

EGFR-targeted Cancer Therapy: Promise, Problems and Potential Solutions

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Deregulation of epidermal growth factor receptor, EGFR, due to gene amplification, somatic mutations and/or transcriptional up-regulation has been reported in almost all types of human cancers. Aberrant EGFR signaling is associated with tumorigenesis, tumor growth and progression [1,2]. Consequently, EGFR has taken center stage of cancer therapeutics and various therapies have been developed to target EGFR-expressing tumors. Presently, five EGFR-targeted agents have been approved by the FDA for treating cancer patients, including, three small molecule tyrosine kinase inhibitors (Gefitinib/ZD1839/Iressa; Erlotinib/OSI-774/Tarceva; Lapatinib/GW572016/Tykerb/Tyverb) and two therapeutic antibodies (Cetuximab/C225/Erbix; Panitumumab/ABX-EGF/Vectibix). The clinical benefits of EGFR-targeted therapy are often modest [2-5] with the exception of the lung cancer carrying specific activation mutations within the EGFR kinase domain which render the receptor highly sensitive to EGFR kinase inhibition [6-8]. These mutations are absent or very rare in other types of cancers. Therefore, it remains an urgent task to gain a deeper understanding of the biology of EGFR-dependent tumors and the drug-resistance phenotype, in order to derive new rationales to maximize the clinical efficacy of EGFR-targeted therapy against cancer.

Atypical modes of the EGFR signaling pathway may underlie the inability of EGFR-targeted therapy to yield substantial clinical benefits. Although EGFR has been defined as a receptor tyrosine kinase that elicits its functions while localized on the plasma membranes, this traditional view has been rapidly revised by compelling evidence that uncovered several long overlooked atypical modes of EGFR signaling. Importantly, evidence to date indicates that these atypical functions of EGFR regulate tumor behaviors and potentially impact tumor cell response to EGFR-targeted therapy, as well as, other anti-cancer therapies, such as, chemotherapy and radiation therapy. The major atypical actions of EGFR that have been revealed to date are: (i) the kinase-independent functions of plasma membrane-bound EGFR that have been shown to regulate cell proliferation, apoptosis and glucose uptake [9-15], (ii) nuclear EGFR that transcriptionally regulates gene expression via its transactivation activity and protein-protein interactions [16-23], as well as, modulates DNA repair by its kinase activity and interactions with nuclear proteins [24-29], and (iii) mitochondrially localized EGFR that modulates apoptosis and autophagy, and consequently drug resistance [30-34].

First, the kinase-independent activity of EGFR could underlie the failure of EGFR kinase inhibitors. Independent of kinase activity or ligand activation, EGFR can mediate cellular processes mostly through its ability to physically interact with other proteins. Loss of EGFR kinase activity did not lead to the phenotypes similar to ablation of EGFR expression [35]. Kinase-dead EGFR mutants retained the ability to stimulate DNA synthesis [9] and promoted survival [11]. In line with these observations, loss of EGFR expression, but not its kinase activity, resulted in autophagic cell death [12]. This was likely attributed to the ability of cell-surface EGFR to physically interact with and stabilize sodium/glucose cotransporter 1 and to maintain high glucose levels in the cells. Interestingly, two recent studies indicate that EGFR activates Akt independent of its kinase activity while localized within the lipid raft microdomain of the plasma membrane, leading to Iressa resistance [14,15]. Conversely, using lovastatin to deplete cholesterol, an essential component of the lipid rafts, sensitized the cells to EGFR kinase inhibition. Most recently, our laboratory reported

that through physical associations but not kinase activity, EGFR can modulate protein subcellular trafficking [13]. In this context, EGFR associates with p53-upregulated modulator of apoptosis (PUMA), a proapoptotic member of the Bcl-2 family of proteins primarily located on the mitochondria [36,37] and sequesters PUMA in the cytoplasm, leading to apoptosis resistance [13]. Rationalized by these notions, dual targeting of the kinase-dependent and -independent functions of EGFR may achieve a greater clinical outcome than inhibiting the kinase activity of the receptor solely.

