Nucl Med Mol Imaging
Vol. 42, No. 3, Jun 2008

Review

Epidermal Growth Factor 수용체 영상을 위한 방사성추적자 기술

성균관대학교 의과대학 삼성서울병원 핵의학과
정경호 · 이경한

Radiotracer Methods for Targeted Imaging of the Epidermal Growth Factor Receptor

Kyung-Ho Jung, M.S. and Kyung-Han Lee, M.D.
Department of Nuclear Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

While indirect targeting strategies using reporter-genes are taking center stage in current molecular imaging research, another vital strategy has long involved direct imaging of specific receptors using radiolabeled ligands. Recently, there is renewal of immense interest in this area with particular attention to the epidermal growth factor receptor (EGFR), a transmembrane glycoprotein critically involved in the regulation of many cellular functions and malignancies. Recently, two novel classes of EGFR-targeting anticancer drugs have entered clinical trials with great expectations. These are monoclonal antibodies such as cetuximab that target the extracellular domain, and small molecule tyrosine kinase inhibitors such as gefitinib (Iressa) and erlotinib (Tarceva) that target the catalytic domain of the receptor. However, early results have showed disappointing survival benefits, disclosing a major challenge for this therapeutic strategy; namely, the need to identify tumors that are most likely to respond to the agents. To address this important clinical issue, several noninvasive imaging techniques are under investigation including radiolabeled probes based on small molecule tyrosine kinase inhibitors, anti-EGFR antibodies, and EGF peptides. This review describes the current status, limitations, and future prospects in the development of radiotracer methods for EGFR imaging. (Nucl Med Mol Imaging 2008;42(3):185-191)

Key Words: epidermal growth factor receptor, molecular imaging, cancer

EGF Receptors and Targeted Cancer Therapies

The epidermal growth factor receptor (EGFR) is a single chain transmembrane glycoprotein whose downstream signaling regulates biological processes closely associated with key features of cancer including tumor growth, survival, angiogenesis, and metastasis. It is also called the ErbB1 or HER1 receptor and belongs to a family of receptor tyrosine kinases that includes ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4 receptors.[1,2] The EGFR consists of an extracellular ligand-binding domain, a short hydrophobic transmembrane region, and an intra-cytoplasmic tyrosine kinase domain. Ligand binding to the ectodomain promotes the formation of receptor homo- and heterodimers, which leads to autophosphorylation-mediated activation through conformational changes. Phosphorylation of key tyrosine residues on the EGFR provides specific docking sites for cytoplasmic proteins that contain Src homology-2 or phosphotyrosine binding domains.[2] A complex formed by adaptor proteins that bind to these docking sites then initiates several specific intracellular signaling pathways. These include the Ras/Raf/mitogen-activated protein kinase (MAPK) pathway, which is critically involved in the regulation of cell proliferation and survival,[3,4] and the phosphatidylinositol 3-kinase (PI3K) pathway, which plays a key role in tumor growth, survival, invasion, and migration

• **Received**: 2008. 4. 30. • **Accepted**: 2008. 5. 9.
• Address for reprints: Kyung-Han Lee, M.D., Department of Nuclear Medicine, Samsung Medical Center, 50 Ilwondong, Kangnamgu, Seoul 135-710, Korea
Tel : 82-2-3410-2630, Fax : 82-2-3410-2639
E-mail: khnm.lee@samsung.com
※This work was supported by the National R&D Program for Cancer Control, Korea (No. 0720190-1).

