Published in final edited form as:

Radiother Oncol. 2013 September ; 108(3): . doi:10.1016/j.radonc.2013.06.010.

Nuclear EGFR as a Molecular Target in Cancer

Toni M. Brand, Mari Iida, Neha Luthar, Megan M. Starr, Evan J. Huppert, and Deric L. Wheeler¹

Department of Human Oncology, University of Wisconsin School of Medicine and Public Health, 1111 Highland Ave., Wisconsin Institute for Medical Research, Madison, Wisconsin, 53705 USA

Abstract

The epidermal growth factor receptor (EGFR) has been one of the most targeted receptors in the field of oncology. While anti-EGFR inhibitors have demonstrated clinical success in specific cancers, most patients demonstrate either intrinsic or acquired resistance within one year of treatment. Many mechanisms of resistance to EGFR inhibitors have been identified, one of these being attributed to alternatively localized EGFR from the cell membrane into the cell's nucleus. Inside the nucleus, EGFR functions as a co-transcription factor for several genes involved in cell proliferation and angiogenesis, and as a tyrosine kinase to activate and stabilize proliferating cell nuclear antigen and DNA dependent protein kinase. Nuclear localized EGFR is highly associated with disease progression, worse overall survival in numerous cancers, and enhanced resistance to radiation, chemotherapy, and the anti-EGFR therapies gefitinib and cetuximab. In this review the current knowledge of how nuclear EGFR enhances resistance to cancer therapeutics is discussed, in addition to highlighting ways to target nuclear EGFR as an anti-cancer strategy in the future.

Keywords

Nuclear EGFR; Cancer; Resistance

Introduction

The epidermal growth factor receptor (EGFR) is one of four members of the HER family of receptor tyrosine kinases [1, 2]. EGFR contains an extracellular ligand-binding domain, a single membrane-spanning region, a juxtamembrane nuclear localization signal (NLS), a tyrosine kinase domain, and a tyrosine-rich C-terminal tail. Ligand binding causes a conformational change in the receptor that allows for both homo- and hetero-dimerization with other activated HER family members [3]. Dimerization activates the intrinsic tyrosine kinase of each receptor, leading to the phosphorylation of tyrosine residues on each

© 2013 Elsevier Ireland Ltd. All rights reserved.

¹To whom requests for reprints should be addressed: Deric L. Wheeler PhD, Department of Human Oncology, University of Wisconsin Comprehensive Cancer Center, 1111 Highland Avenue, WIMR 3159, Madison, Wisconsin 53705. Phone: (608) 262-7837; fax: (608) 263-9947; dlwheeler@wisc.edu.

Competing Interests:

The authors declare no competing interests.

Authors' contributions

TMB and DLW performed the literature search and drafted the manuscript. TMB and MI performed all experimentation depicted in this manuscript. All authors provided critical review of the manuscript and approved the final draft.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

receptor's C-terminal tails. This process serves to activate various growth-promoting signaling cascades such as the RAS/MAPK, PI(3)K/Akt, PLC -PKC, and Jak/STAT pathways.

Several early reports describe the overexpression of EGFR in a variety of epithelial tumors. These findings support the hypothesis that deregulated EGFR expression and signaling may play a critical role in the etiology of several human cancers, including lung, head and neck squamous cell carcinoma (HNSCC), colon, pancreatic, brain and breast [4–8]. Discovery of EGFR overexpression in cancer has led to substantial efforts over the last four decades to target the EGFR as a cancer treatment strategy. One approach uses monoclonal antibodies to target the extracellular domain of the EGFR to block natural ligand binding [9, 10]. Cetuximab (IMC- C225, Erbitux) prevents receptor activation and dimerization, ultimately inducing receptor internalization and down regulation [11]. Cetuximab, either as monotherapy or in combination with chemotherapy and/or radiation, exhibits promising antitumor activity in HNSCC and metastatic colorectal cancer. A second approach utilizes small molecule tyrosine kinase inhibitors (TKIs) that bind to the ATP-binding site in the tyrosine kinase domain of the EGFR. Three anti-EGFR TKIs, erlotinib (OSI-774, Tarceva), gefitinib (ZD1839, Iressa) and lapatinib (GW572016, Tykerb), have been approved by the FDA for use in oncology. Despite intense clinical and preclinical efforts to develop EGFR inhibitors, collectively they have had modest success in curing patients of tumors that express the EGFR. The underwhelming success of these EGFR inhibitors suggests that a more comprehensive understanding of EGFR biology is needed.

Although plasma membrane EGFR signaling has been intensely researched over the last thirty years, new functions of the EGFR are now beginning to unravel. One new prominent mode of EGFR signaling has been found in the cell's nucleus [12–14]. Research over the last decade has deciphered a distinct series of steps for nuclear EGFR transport [15–18]. Activation of the EGFR results in its endocytosis and interaction with importin 1 via its tripartite nuclear localization sequence (NLS) [19]. EGFR is described to undergo COPI-mediated retrograde trafficking from the Golgi to the ER [16]. Once embedded into the ER membrane, EGFR and importin 1 interface with nucleoporins in the nuclear pore complex (NPC) to shuttle EGFR from the outer nuclear membrane (ONM) to the inner nuclear membrane (INM). INM embedded EGFR can be released into the nucleoplasm via association with the Sec61 translocon. This process has been termed the Integral Trafficking from the ER to the Nuclear Envelope Transport model [20].

Upon entry into the nucleus, the EGFR can function in ways distinct from its plasma membrane bound counterpart. Three major functions of nuclear EGFR have been identified (Figure 1). First, nuclear EGFR can function as a co-transcription factor. Although it was shown in 1994 that a kinase dead EGFR could enhance transcriptional expression of the c-fos gene [21], it was not until 2001 that a landmark paper provided direct evidence that EGFR could regulate the cyclin D1 promoter [22]. Since these initial findings, nuclear EGFR has been shown to co-regulate inducible nitric oxide synthase (iNOS), B-Myb, cyclooxygenase-2 (COX-2), aurora Kinase A, c-Myc, breast cancer resistant protein (BCRP), and Stat1 [23–28]. Second, nuclear EGFR has been shown to phosphorylate proliferating cell nuclear antigen (PCNA) on Y211, thereby increasing PCNA stability and ultimately enhancing cellular proliferation. Lastly, EGFR has been shown to enter the nucleus upon radiation treatment and interact with DNA-dependent protein kinase (DNA-PK) leading to repair of radiation-induced DNA double strand breaks [29, 30]. Importantly, these nuclear functions have been inversely correlated with overall patient survival in breast, ovarian, oropharyngeal, gallbladder, and lung cancer providing a strong rationale for the molecular targeting of this nuclear receptor [31–36].

