeted therapy aims specifically at the relevant renegade protein. Although tumors harbor multiple genetic abnormalities, according to the “oncogene addiction” theory, their tumorigenic drive may be attributed to a single pathway, to which the tumor became “addicted” [53]. Indeed, in many cases, when this pathway is pharmacologically blocked, cancer cells die, leading to tumor regression. A related theory, termed the “oncogenic shock”, proposes that short-lived pro-survival signals are depleted due to treatment, leaving the cell with long-lived pro-apoptotic elements that drive cell death [54]. Regardless of the mechanism, RTK targeting has been proven beneficial across a wide

spectrum of malignancies. Accordingly, ErbB proteins and other RTKs are currently directly targeted using two distinct strategies (Fig. 3):

Tyrosine kinase inhibitors

One of the outcomes of the pursuit for EGFR inhibiting agents was the development of small molecules that bind to the ATP-binding pocket of the kinase domain, thereby inhibiting its enzymatic activity. These Tyrosine Kinase Inhibitors (TKIs) mimic ATP, which serves as phosphate source for the phosphorylation process [55]. TKIs may bind either reversibly or irreversibly, and their specificity can be designed to target EGFR alone, several ErbB family members, or even other tyrosine kinases as well. Two such molecules, Erlotinib (OSI-774, Tarceva®) and Gefitinib (ZD 1839, Iressa®) are reversible EGFR-specific TKIs, which are already in clinical use for treatment of NSCLC and pancreatic adenocarcinomas [56–59]. A third approved compound, lapatinib/Tykerb® inhibits both EGFR and its closest kin, HER2/ErbB-2, and is successfully used in patients with HER2-overexpressing metastatic breast cancer [60,61].

Monoclonal anti-receptor antibodies

The first broadly successful monoclonal antibody (mAb) to target the ErbB system in human malignancies was trastuzumab, a humanized mAb targeting ErbB-2/HER2, a receptor frequently over expressed in breast cancer [62]. Trastuzumab (Herceptin®), is widely used now as a common treatment for patients with ErbB-2 overexpressing breast cancer [63–65]. EGFR's extracellular domain is similarly targeted using specific antibodies, with the aim of inhibiting ligand binding and accelerating receptor down-regulation, following antibody-directed clustering and aggregation [66]. In addition, recruitment of the patient's immune system to the cancer cells, via the antibody's Fc region, to promote antibody-dependent cell-mediated cytotoxicity, is considered another advantage of this approach [67]. Cetuximab (C225, Erbitux®) is a humanized mouse antibody that is approved for treatment of two clinical indications: patients with metastatic colorectal cancer (harboring wild-type KRAS, see below), or patients with advanced or recurrent head and neck cancer. The antibody has also shown some activity in NSCLC [68]. Panitumumab (Vectibix®) is a fully human EGFR antibody approved for treatment of advanced colorectal cancers that express wild-type KRAS and progressed during or after previous lines of therapy [69,70].

Because EGFRvIII exhibits a novel epitope at its extracellular domain, which is exclusive to cancer cells, antibodies specifically targeting this mutated form were generated and showed encouraging initial results in clinical trials [71]. Other therapies also utilize the epitope differences between EGFRvIII and its wild type counterpart. Active immunization with peptides based on the fusion junction of EGFRvIII was proven effective in animal models [72]. Additionally, a re-targeted oncolytic measles strain, that specifically identifies EGFRvIII, exhibited host cell specificity both in vitro and in animals, adding virus targeted therapy to the battery of future potential therapies [73].

Games of cat and mouse - EGFR secondary mutations

A unique opportunity to grasp the mutational micro-evolution of cancer progression has arisen through the treatment of NSCLC patients with gefitinib or erlotinib. Clinical trials have shown that only a relatively small portion of patients responded to the TKI treatment, but with impressive clinical improvement [24]. Further studies discovered that TKI responsiveness was dependent on point mutations or small deletions within the kinase domain. These mutations, while activating the kinase, also conferred sensitivity to treatment [52,74]. However, soon afterward, many of the responsive tumors relapsed under ongoing TKI treatment. Sequencing of the kinase domain from these tumors revealed a secondary mutation (T790M) that not only increases kinase activity, but grants TKI resistance as well [75,76]. Later, this phenomenon

was repeated in mouse models establishing it as a secondary, resistance providing mutation [76], although it has been identified as a germ-line mutation in families with inherited cancer susceptibility as well [77]. This is an outstanding demonstration of cancer mechanisms relentlessly seeking for active signaling despite external perturbations.