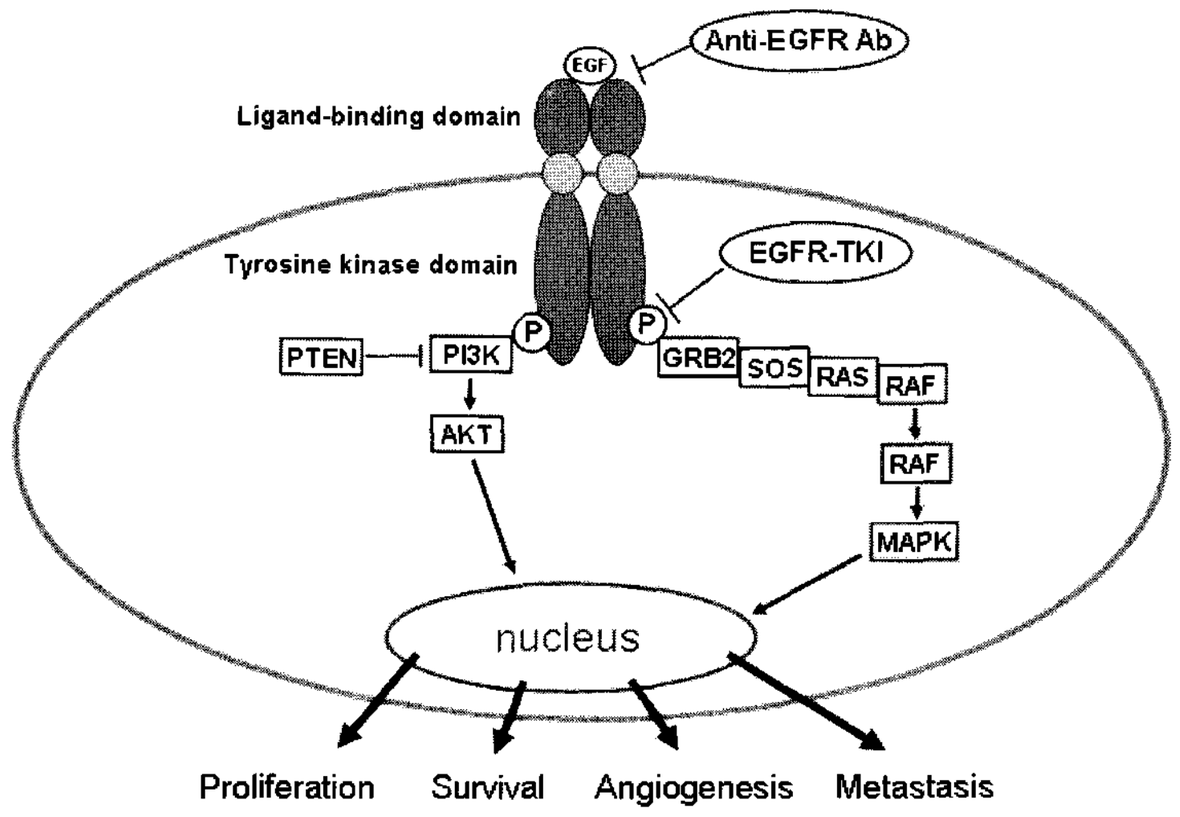

Figure 1. Schematic illustration of the epidermal growth factor receptor (EGFR) and targets for inhibition. Potential downstream signal transduction pathways are shown including the mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3-K) pathways, and the resultant biological effects are highlighted. Also shown are the action sites for EGFR inhibition by antibodies (Ab) and tyrosine kinase inhibitors (TKI).

(Fig. 1).[5,6] There is also activation of phospholipase Cg, which produces inositol 1,3,5-triphosphate (important for intracellular calcium release) and 1,2-diacylglycerol (a cofactor for protein kinase C activation).[7]

Many cancers have upregulated EGFR signaling either through increased ligand-binding or receptor expression, or by ligand-independent activation mechanisms. EGFR overexpression is of particular importance, with most non-small cell lung cancers having about 10-fold, and 30-60% of breast cancers having up to 100-fold increases in expression levels. Malignancies of the colon, bladder, and brain also often show increased levels of EGFR expression. Furthermore, high EGFR expression levels have been correlated with poor patient survival in certain cancers. These findings form the basis for the strategy to target the EGFR and its downstream effectors for the treatment of cancer.

Recent advances in our understanding in the mechanisms of EGFR function have led to the development of two promising classes of agents that achieved regulatory approval and entered the clinics. These are monoclonal antibodies directed at the extracellular domain and low molecular weight ATP-competitive inhibitors against the intracellular domain tyrosine kinase (Fig. 1).[8] Since an early report on their preclinical activity,[9] anti-EGFR monoclonal antibodies have been shown to prevent EGFR activation through steric hindrance between their Fab-domain and the extracellular portion of the receptor. This mechanism could be important when EGFR activation occurs via stabilization of the extended configuration without ligand binding, such as may occur with high receptor expression. The clinical use of anti-EGFR antibodies is presently approved for the treatment of advanced colorectal and head and neck tumors. Another approach to disrupt EGFR signaling is based on the observation that EGFR with mutations on its tyrosine kinase site lacks ligand-i nduced biologic responses. Thus, small molecules were developed that compete for the ATP binding site of the catalytic domain of the EGFR tyrosine kinase. These include gefitinib (Iressa) and erlotinib (Tarceva), which have been clinically approved for the treatment of advanced non-small cell lung cancer and pancreatic carcinoma. Tyrosine kinase inhibitors also have activity in head and neck tumors and in glioblastoma brain tumors.