The Role of Nuclear EGFR in Resistance to Cancer Therapeutics

Intrinsic and acquired tumor cell resistance to both conventional and targeted cancer therapies remains one of the largest obstacles to overcome clinically. While nuclear EGFR is observed in cells of high proliferative origin, numerous reports describe increased nuclear localization of EGFR in models of cancer resistance to different therapeutic regimes [28, 29, 37–39]. These studies identified that nuclear EGFR could enhance resistance by influencing DNA damage repair, DNA replication, and transcription of oncogenes [28, 29, 37–39]. Thus, nuclear EGFR is now emerging as a potent biomarker for response to numerous cancer therapies. In the following paragraphs, we will discuss the role of nuclear EGFR in resistance to radiation, chemotherapy, and the anti-EGFR targeted therapies gefitinib and cetuximab.

Nuclear EGFR and Radiation Resistance

Radiation therapy is one of the most common anti-cancer treatments used due to its ability to induce widespread DNA damage in tumor cells. Enhanced tumor cell DNA damage repair can lead to radiation resistance, a process that is mediated by DNA-PK [40]. In 1997, Schmidt-Ullrich and colleagues observed that radiation treatment of tumor cells led to EGFR activation and internalization similar to growth factor stimulation of the EGFR [41]. Further research demonstrated that tumor cells could be radiosensitized upon inhibition of EGFR activation with cetuximab [42, 43], suggesting that EGFR plays a role in promoting DNA damage repair pathways. Pioneering studies by Dittmann et al. demonstrated that EGFR and DNA-PK form a complex in the nucleus upon radiation treatment, and that this interaction led to enhanced DNA-PK activity and DNA repair [30, 44]. Importantly, inhibition of nuclear EGFR localization led to the inactivation of DNA-PK, resulting in less DNA damage repair and increased radiation response [29, 30].

To further understand the molecular requirements of nuclear EGFR transport, Dittmann et al. demonstrated that the phosphorylation of EGFR at Tyrosine 654 was necessary for radiation induced nuclear transport and DNA damage repair [45, 46]. Additional studies showed that nuclear EGFR participates in chromatin relaxation, a necessary step for recruitment of repair proteins to DNA double strand breaks [47]. A recent study by Liccardi et al. elaborated on these findings by demonstrating that EGFR mutants lacking nuclear localization (constitutively active EGFR L858R and EGFR lacking its NLS) had decreased repair of radiation induced DNA double strand breaks [37]. Collectively, this body of work supports the important role of nuclear EGFR in enhancing DNA-PK induced DNA damage repair upon treatment with radiation therapy.

Interestingly, two reports have identified two radioprotectors that enhance the nuclear transport of EGFR in tissues with wild-type p53. O-phospho-tyrosine and Bowman-Birk proteinase inhibitor were shown to induce nuclear EGFR localization and activation of DNA-PK in p53 wild-type cells, deeming these cells more resistant to radiation [46, 48]. These two radioprotectors may protect normal tissues that are wild-type p53 from the deleterious effects of radiation therapy. PKC was also shown to play a role in the nuclear translocation of EGFR and O-phospho-tyrosine radioprotection, both of which were lost upon PKC knockdown [49]. Overall, these studies support the role of nuclear EGFR in resistance to radiation therapy.

Most recently, a study identified that nuclear EGFR plays a key role in regulating the activity of an exoribonuclease termed polynucleotide phosphorylase (PNPase), which can function to degrade c-MYC mRNA in the cytoplasm [50]. Researchers show that radiation treatment of breast cancer cells promotes the nuclear association of EGFR and PNPase, and that this association was linked via DNA-PK phosphorylation of PNPase at Serine 776. The

phosphorylation of PNPase at Serine 776 abolished its ribonuclease activity and led to the upregulation of c-MYC expression, thereby enhancing radioresistance. Upon inhibition of EGFR or DNA-PK activity, PNPase was no longer activated and c-MYC levels were downregulated, increasing radiosensitivity [50]. Overall, this study highlights a novel role of nuclear EGFR in the regulation of PNPase and augmentation of radiation response.

Nuclear EGFR and Cisplatin Resistance

Cisplatin is a mainstay chemotherapy used to treat a variety of cancers. Cisplatin elicits DNA damage through crosslinking DNA, preventing replication and cell division and thereby triggering apoptosis [51]. Treatment of tumor cells with cisplatin has also been shown to induce nuclear EGFR translocation much like radiation treatment. In 2009, Hsu et al. demonstrated that wild-type EGFR stable cells were resistant to cisplatin and had enhanced DNA repair upon treatment, while EGFR deleted of its NLS exhibited hindered DNA repair capabilities and sensitivity [39]. Liccardi et al. further supported these findings by showing that stable cell lines deficient in nuclear EGFR localization lacked DNA crosslinking repair mechanisms and association/activation of DNA-PK [37].

Nuclear EGFR and Anti-EGFR Therapy Resistance

The EGFR is commonly overexpressed and/or aberrantly activated in tumors, and therefore a large-scale clinical effort has been made to inhibit the activation of this receptor. Similar to radiation and chemotherapy, *in-vitro* models studying cancer cell resistance to both gefitinib and cetuximab have demonstrated that resistant cells often retain dependency on the EGFR for enhanced growth potential and contain high levels of nuclear localized EGFR [28, 38, 52]. In the case of gefitinib resistance, nuclear EGFR was shown to function as a co-transcriptional activator for breast cancer resistant protein (BCRP/ABCG2), a plasma-membrane bound ATP dependent transporter that can extrude anti-cancer drugs from cells and thereby diminish their effects [28]. Authors hypothesize that this ATP dependent transporter may function to remove gefitinib from cells and thereby enhance resistance [28].

Cetuximab resistance has also been attributed to nuclear EGFR. Various researchers have demonstrated that cetuximab treatment can enhance the nuclear localization of EGFR [38, 53, 54], and that cell lines with intrinsic resistance to cetuximab contain high levels of nuclear EGFR [38]. In the setting of acquired resistance to cetuximab, our lab demonstrated that resistant cells have enhanced nuclear EGFR levels, which were attributed to increases in Src Family Kinase (SFK) activity [38, 52, 55]. Inhibition of SFKs with the small molecule inhibitor dasatinib decreased nuclear EGFR and enhanced plasma membrane bound EGFR levels [38]. Importantly, treatment of resistant cells with dasatinib resensitized them to cetuximab. These findings were further validated via the use of a nuclear localization sequence-tagged EGFR, which enhanced cetuximab resistance in sensitive parental cells [38]. Collectively, this body of work demonstrates that nuclear EGFR plays a role in resistance to both gefitinib and cetuximab therapies.