Mutations downstream to EGFR

Apart from additional mutations that evolve to rescue EGFR activity and tumor progression, as seen in NSCLC, further mutations may preexist or appear in other components of signaling pathways, thus shifting the oncogenic addiction downstream, and rendering EGFR targeting insufficient. In glioblastomas, only a fraction of patients respond to EGFR TKI treatment. Thorough analysis revealed that some non-responsive tumors, although expressing the mutated EGFRvIII, displayed decreased expression of PTEN (phosphatase and tensin homolog) [78]. PTEN is a tumor suppressor (Fig. 1) that reverses the action of PI3K, transforming the second messenger PIP3, which is responsible for AKT activation, back to PIP2, therefore attenuating this signaling cascade. Tumors that lost PTEN expression often exhibit a constitutively active PI3K pathway, regardless of the status of EGFR. Indeed, reintroduction of PTEN into glioblastoma cells *in vitro* re-sensitized them to TKI treatment [78]. The PI3K pathway is mutationally altered in colorectal cancer (CRC) as well, in which additionally to PTEN deletion, PI3K activating mutations have been reported [79]. Additional downstream mutated proteins that confer resistance to EGFR-targeted therapy in colorectal cancer are KRAS and BRAF (see Fig. 1). Although EGFR is ubiquitously expressed in CRC, tests have shown that only patients expressing the wild type KRAS and BRAF, which are major mediators of the MAPK pathway, will benefit from anti-EGFR treatment [80,81]. Moreover, several reports concluded that occurrence of either EGFR or KRAS mutations are mutually exclusive in NSCLC [82], highlighting the apparent functional redundancy of these mutations.

The least explored route - recruitment of Heat Shock Protein (HSP) 90

HSP90 is a molecular chaperone that facilitates proper folding of specific client proteins in an ATP-dependent manner [83], and has a significant role in buffering genetic variation, contributing to evolutionary processes [84]. However, HSP90 also stabilizes viral kinases and mutated oncoproteins, such as v-SRC [85] and Bcr-Abl [86], revealing an inevitable darker side. While the constitutively active ErbB-2/HER2 is yet another HSP90 client [87], mutant forms of EGFR, probably due to their constitutively-active kinases, are dependent on the chaperoning function of HSP90 through direct interaction, which warrants their stability [88–91]. Indeed HSP90 inhibition, using benzoquinoid ansamycins such as geldanamycin, reduces mutant EGFR levels and activity, suggesting an alternative EGFR inhibition strategy [92].

Concluding remarks

Evolution is usually referred to natural selection of species, with survival of the fittest to a changing environment. However, the same concept applies to cancer progression [93,94]: A tumor is composed of heterogeneous sub-clones, with each harboring slightly different sets of mutations [95]. Environmental stress, such as hypoxia, immune mediators and therapeutic interventions, selects the fittest clones for survival, thus pushing the tumor further to aggressiveness, and to finding new solutions for new pressures, such as in the case of secondary NSCLC mutations. Recently, a new generations of irreversible TKIs have been shown to exhibit activity against the secondary T790M mutation [96], thus raising the “arms race” to a new level. Time will tell whether new, tertiary resistant mutations will emerge, or whether this will drive tumors into switching to other kinases in their thrive for growth signals, such as ErbB-3 and MET, the receptor for the hepatocyte growth factor [97,98].

The discovery of the first oncogenes and tumor-suppressors helped in understanding the nature of cancer [99]. The acquisition of gain-of-function and loss-of-function mutations seems to similarly apply to the smaller scale of single proteins, such as EGFR: gain-of-function mutations affect the kinase domain, whereas regulatory safe-guards embedded within the EGFR, such as the ligand binding domain and the carboxyl tail, are the targets of loss-of-function mutations. Interestingly, specific cancer types choose different strategies.

Drug development, in its constant pursuit for maximizing clinical efficacy, while minimizing toxicity, led to a major effort concentrating on EGFR-targeted therapies. However, according to the lessons learnt from RAS mutations and PTEN deletions, in order to predict which patient will benefit from such treatments, the whole genomic and proteomic status of a tumor should be mapped and analyzed. With deep sequencing, microarray technology and high throughput proteomics, such an ambitious task may be plausible sooner than expected. Patient-specific drug combinations will have to consider not only the aberrant gene itself, but also common compensatory pathways to be simultaneously targeted, such as MET, AKT and ErbB-3, in the case of EGFR. Moreover, a third circle of inhibitors may be required, such as modifiers of the tumor's stroma [100], blockers of angiogenesis [101], and HSP90 inhibitors. In lung tumors harboring mutant forms of EGFR, specific TKIs have already demonstrated superior efficacy and tolerability compared to conventional cytotoxic agents [102,103]. In the foreseeable future, similar approaches along with rationally designed combinations of targeted therapies, will hopefully achieve the goal also in brain and other tumors expressing mutant forms of EGFR.