Second, EGFR nuclear translocalization contributes to tumor cell growth and resistance to anti-cancer therapy. Nuclear presence of full-length EGFR has been reported for over 20 years; however, its impact on tumor behavior was not elucidated until the past decade. EGFRvIII, a constitutively active variant of EGFR, can be detected in prostate cancer [38] and in malignant gliomas [20,39]. Within the cell nucleus, EGFR functions as a transcriptional regulator via its own transactivation domain [19] and through its interactions with RNA helicase A [18] or with DNA-binding transcription factors that are highly expressed in cancer cells, including STAT3 [20,21,23], E2F1 [16] and STAT5 [17]. Nuclear accumulation of EGFR is linked to poor patient survival, tumor aggressiveness and tumor drug resistance to EGFR-targeted therapy and other treatments [40-46]. In line with these associations, nuclear EGFR activates expression of cyclin D1 [19], inducible nitric oxide synthase [21], B-Myb [16], COX-2 [20], aurora A [17], c-Myc [23], and breast cancer resistance protein [22]. In addition to acting as a transcriptional regulator, nuclear EGFR retains its tyrosine kinase activity and phosphorylates/stabilizes proliferating cell nuclear antigen to promote cell proliferation and DNA repair [29]. Furthermore, nuclear EGFR has been shown to facilitate repair of the DNA damaged by radiation therapy and anti-cancer alkylating agents by binding to DNA-dependent protein kinase [25,26,28,47]. The impact of nuclear EGFR on tumor response to EGFR-targeted therapy remains unclear. Nevertheless, several studies suggest that nuclear existence of EGFR may be beneficial to the tumors encountering EGFR-targeted therapeutic antibodies and small molecule inhibitors [5,46,48]. Cetuximab has been shown to inhibit ionizing radiation-induced EGFR nuclear transport [49]. However, another study reported that cetuximab activates EGFR nuclear transport [50]. Lapatinib has been shown to block EGFR nuclear entry [51]. Overall, current evidence suggests that blocking EGFR nuclear functions may maximize the efficacy of EGFR-targeted agents and other anti-cancer therapies. In this regard, several agents have been shown to block EGFR nuclear import, such as, the Src family kinase inhibitor, dasatinib [46], Celecoxib [52] and Akt inhibitors [22].

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Received December 03, 2011; **Accepted** December 06, 2011; **Published** December 08, 2011

Citation: Lo HW (2011) EGFR-targeted Cancer Therapy: Promise, Problems and Potential Solutions. *Translational Medicine* 1:105e. doi:10.4172/2161-1025.1000105e

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Third, emerging evidence indicates that EGFR can be mislocated in the mitochondria where EGFR promotes tumor cell survival and renders tumor cells more resistant to EGFR inhibition. Mitochondrial detection of EGFR was first reported in 2004 [31], in which EGFR translocates into the mitochondria after EGF stimulation and interacts with and phosphorylates cytochrome c oxidase subunit II, a mitochondrion-encoded protein and a critical component of the oxidative phosphorylation pathway. This group further reported that c-Src translocated to the mitochondria with similar kinetics as EGFR after EGF stimulation and that c-Src kinase activity/overexpression enhanced EGFR localization to the mitochondria [32]. In addition to EGF, mitochondrial import of EGFR can be enhanced by mTOR inhibitor rapamycin [34], EGFR kinase inhibitor Iressa [30], and apoptosis inducers, staurosporine and anisomycin [30]. EGFRvIII mitochondrial import has been shown to be induced by Iressa [30], staurosporine and anisomycin [30] and cetuximab [53]. Conversely, EGFR mitochondrial import can be inhibited by autophagy inhibition using 3-methyladenine (an inhibitor of autophagy and a PI-3K inhibitor) and by etoposide [34]. Importantly, our laboratory reported that tumor cells with mutant EGFR/EGFRvIII receptors engineered to undergo enriched intracellular trafficking into the mitochondria were more resistant to staurosporine- and anisomycin-induced growth suppression and apoptosis [30]. We also found that the tumor cells with mitochondrial EGFRvIII accumulation were highly resistant to Iressa-mediated growth inhibition [30]. Taken together, accumulation of EGFR and EGFRvIII in the tumor mitochondria could contribute to tumor resistance to apoptosis although the underlying mechanisms are still unknown. Blocking mitochondrial import and/or functions of EGFR may sensitize tumor cells to EGFR-targeted therapy and other anti-cancer treatments that initiate intrinsic apoptosis.

In summary, the EGFR signaling pathway remains a promising target of anti-cancer therapy despite the disappointing fact that current EGFR-targeted therapy has only yielded modest clinical effects. In addressing this medical challenge, researchers have uncovered several atypical modes of EGFR signaling that were overlooked for over 30 years. First, EGFR elicits prosurvival signals independent of its tyrosine kinase activity; this atypical mode of EGFR signaling could help explain why EGFR kinase inhibitors did not yield substantial therapeutic effects. This important discovery also provides novel rationales to simultaneously target the kinase-dependent and -independent inadvertently activates of EGFR in order to maximize clinical efficacy of EGFR-targeted therapy. Second, constitutive presence of EGFR in the tumor nuclei may help tumor cells to undergo more aggressive growth and to resist therapy-induced cell death. Furthermore, some anti-cancer treatments have been reported to stimulate EGFR nuclear transport, leading to therapeutic resistance. Based on these compelling findings, using the agents that block EGFR nuclear transport could potentially maximize the effects of EGFR-targeted therapy that inadvertently activates EGFR nuclear import. Third, tumor cells shuttle EGFR into the mitochondria when encountering EGFR kinase inhibition, apoptotic signals and autophagic stress; mitochondrially localized EGFR can promote tumor cell survival. In light of these observations, inhibiting EGFR mitochondrial transport may sensitize tumor cells to EGFR-targeted therapy and other therapies that induce apoptosis.

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