Targeting Nuclear EGFR in Cancer: Where Are We Now?

The current body of work focused on the roles of nuclear EGFR in cancer provides a strong rationale for learning how to target this subcellular receptor. Targeting nuclear EGFR may also enhance a cancer cell's dependency on classical membrane-bound functions of EGFR (such as activation of traditional signaling pathways) and thereby sensitize these cells to established targeting agents. Over the past decade numerous studies have focused on the specific proteins and post-translational modifications of EGFR necessary for its nuclear translocation and function. In the following paragraphs we will discuss these molecular determinants and how they have been used to target nuclear EGFR in cancer cells.

Targeting nuclear EGFR with anti-EGFR therapies

Current anti-EGFR therapies inhibit the activation of the EGFR via prevention of ligand binding, receptor dimerization, and through association with the ATP binding pocket of the kinase domain [56, 57]. In 2009, Kim et al. demonstrated that the small molecule EGFR inhibitor lapatinib could inhibit EGF induced nuclear EGFR translocation in two breast cancer cell lines; however endogenous levels of nuclear EGFR were not changed [58]. While this study provided evidence that anti-EGFR inhibitors may prevent nuclear EGFR translocation, the majority of current research suggests that these therapies enhance EGFR endocytosis and nuclear translocation, especially in the setting of acquired resistance [28, 38, 53, 59, 60]. In Figure 2 a panel of HNSCC and breast cancer cell lines were treated with the anti-EGFR inhibitors erlotinib and lapatinib for 24 hours and then harvested for whole cell, non-nuclear, and nuclear proteins. While both inhibitors prevented the activation of EGFR at Tyrosine 1173 (Figure 2A), they did not effect, and in some cases enhanced, nuclear EGFR levels (Figure 2B). In the HNSCC cell lines in particular, there is an enhancement of non-nuclear EGFR levels as well. This may be due to increased EGFR internalization upon TKI treatment, a phenomenon observed in cells treated with cetuximab and gefitinib [28, 38, 53]. This phenomenon may be a rescue mechanism by which a cell becomes more reliant on internal kinase-independent functions of EGFR. Additionally, Weihua et al. further showed that a kinase-dead EGFR is capable of inhibiting autophagic cell death in cancer cell lines, demonstrating that EGFR induced tumorigenesis is independent of its kinase activity [61]. Researchers have further demonstrated that a kinase-dead EGFR can undergo endocytosis [62], and work from our lab has indicated that kinase-dead EGFR can effectively translocate to the nucleus (Figure 3). Collectively, these data suggest that 1) nuclear EGFR is not accurately targeted by kinase inhibiting anti-EGFR therapeutics, and 2) that nuclear EGFR translocation and function may be independent of kinase activity, a mechanism by which a cancer cell can sustain enhanced growth and survival.

Targeting nuclear EGFR via AKT Inhibition

The initial studies aimed at targeting nuclear EGFR were focused in cell lines that demonstrated resistance to both cetuximab and gefitinib therapies [28, 38]. These cell lines were established by treating cells *in-vitro* with increasing amounts of drug over a several month time period until resistance was observed. Cell lines in both models of resistance demonstrated enhanced nuclear localization of the EGFR as compared to sensitive parental lines [28, 38]. In the gefitinib resistant setting, Huang et al. demonstrated that the activity of AKT was enhanced, and that EGFR was specifically phosphorylated by AKT on Serine 229 to promote EGFR nuclear translocation [28]. Importantly, the overexpression of EGFR mutated at Serine 229 or the use of an AKT inhibitor rendered resistant cells more sensitive to gefitinib. Collectively, these data suggest that AKT inhibition may successfully inhibit nuclear EGFR transport and thereby sensitize cells to anti-EGFR therapies such as gefitinib [28].

Targeting nuclear EGFR via Src Family Kinase Inhibition

In the setting of cetuximab resistance, work from our laboratory has shown that resistant cells have both enhanced nuclear EGFR and SFK activity [38, 55]. Initial studies demonstrated that SFK inhibition of nuclear EGFR transport could enhance sensitivity of cells to cetuximab therapy [38]. Further research identified that the SFK family members Yes and Lyn were overexpressed in cetuximab resistant clones and that these SFKs directly phosphorylated EGFR at Tyrosine 1101 to initiate EGFR nuclear translocation [63]. These data demonstrate that nuclear EGFR may be accurately targeted via SFK inhibition of phospho-Tyrosine 1101. Interestingly, current anti-EGFR therapies do not inhibit the activation of EGFR at Tyrosine 1101 (Figure 4), which may further explain why these

agents do not accurately target nuclear EGFR. Collectively, the inhibition of EGFR at Tyrosine 1101 and Serine 229 activity (Figure 5) with both SFK and AKT inhibitors may lead to the complete inhibition of nuclear EGFR translocation, and sensitization of cells to current anti-EGFR agents [28, 38, 63].

Targeting nuclear EGFR via the selective COX-2 Inhibitor Celecoxib

The COX-2 inhibitor Celecoxib has demonstrated radiosensitizing effects in various tumors [64, 65]. Interestingly, anti-tumor effects of Celecoxib treatment have been observed in cell lines that did not express COX-2 to a high degree [66, 67]. To elucidate the mechanism of Celecoxib radiosensitization, Dittmann et al. demonstrated that Celecoxib could inhibit both basal and radiation-induced nuclear EGFR translocation in various cancer cell lines [68]. Celecoxib also inhibited radiation-induced phosphorylation of DNA-PK at Threonine 2609 only in cell lines that were radiosensitized with this drug. Overall, this study highlights a COX-2 independent mechanism of Celecoxib radiosensitization through the direct inhibition of nuclear EGFR translocation [68]. Thus, this inhibitor may prove to be useful for treatment of nuclear EGFR expressing tumors, even for tumors lacking COX-2 expression.