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Figure 1. Activation mechanisms and signaling pathways engaged by EGFR

In the absence of ligand, EGFR predominantly exists in a monomeric form, in which the kinase domain is auto-inhibited by the carboxyl-terminal tail of the receptor. Ligand binding traps the extracellular domain of EGFR in a conformation poised to form receptor dimers, which are mediated by mutual interactions of the dimerization loops. The resulting conformational change is relayed into the intracellular receptor portion, resulting in the formation of an asymmetric dimer of kinase domains. In this asymmetric dimer, the C-lobe of the kinase domain of the “activator kinase” buttresses the kinase domain N-lobe of the “receiver kinase”, resulting in catalytic activation of the latter. The receiver kinase then phosphorylates intracellular tyrosine residues of the activator kinase, which cluster in the carboxyl-terminal tail. Phosphorylated tyrosine residues function as docking sites for signaling adaptors (e.g., Grb2), intracellular enzymes (e.g., PLCgamma) or transcription factors (e.g., STAT3). Thereby, activated EGFR couples to a myriad of intracellular signaling pathways mediating such diverse cellular responses as cell lineage determination, proliferation, survival, or migration. The abbreviations used are: PI3K, phosphoinositide-3-kinase; PIP₃, phosphatidylinositol-3,4,5-trisphosphate; Akt, v-akt murine thymoma viral oncogene homolog; mTOR, mechanistic target of rapamycin; STAT, signal transducer and activator of

transcription; PLCgamma, phospholipase C gamma; IP₃, inositol 1,4,5-triphosphate; DAG, diacylglycerol; PKC, protein kinase C; CAMK, calcium/calmodulin-dependent protein kinase; Grb2, growth factor receptor-bound protein 2; Shc, Src homology 2 domain containing transforming protein; SOS, son of sevenless homolog; Ras, Ras viral oncogene homolog; Raf, Raf murine viral oncogene homolog; MEK, mitogen-activated protein kinase kinase; ERK, mitogen-activated protein kinase (extracellular signal-regulated kinase)

Figure 2. Receptor aberrations cluster in functionally relevant hotspots of EGFR

Structural constraints impose ligand binding for receptor activation. Brain tumors such as glioblastoma multiforme (GBM) circumvent this requirement by deletions or point mutations in the ligand-binding domain (e.g., EGFRvIII) of EGFR, which promote receptor dimerization and activation. Alternately, GBMs may enhance the EGFR catalytic activity through duplications of its kinase domain, as well as by deletions in the carboxyl-terminal tail, which may otherwise execute an auto-inhibitory role on the kinase activity. Conversely, non-small cell lung cancers (NSCLC) often exhibit prototypical point mutations or small insertions/deletions in kinase domain regions regulating the basal catalytic activity of EGFR.

Figure 3. Therapies targeting EGFR signaling

Oncogenic aberrations of the EGFR extracellular domain may result in exposure of novel epitopes not displayed by the wild-type receptor. In context of the EGFRvIII deletion, monoclonal antibodies targeting such epitopes (e.g., mAb 806) have already demonstrated clinical activity. Monoclonal antibodies targeting the wild type EGFR have demonstrated substantial activity in patients with colorectal cancer, head and neck cancer, and non-small cell lung cancer (NSCLC). Tyrosine kinase inhibitors (TKIs) represent another class of anti-EGFR agents. These small ATP-mimetics readily penetrate into the cell, with gefitinib and erlotinib binding to the kinase domain in its active conformation, and inhibiting transfer of phosphate moieties. Patients with NSCLC harboring mutations in the EGFR kinase domain are particularly sensitive to TKIs (kinase marked in red). Erlotinib is approved also for patients with pancreatic cancer. Another, non-direct strategy entails HSP90 inhibition; mutated and activated EGF receptors have been shown to serve as HSP90 clients, demonstrating significantly reduced activity upon its inhibition by benzoquinoid ansamycins, such as geldanamycin derivatives.

Table 1

Common EGFR mutations

Cancer type	Name	Domain affected	Mutation type	Biochemical traits	reference
GBM	EGFRvI	extracellular	deletion	ND	[34]
	EGFRvII	extracellular	deletion	ND	[28,104]
	EGFRvIII*	extracellular	deletion	Basal dimer formation, basal kinase activity	[20,28,34,38]
	EGFRvIV	Carboxyl tail	deletion	Predicted basal kinase activity	[20,28,30,31]
	EGFRvV	Carboxyl tail	deletion	ND	[20,28]
NSCLC	EGFR TDM	Kinase	duplication	Basal kinase activity, escapes down regulation	[28,32]
	746-759&	Kinase	deletion	Basal kinase activity	[105,106]
	L858R#	Kinase-activation loop	Point mutation	Basal kinase activity	[105,106]
	G719S#	Kinase-nucleotide-binding loop	Point mutation	Basal kinase activity	[105,106]
	T790M	Kinase	Point mutation	Confers reversible TKI resistance, increases basal activity	[76]
	D770InsNPG	kinase	insertion	Basal kinase activity, confers reversible TKI resistance	[106]

ND-Not Determined.

* EGFRvIII has been identified in lung, breast, ovarian and other tumors [35,36]

& Various deletions within the indicated range have been reported

G719S and L858R are representative point-mutations in exons 18 and 21, respectively