To date, however, only a portion of cancer patients

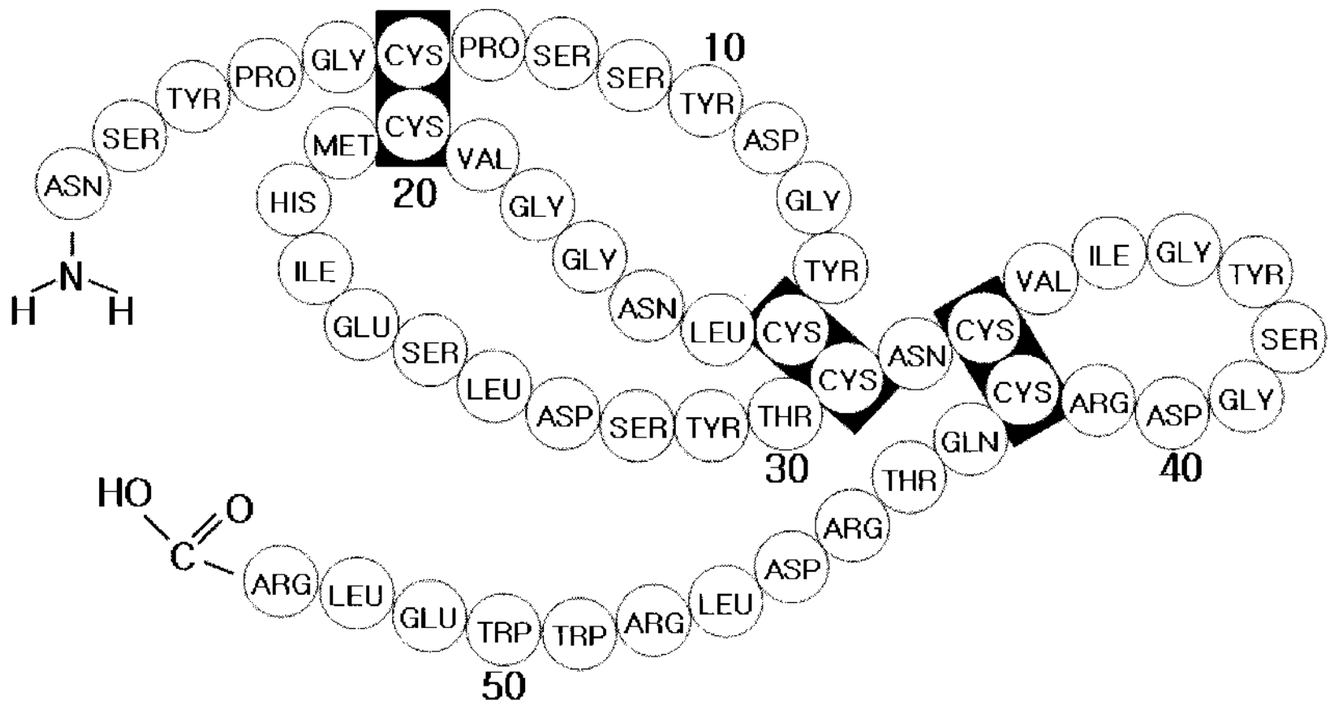

Figure 2. Schematic illustration of the epidermal growth factor. The aminoacid sequence is shown and disulfide bonds are depicted in black boxes. Tyrosine residues (TYR) can be used for direct radioiodination. Lysine residues and the amino-terminal provide sites for indirect labeling with radioiodine, radioindium, or radiotechnetium.