Targeting nuclear EGFR via PCNA inhibition

Another method of targeting nuclear EGFR is to directly inhibit the activity of its effector molecules. One main effector molecule of nuclear EGFR is PCNA. Nuclear EGFR was initially shown to associate and phosphorylate PCNA at tyrosine 211, which resulted in PCNA stabilization on chromatin and decreased proteosomal degradation, ultimately enhancing DNA replication and repair [69]. In a recent study by Yu et al. researchers demonstrate that treatment of triple negative breast cancer (TNBC) cells with an anti-PCNA peptide (targeting Tyrosine 211) can inhibit growth of cells *in-vitro* and *in-vivo* mouse models [59]. Additionally, researchers established both gefitinib and erlotinib resistant cell lines, both of which contained enhanced nuclear EGFR and activated PCNA levels. Treatment of these resistant cell lines with the anti-PCNA peptide sensitized all cell lines to their respective therapies. These studies suggest that the inhibition of the nuclear EGFR effector molecule PCNA may enhance the dependency of cells on classical EGFR signaling pathways and thereby re-sensitize them to EGFR inhibitors.

Prospective Targets of Nuclear EGFR Translocation and/or Function

Over the past five years various studies have identified key proteins that play a role in the regulation of nuclear EGFR translocation and function. The transmembrane protein mucin-1 (MUC1) [70] and RNA helicase A (RHA) [71] have been shown to be instrumental for EGFR association with target gene promoters in the nucleus. Additionally, the transcriptional co-factor Tat interacting protein (TIP3) has been shown to suppress nuclear EGFR translocation, while conversely its loss enhances nuclear EGFR translocation and function [72]. Finally, the latent membrane protein 1 (LMP1) has been shown to promote the interaction of nuclear EGFR with transcriptional intermediary factor 2 (TIF2) to upregulate cyclin D1 expression [73]. Finally, Wanner et al. demonstrated that PKC could phosphorylate EGFR at Threonine 654 to induce EGFR's nuclear translocation in response to radiation therapy [49]. PKC inhibitors such as midostaurin (PKC 412) and enzastaurin (LY317615) have been available since the early 2000s and have shown radiosensitizing capabilities, however, their ability to target nuclear EGFR has yet to be examined [74]. Collectively, the proteins MUC1, RHA, TIP2, LMP1, and PKC may be potential future targets for the inhibition of both nuclear EGFR translocation and function.

Future Prospective of the Nuclear RTK Field

From the identification of nuclear localized EGFR in highly proliferative tissues to the uncovering of its vibrant roles in enhancing tumorigenic processes, the field of nuclear HER family receptors has blossomed over the past ten years. Even with the expose of over 50 articles citing the presence and/or functions of nuclear EGFR in cancer, this field is still in its infancy. Studies have yet to show that nuclear EGFR functions as a true oncogene separate from its membrane-localized counterpart. The answer to this question lies in the ability to isolate nuclear EGFR and demonstrate that it can lead to the formation and/or progression of cancer on its own. This feat is extremely hard to combat in the laboratory, since membrane-, cytoplasmic-, and nuclear localized EGFR function simultaneously to elicit downstream oncogenic effects. With the knowledge that nuclear EGFR plays a role in resistance to various cancer therapeutics, and that it is correlated with worse overall survival in numerous cancers, there is an over-arching need to target this nuclear receptor and potentially use it as a biomarker to predict therapeutic outcome in the future.

Materials and Methods

Cell Lines

The human HNSCC cell lines SCC1, SCC6, and SCC1483 were kindly supplied by Dr. T. Carey (University of Michigan, MI, USA) [75]. The human breast cancer cell lines SKBr3, MDAMB468, and SUM229 were kindly supplied by Dr. J. Boerner (Wayne State University School of Medicine, Karmanos Cancer Institute, MI, USA) [76]. The human MCF-7 and hamster CHOK1 cells were purchased from ATCC (Manassas, VA, USA). All cell lines were maintained in their respective media with 10% fetal bovine serum and 1% penicillin and streptomycin; SCC1, SCC6, SCC1483, SKBR3, and SUM229 cells were maintained in Dulbecco's modified Eagle's medium (Mediatech Inc., Manassas, VA, USA); MDAMB468 and MCF-7 cells were maintained in DMEM/F12K medium (Mediatech Inc.); and CHOK1 cells were maintained in F12K medium (Mediatech Inc.).

Antibodies and Compounds

All antibodies were obtained from the following sources: EGFR (SC-03), Histone H3, and HRP-conjugated goat-anti-rabbit IgG, goat-anti mouse IgG and donkey-anti-goat IgG were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). pEGFR-Y1101 was obtained from Abcam (Cambridge, MA, USA). α -tubulin was purchased from Calbiochem (San Diego, CA, USA). Cetuximab (C225, Erbitux) was generously provided by ImClone Systems Inc (New York, NY, USA), erlotinib (OSI-774, Tarceva) was kindly provided by OSI Pharmaceuticals (Farmingdale, NY, USA) and lapatinib (GW572016, Tykerb) was purchased from LC Laboratories (Woburn, MA, USA). EGF was purchased from R&D systems (Minneapolis, MN, USA).

Cellular Fractionation and Immunoblotting Analysis

To obtain nuclear proteins, cells were plated in 15 cm dishes. At ~80–90% confluency, cells were scraped in PBS and swelled in cytoplasmic lysis buffer (20 mM HEPES, pH 7.0, 10 mM KCl, 2 mM MgCl₂, 0.5% NP40, 1 mM Na₃VO₄, 1 mM PMSF, 1 mM beta-glycerophosphate (BGP), 10 ug/ml of leupeptin and aprotinin) for 15 min on ice. Cells were then homogenized by 30–40 strokes in a tightly fitting Dounce homogenizer and checked under microscope for intact nuclei. The homogenate was centrifuged at 1,500 g for 5 min at 4°C to sediment the nuclei. The nuclear pellet was washed 5 times in cytoplasmic lysis buffer to ensure complete removal of cytosolic membranes. After washes, the nuclear pellet was lysed in the same buffer with the addition of 0.5 M NaCl. Nuclear pellets were sonicated for 10 sec, and vortexed for 30 sec 3 times. The extracted nuclear lysate was

centrifuged at 15,000 g for 10 min at 4°C, and the supernatants were collected as nuclear lysate. Whole cell protein lysate was obtained through lysis with RIPA buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 1% Tween-20, 10% glycerol, 2.5 mM EGTA, 1 mM EDTA, 1 mM DTT, 1 mM Na₃VO₄, 1 mM PMSF, 1 mM BGP, and 10 µg/ml of leupeptin and aprotinin). Samples were sonicated for 10 sec, and then centrifuged at 15,000 g for 10 min at 4°C. All protein lysates were quantified via Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of protein were fractionated by SDA-PAGE, transferred to a PVDF membrane (Millipore, Billerica, MA, USA), and analyzed by incubation with the appropriate primary antibody overnight at 4°C. Membranes were then subjected to incubation with HRP-conjugated secondary antibodies for 1 hr at room temperature. ECL chemiluminescence detection system was used to visual proteins with ECL Western Blotting Substrate (Promega Cooperation, Madison, WI, USA).

Plasmid Construction and Transfection

pcDNA3-Wild-Type EGFR was kindly provided by Dr. J. Boerner (Wayne State University School of Medicine, Karmanos Cancer Institute, MI, USA). Kinase-dead EGFR K721A, EGFR-Y1101F, and EGFR-S229A were created via QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) following the manufacturer's instructions. All mutations were verified for correct orientation and integrity via sequencing. All plasmid transfections were performed using Lipofectamine LTX and Opti-MEM I (Invitrogen) according to the manufacturer's instructions. Cells were analyzed 48 hr post transfection for expression and nuclear localization of EGFR.

Abbreviations

EGFR	epidermal growth factor receptor
NLS	nuclear localization signal
DNA-PK	DNA protein kinase
PCNA	proliferating cell nuclear antigen
NPC	nuclear pore complex
ONM	outer nuclear membrane
INM	inner nuclear membrane
iNOS	nitric oxide synthase
COX-2	cyclooxygenase-2
BCRP	breast cancer resistant protein
HNSCC	head and neck squamous cell carcinoma
NLS	nuclear localization sequence
NSLC	non-small cell lung cancer
TKI	tyrosine kinase inhibitor
PNPase	polynucleotide phosphorylase
TNBC	triple negative breast cancer
MUC1	mucin-1
RHA	RNA helicase A
TIP3	TAT interacting protein

LMP1 latent membrane protein 1
TIF2 transcriptional intermediary factor 2

References

1. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol.* 2001; 2:127–137. [PubMed: 11252954]
2. The scent of Nobel Prize success. *Funct Neurol.* 2004; 19:217–218. [PubMed: 15776788]
3. Marmor MD, Skaria KB, Yarden Y. Signal transduction and oncogenesis by ErbB/HER receptors. *Int J Radiat Oncol Biol Phys.* 2004; 58:903–913. [PubMed: 14967450]
4. Libermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, Whittle N, Waterfield MD, Ullrich A, Schlessinger J. Amplification and overexpression of the EGF receptor gene in primary human glioblastomas. *J Cell Sci Suppl.* 1985; 3:161–172. [PubMed: 3011820]
5. Libermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, Whittle N, Waterfield MD, Ullrich A, Schlessinger J. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. *Nature.* 1985; 313:144–147. [PubMed: 2981413]
6. Libermann TA, Razon N, Bartal AD, Yarden Y, Schlessinger J, Soreq H. Expression of epidermal growth factor receptors in human brain tumors. *Cancer Res.* 1984; 44:753–760. [PubMed: 6318976]
7. Veale D, Ashcroft T, Marsh C, Gibson GJ, Harris AL. Epidermal growth factor receptors in non-small cell lung cancer. *Br J Cancer.* 1987; 55:513–516. [PubMed: 3038157]
8. Weichselbaum RR, Dunphy EJ, Beckett MA, Tybor AG, Moran WJ, Goldman ME, Vokes EE, Panje WR. Epidermal growth factor receptor gene amplification and expression in head and neck cancer cell lines. *Head Neck.* 1989; 11:437–442. [PubMed: 2807884]
9. Sato JD, Kawamoto T, Le AD, Mendelsohn J, Polikoff J, Sato GH. Biological effects in vitro of monoclonal antibodies to human epidermal growth factor receptors. *Mol Biol Med.* 1983; 1:511–529. [PubMed: 6094961]
10. Kawamoto T, Sato JD, Le A, Polikoff J, Sato GH, Mendelsohn J. Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. *Proc Natl Acad Sci U S A.* 1983; 80:1337–1341. [PubMed: 6298788]
11. Sunada H, Magun BE, Mendelsohn J, MacLeod CL. Monoclonal antibody against epidermal growth factor receptor is internalized without stimulating receptor phosphorylation. *Proc Natl Acad Sci U S A.* 1986; 83:3825–3829. [PubMed: 2424012]
12. Brand TM, Iida M, Li C, Wheeler DL. The nuclear epidermal growth factor receptor signaling network and its role in cancer. *Discovery medicine.* 2011; 12:419–432. [PubMed: 22127113]
13. Wang YN, Yamaguchi H, Hsu JM, Hung MC. Nuclear trafficking of the epidermal growth factor receptor family membrane proteins. *Oncogene.* 2010; 29:3997–4006. [PubMed: 20473332]
14. Han W, Lo HW. Landscape of EGFR signaling network in human cancers: biology and therapeutic response in relation to receptor subcellular locations. *Cancer letters.* 2012; 318:124–134. [PubMed: 22261334]
15. Wang YN, Yamaguchi H, Huo L, Du Y, Lee HJ, Lee HH, Wang H, Hsu JM, Hung MC. The translocon Sec61beta localized in the inner nuclear membrane transports membrane-embedded EGF receptor to the nucleus. *J Biol Chem.* 2010; 285:38720–38729. [PubMed: 20937808]
16. Wang YN, Wang H, Yamaguchi H, Lee HJ, Lee HH, Hung MC. COPI-mediated retrograde trafficking from the Golgi to the ER regulates EGFR nuclear transport. *Biochemical and biophysical research communications.* 2010; 399:498–504. [PubMed: 20674546]
17. Lo HW, Ali-Sayed M, Wu Y, Bartholomeusz G, Hsu SC, Hung MC. Nuclear-cytoplasmic transport of EGFR involves receptor endocytosis, importin beta1 and CRM1. *J Cell Biochem.* 2006; 98:1570–1583. [PubMed: 16552725]
18. Liao HJ, Carpenter G. Role of the Sec61 translocon in EGF receptor trafficking to the nucleus and gene expression. *Mol Biol Cell.* 2007; 18:1064–1072. [PubMed: 17215517]