treated with EGFR inhibitors has shown therapeutic efficacy. In these early clinical trials, subjects were selected without consideration of the status of the receptor being targeted. But previous experience with targeted hormone therapy of breast cancer clearly illustrates the importance of integrating the status of the target receptor for patient selection.[10] Therefore, an important challenge for EGFR targeted molecular therapy is to identify treatment response effectors that can help predict tumors more sensitive to the agents. Several somatic mutations of the EGFR gene in non-small cell lung cancer patients have been found to be linked with favorable response to anti-EGFR tyrosine kinase inhibitors.[11,12] However, patients with such mutations are more likely to also have favorable prognostic factors, which include female gender, Asian, adenocarcinoma, and absent smoking history. Since patients with favorable prognostic factors would show better outcomes to any form of treatment, confusion of prognostic biomarkers as therapeutic response biomarkers is problematic. Indeed, several clinical trials in Caucasians have failed to demonstrate a significant correlation between EGFR mutation and patient survival.[13,14] EGFR mutations are further limited as a useful parameter for patient selection because they can be relatively infrequent depending on tumor type, and clinical benefit with anti-EGFR agents is not restricted to patients harboring such mutations. Therefore, it would be most useful to incorporate, in addition to EGFR gene mutations, other biomarkers of drug sensitivity, and a large amount of interest is focused on the level of EGFR expression.[8]

EGFR gene copy numbers are more straightforward in assessing its value as a predictor of treatment response since it is not a clinical prognostic factor.[15] A few phase-2 clinical trials using EGFR inhibitors for the treatment of lung cancer demonstrated better responses and longer survival with higher EGFR gene copy numbers.[13] The randomized BR21 Trial and Iressa Survival Evaluation in Lung Cancer Study also showed lung cancer patients with high EGFR gene copy numbers to have better survival after treatment. In addition to lung cancer, colon cancers with high EGFR gene copy numbers also showed significant correlation to cetuximab and panitumumab treatment response.[16] In a study where EGFR expression levels were graded by staining intensity with a score from 0 to 4 multiplied by % of positive cells, response to gefitinib treatment was observed to correlate with patient survival. This suggests that quantitative assessment of EGFR expression levels may be useful for selecting subjects for EGFR targeted therapies and predicting treatment response. Histological methods of assessment suffer from

limitations of invasiveness, sampling error from inhomogeneous expression levels, lack of quantitative assessment, and difficulty in repeated testing after treatment. To overcome these obstacles, there is increasing interest in molecular imaging techniques that may allow repeated assessment of EGFR expression levels.

Radiolabeled Small Molecule Tyrosine Kinase Inhibitors

One recent strategy under development focuses on the utilization of reversible and irreversible inhibitors with high affinity and specificity for the EGFR tyrosine kinase. Among irreversible binding inhibitors, the 4-dimethylamino-but-2-enoic acid [4-(phenylamino)-quinazoline-6-yl]-amide group stands out from others by a remarkable inhibitory potency toward the EGFR, sufficient receptor selectivity, and elevated chemical and biological stabilities.[17] The lead compound in this group, ML04, has been labeled with ^{11}C and ^{18}F and evaluated for its potential as an EGFR-PET imaging agent. Although the biological stability of ML04 was improved relative to previously described irreversible labeled inhibitors, it did not yield adequate PET images in tumor-bearing animal models, probably due to low bioavailability and solubility under physiological conditions, and excessively fast clearance from the blood. Reversible inhibitors have shown even less favorable characteristics. These compounds are mostly based on the potent inhibitor 4-(3-bromoanilino)-6,7-dimethoxyquinazoline (PD153035), which exhibits antitumor activity and selectivity for the EGFR tyrosine kinase with a Ki of 65 pM. The lead molecule has been labeled with ^{11}C, ^{125}I, and ^{18}F. These tracers, however, showed low tumor accumulation in vivo and ex vivo, due to insufficient receptor affinity and significant probe metabolism.

Hence, while radiolabeled EGFR tyrosine kinase inhibitors are quite attractive as imaging probes, efforts to date have failed to show their feasibility because of inadequate in vivo properties despite favorable in vitro results. Various explanations for this inadequacy include suboptimal hydrophilicity, in vivo metabolism, and poor permeation through cellular membranes.