19. Hsu SC, Hung MC. Characterization of a novel tripartite nuclear localization sequence in the EGFR family. *J Biol Chem.* 2007; 282:10432–10440. [PubMed: 17283074]
20. Wang YN, Lee HH, Lee HJ, Du Y, Yamaguchi H, Hung MC. Membrane-bound Trafficking Regulates Nuclear Transport of Integral Epidermal Growth Factor Receptor (EGFR) and ErbB-2. *Journal of Biological Chemistry.* 2012; 287:16869–16879. [PubMed: 22451678]
21. Eldredge ER, Korf GM, Christensen TA, Connolly DC, Getz MJ, Maihle NJ. Activation of c-fos gene expression by a kinase-deficient epidermal growth factor receptor. *Molecular and cellular biology.* 1994; 14:7527–7534. [PubMed: 7935468]
22. Lin SY, Makino K, Xia W, Martin A, Wen Y, Kwong KY, Bourguignon L, Hung MC. Nuclear localization of EGF receptor and its potential new role as a transcription factor. *Nat Cell Biol.* 2001; 3:802–808. [PubMed: 11533659]
23. Lo HW, Hsu SC, Ali-Sayed M, Gunduz M, Xia W, Wei Y, Bartholomeusz G, Shih JY, Hung MC. Nuclear interaction of EGFR and STAT3 in the activation of the iNOS/NO pathway. *Cancer Cell.* 2005; 7:575–589. [PubMed: 15950906]
24. Hanada N, Lo HW, Day CP, Pan Y, Nakajima Y, Hung MC. Co-regulation of B-Myb expression by E2F1 and EGF receptor. *Mol Carcinog.* 2006; 45:10–17. [PubMed: 16299810]
25. Lo HW, Cao X, Zhu H, Ali-Osman F. Cyclooxygenase-2 is a novel transcriptional target of the nuclear EGFR-STAT3 and EGFRvIII-STAT3 signaling axes. *Mol Cancer Res.* 2010; 8:232–245. [PubMed: 20145033]
26. Hung LY, Tseng JT, Lee YC, Xia W, Wang YN, Wu ML, Chuang YH, Lai CH, Chang WC. Nuclear epidermal growth factor receptor (EGFR) interacts with signal transducer and activator of transcription 5 (STAT5) in activating Aurora-A gene expression. *Nucleic Acids Res.* 2008; 36:4337–4351. [PubMed: 18586824]
27. Jaganathan S, Yue P, Paladino DC, Bogdanovic J, Huo Q, Turkson J. A functional nuclear epidermal growth factor receptor, SRC and stat3 heteromeric complex in pancreatic cancer cells. *Plos One.* 2011; 6:e19605. [PubMed: 21573184]
28. Huang WC, Chen YJ, Li LY, Wei YL, Hsu SC, Tsai SL, Chiu PC, Huang WP, Wang YN, Chen CH, et al. Nuclear Translocation of Epidermal Growth Factor Receptor by Akt-dependent Phosphorylation Enhances Breast Cancer-resistant Protein Expression in Gefitinib-resistant Cells. *J Biol Chem.* 2011; 286:20558–20568. [PubMed: 21487020]
29. Dittmann K, Mayer C, Rodemann HP. Inhibition of radiation-induced EGFR nuclear import by C225 (Cetuximab) suppresses DNA-PK activity. *Radiother Oncol.* 2005; 76:157–161. [PubMed: 16024112]
30. Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Raju U, Milas L, Chen DJ, Kehlbach R, Rodemann HP. Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. *J Biol Chem.* 2005; 280:31182–31189. [PubMed: 16000298]
31. Psyrri A, Yu Z, Weinberger PM, Sasaki C, Haffty B, Camp R, Rimm D, Burtneess BA. Quantitative determination of nuclear and cytoplasmic epidermal growth factor receptor expression in oropharyngeal squamous cell cancer by using automated quantitative analysis. *Clin Cancer Res.* 2005; 11:5856–5862. [PubMed: 16115926]
32. Lo HW, Xia W, Wei Y, Ali-Sayed M, Huang SF, Hung MC. Novel prognostic value of nuclear epidermal growth factor receptor in breast cancer. *Cancer Res.* 2005; 65:338–348. [PubMed: 15665312]
33. Xia W, Wei Y, Du Y, Liu J, Chang B, Yu YL, Huo LF, Miller S, Hung MC. Nuclear expression of epidermal growth factor receptor is a novel prognostic value in patients with ovarian cancer. *Mol Carcinog.* 2009; 48:610–617. [PubMed: 19058255]
34. Hoshino M, Fukui H, Ono Y, Sekikawa A, Ichikawa K, Tomita S, Imai Y, Imura J, Hiraishi H, Fujimori T. Nuclear expression of phosphorylated EGFR is associated with poor prognosis of patients with esophageal squamous cell carcinoma. *Pathobiology.* 2007; 74:15–21. [PubMed: 17496429]
35. Li CF, Fang FM, Wang JM, Tzeng CC, Tai HC, Wei YC, Li SH, Lee YT, Wang YH, Yu SC, et al. EGFR Nuclear Import in Gallbladder Carcinoma: Nuclear Phosphorylated EGFR Upregulates iNOS Expression and Confers Independent Prognostic Impact. *Annals of surgical oncology.* 2011

36. Traynor AM, Weigel TM, Oettel KR, Yang DT, Zhang C, Kim K, Salgia R, Iida M, Brand TM, Hoang T, et al. Nuclear EGFR expression predicts poor survival in resected non-small cell lung cancer. *Journal of thoracic oncology: official publication of the International Association for the Study of Lung Cancer*. 2012
37. Liccardi G, Hartley JA, Hochhauser D. EGFR nuclear translocation modulates DNA repair following cisplatin and ionizing radiation treatment. *Cancer Res*. 2011; 71:1103–1114. [PubMed: 21266349]
38. Li C, Iida M, Dunn EF, Ghia AJ, Wheeler DL. Nuclear EGFR contributes to acquired resistance to cetuximab. *Oncogene*. 2009; 28:3801–3813. [PubMed: 19684613]
39. Hsu SC, Miller SA, Wang Y, Hung MC. Nuclear EGFR is required for cisplatin resistance and DNA repair. *American journal of translational research*. 2009; 1:249–258. [PubMed: 19956435]
40. Curtin NJ. DNA repair dysregulation from cancer driver to therapeutic target. *Nat Rev Cancer*. 2012; 12:801–817. [PubMed: 23175119]
41. SchmidtUllrich RK, Mikkelsen RB, Dent P, Todd DG, Valerie K, Kavanagh BD, Contessa JN, Rorrer WK, Chen PB. Radiation-induced proliferation of the human A431 squamous carcinoma cells is dependent on EGFR tyrosine phosphorylation. *Oncogene*. 1997; 15:1191–1197. [PubMed: 9294612]
42. Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. *Cancer Res*. 1999; 59:1935–1940. [PubMed: 10213503]
43. Huang SM, Li J, Harari PM. Monoclonal antibody blockade of the epidermal growth factor receptor in cancer therapy. *Cancer Chemother Biol Response Modif*. 2001; 19:339–352. [PubMed: 11686022]
44. Dittmann K, Mayer C, Kehlbach R, Rodemann HP. Radiation-induced caveolin-1 associated EGFR internalization is linked with nuclear EGFR transport and activation of DNA-PK. *Mol Cancer*. 2008; 7:69. [PubMed: 18789131]
45. Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Kehlbach R, Rodemann HP. Nuclear EGFR shuttling induced by ionizing radiation is regulated by phosphorylation at residue Thr654. *FEBS Lett*. 2010; 584:3878–3884. [PubMed: 20692258]
46. Dittmann K, Mayer C, Wanner G, Kehlbach R, Rodemann HP. The radioprotector O-phosphotyrosine stimulates DNA-repair via epidermal growth factor receptor- and DNA-dependent kinase phosphorylation. *Radiother Oncol*. 2007; 84:328–334. [PubMed: 17714814]
47. Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Kehlbach R, Rodemann HP. Nuclear epidermal growth factor receptor modulates cellular radio-sensitivity by regulation of chromatin access. *Radiother Oncol*. 2011
48. Dittmann K, Mayer C, Kehlbach R, Rodemann HP. The radioprotector Bowman- Birk proteinase inhibitor stimulates DNA repair via epidermal growth factor receptor phosphorylation and nuclear transport. *Radiother Oncol*. 2008; 86:375–382. [PubMed: 18237807]
49. Wanner G, Mayer C, Kehlbach R, Rodemann HP, Dittmann K. Activation of protein kinase C epsilon stimulates DNA-repair via epidermal growth factor receptor nuclear accumulation. *Radiotherapy and Oncology*. 2008; 86:383–390. [PubMed: 18037521]
50. Yu YL, Chou RH, Wu CH, Wang YN, Chang WJ, Tseng YJ, Chang WC, Lai CC, Lee HJ, Huo LF, et al. Nuclear EGFR Suppresses Ribonuclease Activity of Polynucleotide Phosphorylase through DNAPK-mediated Phosphorylation at Serine 776. *Journal of Biological Chemistry*. 2012; 287:31015–31026. [PubMed: 22815474]
51. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov*. 2005; 4:307–320. [PubMed: 15789122]
52. Wheeler DL, Huang S, Kruser TJ, Nechrebecki MM, Armstrong EA, Benavente S, Gondi V, Hsu KT, Harari PM. Mechanisms of acquired resistance to cetuximab: role of HER (ErbB) family members. *Oncogene*. 2008; 27:3944–3956. [PubMed: 18297114]
53. Liao HJ, Carpenter G. Cetuximab/C225-induced intracellular trafficking of epidermal growth factor receptor. *Cancer Res*. 2009; 69:6179–6183. [PubMed: 19602593]

54. Li C, Iida M, Dunn EF, Wheeler DL. Dasatinib blocks cetuximab- and radiation-induced nuclear translocation of the epidermal growth factor receptor in head and neck squamous cell carcinoma. *Radiother Oncol.* 2010; 97:330–337. [PubMed: 20667610]
55. Wheeler DL, Iida M, Kruser TJ, Nechrebecki MM, Dunn EF, Armstrong EA, Huang S, Harari PM. Epidermal growth factor receptor cooperates with Src family kinases in acquired resistance to cetuximab. *Cancer Biol Ther.* 2009; 8:696–703. [PubMed: 19276677]
56. Wheeler DL, Dunn EF, Harari PM. Understanding resistance to EGFR inhibitors-impact on future treatment strategies. *Nat Rev Clin Oncol.* 2010; 7:493–507. [PubMed: 20551942]
57. Brand TM, Iida M, Wheeler DL. Molecular mechanisms of resistance to the EGFR monoclonal antibody cetuximab. *Cancer biology & therapy.* 2011:11.
58. Kim HP, Yoon YK, Kim JW, Han SW, Hur HS, Park J, Lee JH, Oh DY, Im SA, Bang YJ, Kim TY. Lapatinib, a Dual EGFR and HER2 Tyrosine Kinase Inhibitor, Downregulates Thymidylate Synthase by Inhibiting the Nuclear Translocation of EGFR and HER2. *Plos One.* 2009:4.
59. Yu YL, Chou RH, Liang JH, Chang WJ, Su KJ, Tseng YJ, Huang WC, Wang SC, Hung MC. Targeting the EGFR/PCNA Signaling Suppresses Tumor Growth of Triple-Negative Breast Cancer Cells with Cell-Penetrating PCNA Peptides. *Plos One.* :8.
60. Oliveras-Ferreros C, Vazquez-Martin A, Lopez-Bonet E, Martin-Castillo B, Del Barco S, Brunet J, Menendez JA. Growth and molecular interactions of the anti-EGFR antibody cetuximab and the DNA cross-linking agent cisplatin in gefitinib-resistant MDA-MB-468 cells: new prospects in the treatment of triple-negative/basal-like breast cancer. *Int J Oncol.* 2008; 33:1165–1176. [PubMed: 19020749]
61. Weihua Z, Tsan R, Huang WC, Wu Q, Chiu CH, Fidler IJ, Hung MC. Survival of cancer cells is maintained by EGFR independent of its kinase activity. *Cancer Cell.* 2008; 13:385–393. [PubMed: 18455122]
62. Wang Q, Villeneuve G, Wang Z. Control of epidermal growth factor receptor endocytosis by receptor dimerization, rather than receptor kinase activation. *EMBO Rep.* 2005; 6:942–948. [PubMed: 16113650]
63. Iida M, Brand TM, Campbell DA, Li C, Wheeler DL. Yes and Lyn play a role in nuclear translocation of the epidermal growth factor receptor. *Oncogene.* 2012
64. Milas L. Cyclooxygenase-2 (COX-2) enzyme inhibitors as potential enhancers of tumor radioresponse. *Semin Radiat Oncol.* 2001; 11:290–299. [PubMed: 11677654]
65. Choy H, Milas L. Enhancing radiotherapy with cyclooxygenase-2 enzyme inhibitors: A rational advance? *J Natl Cancer I.* 2003; 95:1440–1452.
66. Maier TJ, Schilling K, Schmidt R, Geisslinger G, Grosch S. Cyclooxygenase-2 (COX- 2)-dependent and -independent anticarcinogenic effects of celecoxib in human colon carcinoma cells. *Biochem Pharmacol.* 2004; 67:1469–1478. [PubMed: 15041464]
67. Patel MI, Subbaramaiah K, Du BH, Chang M, Yang PY, Newman RA, Cordon-Cardo C, Thaler HT, Dannenberg AJ. Celecoxib inhibits prostate cancer growth: Evidence of a cyclooxygenase-2-independent mechanism. *Clin Cancer Res.* 2005; 11:1999–2007. [PubMed: 15756026]
68. Dittmann KH, Mayer C, Ohneseit PA, Raju U, Andratschke NH, Milas L, Rodemann HP. Celecoxib induced tumor cell radiosensitization by inhibiting radiation induced nuclear EGFR transport and DNA-repair: a COX-2 independent mechanism. *Int J Radiat Oncol Biol Phys.* 2008; 70:203–212. [PubMed: 17996386]
69. Wang SC, Nakajima Y, Yu YL, Xia W, Chen CT, Yang CC, McIntush EW, Li LY, Hawke DH, Kobayashi R, Hung MC. Tyrosine phosphorylation controls PCNA function through protein stability. *Nat Cell Biol.* 2006; 8:1359–1368. [PubMed: 17115032]
70. Bitler BG, Goverdhan A, Schroeder JA. MUC1 regulates nuclear localization and function of the epidermal growth factor receptor. *J Cell Sci.* 2010; 123:1716–1723. [PubMed: 20406885]
71. Huo LF, Wang YN, Xia WY, Hsu SC, Lai CC, Li LY, Chang WC, Wang Y, Hsu MC, Yu YL, et al. RNA helicase A is a DNA-binding partner for EGFR-mediated transcriptional activation in the nucleus. *P Natl Acad Sci USA.* 2010; 107:16125–16130.
72. Li A, Zhang C, Gao S, Chen F, Yang C, Luo R, Xiao H. TIP30 loss enhances cytoplasmic and nuclear EGFR signaling and promotes lung adenocarcinogenesis in mice. *Oncogene.* 2012

73. Shi Y, Tao Y, Jiang Y, Xu Y, Yan B, Chen X, Xiao L, Cao Y. Nuclear epidermal growth factor receptor interacts with transcriptional intermediary factor 2 to activate cyclin D1 gene expression triggered by the oncoprotein latent membrane protein 1. *Carcinogenesis*. 2012; 33:1468–1478. [PubMed: 22581837]
74. Mackay HJ, Twelves CJ. Targeting the protein kinase C family: are we there yet? *Nature Reviews Cancer*. 2008; 8:255–255.
75. Brenner JC, Graham MP, Kumar B, Saunders LM, Kupfer R, Lyons RH, Bradford CR, Carey TE. Genotyping of 73 Um-Scc Head and Neck Squamous Cell Carcinoma Cell Lines. *Head Neck-J Sci Spec*. 2010; 32:417–426.
76. Irwin ME, Mueller KL, Bohin N, Ge YB, Boerner JL. Lipid Raft Localization of EGFR Alters the Response of Cancer Cells to the EGFR Tyrosine Kinase Inhibitor Gefitinib. *J Cell Physiol*. 2011; 226:2316–2328. [PubMed: 21660955]

Figure 1. Nuclear EGFR Translocation and Function

The nuclear translocation of EGFR has been shown to be dependent on specific phosphorylation events by various intracellular kinases. EGFR phosphorylation at Tyrosine 1101 by SFKs, Serine 229 by AKT, and Threonine 654 by PKC, have all been shown to stimulate nuclear EGFR translocation. In the nucleus, EGFR has been shown to function as a co-transcription factor alongside STAT3, E2F1, and STAT5 to enhance the transcription of eight gene targets. Nuclear EGFR can also activate and stabilize DNA-PK and PCNA to enhance DNA repair and replication. Collectively, these functions may be inhibited through drugs that target the intracellular kinases identified to influence nuclear EGFR translocation, and thereby sensitize cancer cells to radiation, chemotherapy, and anti-EGFR therapies such as cetuximab and gefitinib.

Figure 2. Current anti-EGFR therapies do not inhibit nuclear EGFR localization

A) Whole cell lysate was harvested from the HNSCC cell lines SCC1, SCC6, and SCC1483, and from the breast cancer cell lines SKBr3, MDAMB468, and SUM229 24 hr post treatment with 100nM erlotinib or lapatinib therapies. Lysates were fractionated on SDS-PAGE followed by immunoblotting for EGFR, pEGFR-Y1173, and α -tubulin as a loading control. **B)** Non-nuclear and nuclear proteins were harvested from the same cell lines 24 hr post treatment with 100nM erlotinib or lapatinib therapies. Lysates were fractionated on SDS-PAGE followed by immunoblotting for EGFR. α -tubulin and Histone H3 were used as loading and purity controls for the non-nuclear and nuclear fractions, respectively.

Figure 3. Kinase dead EGFR can effectively translocate to the nucleus

CHOK1 cells were transfected with vector control, EGFR-wild type (WT) or EGFR-kinase dead (KD) for 48 hr prior to harvesting non-nuclear and nuclear protein. Lysates were fractionated on SDS-PAGE followed by immunoblotting for EGFR. α -tubulin and Histone H3 were used as loading and purity controls for the non-nuclear and nuclear fractions, respectively.

Figure 4. Current anti-EGFR targeted therapies do not inhibit the phosphorylation of EGFR at Tyrosine 1101

SUM149 and MDAMB468 cells were treated with 100nM cetuximab or erlotinib for 24 hr. Whole cell lysate was fractionated on SDS-PAGE followed by immunoblotting for EGFR and phospho-EGFR Y1101. β -tubulin was used as a loading control. V; vector control, CTX; cetuximab, ERL; erlotinib

Figure 5. Phosphorylation of Tyrosine 1101 and Serine 229 influence nuclear EGFR translocation

CHOK1 cells were transfected with vector control, EGFR-WT, EGFR-Y1101F, or EGFR-S229A for 48 hr prior to harvesting non-nuclear and nuclear protein. Lysates were fractionated on SDS-PAGE followed by immunoblotting for EGFR. α -tubulin and Histone H3 were used as loading and purity controls for the non-nuclear and nuclear fractions, respectively.