Radiolabeled Anti-EGFR Antibodies

Radiolabeled antibodies were the first to be investigated as EGFR targeted imaging probes. In a study at the Memorial Sloan-Kettering Cancer Center in 1989, Goldenberg and coworkers labeled with ^{111}In-DTPA a mouse monoclonal antibody (225 IgG1) that binds and blocks EGFR. Intraperitoneal administration of ^{111}In-DTPA-mAb-225 into nude mice xenograted with A431 human vulvar squamous cancer cells that have high EGFR expression revealed promising tumor uptake (12 %ID/g) and image-contrast at day 7.[18] Two years later, the same ^{111}In-DTPA-mAb-225 was tested for applicability in inoperable squamous lung cancer patients undergoing a phase I trial with the antibody. As a result, scintigraphy visualization of the tumors was demonstrated and uptake levels were shown to relate to antibody dose.[19]

Soon thereafter, a group in Pennsylvania prepared a different anti-EGFR antibody (mAb-425) labeled with ^{111}In, and SPECT imaging after injection in 28 patients with intracranial glioma demonstrated a detection sensitivity of 96% and specificity of 60%.[20] Further endeavors for improvement led to the development of PEGylated ^{111}In-mAb-C225 to allow reduced liver accumulation.[21] Also a ^{99m}Tc labeled anti-EGF antibody tracer was synthesized and tested for pharmacokinetic analysis in 9 patients, demonstrating a biexponential pattern of plasma disappearance with distribution and elimination half-lives of 0.14 and 20.3 hours, respectively.[22] However, antibody radiotracers suffer from poor tumor-to-background count ratios due to slow clearance of plasma activity. Consequently, these tracers are more suitable for studying the in vivo pharmacokinetics of antibody based anti-EGFR agents than for tumor imaging.

Radiolabeled EGF Ligands

As a natural ligand, EGF is an obvious candidate as an EGFR imaging probe with the added advantages of better intratumoral penetration and faster background clearance compared to antibody tracers (Fig. 2). The first study utilizing radiolabeled EGF for imaging was done by Schatten et al. in which 14 patients with uterine cervix

cancer were subcutaneously injected in the feet with ^{123}I-EGF. Lymphoscintigraphy of the lower extremities showed abnormal findings in 11 of these subjects and lymphatic abnormalities were verified in 4 of the cases.[23] Soon afterwards, the in vivo pharmacokinetics and biodistribution of intravenously administered ^{131}I-EGF was evaluated in normal pigs, revealing a circulating half-life of 0.4-0.7 min and high hepatic and renal activity.[24] Pharmacokinetics and imaging in human subjects was studied following intravenous injection of ^{131}I-EGF in 9 patients with squamous cell lung cancer. The results showed predominant renal elimination and tumor visualization in 6 cases with peak tumor-to- background uptake ratios at 2-3 days.[25] Senekowitsch- Schmidtke et al. compared the in vivo findings of ^{125}I-EGF and ^{125}I-mAb-425 and showed that whereas both were taken up by tumors in nude mice, EGFR concentration was better represented by ^{125}I-EGF uptake. ^{125}I-EGF also had the advantage of faster blood clearance. However, in vivo deiodination was a potential problem of this radiotracer.[26]

^{99m}Tc labeled EGF tracers have also been developed because of the practical advantages of ^{99m}Tc over ^{123}I. Rusckowski et al. labeled EGF with ^{99m}Tc using N-hydroxysuccinimide ester MAG3 and showed specific binding to EGFR expressed by cancer cells. When injected into tumor bearing nude mice, ^{99m}Tc-EGF was taken up by EGFR+ A431 and LS-174T xenografts with 12 hour uptake levels of 0.44 %ID/g-tissue.[27] In a recent study, Babaei et al. prepared a different ^{99m}Tc-EGF tracer by hydrazinopyridine-3-carboxylic acid (HYNIC) conjugation using tricine and ethylenediamine-N,N'-diacetic acid (EDDA) as co-ligands. ^{99m}Tc-EDDA-HYNIC-EGF bound to EGFR+ cells with nanomolar affinity in vitro, and demonstrated high serum stability, fast blood clearance, and EGFR targeting ability in vivo.[28]

Perhaps the best characterized EGF tracer to date is ^{111}In-DTPA-EGF, first introduced by Reilly and coworkers at the University of Toronto. When injected into nude mice xenografted with various breast cancer cells that express different levels of EGFR, ^{111}In-DTPA-EGF was eliminated from the blood much faster (< 0.2 %ID/g at 72 hr) than ^{111}In-DTPA-MAb528, while both tracers showed high activity in liver and kidneys. In MDA-MB-468 tumors, maximum localization was 10-fold higher for ^{111}In-DTPA-MAb528 (21.6 %ID/g), but tumor-to-blood ratios were greater for ^{111}In-DTPA-EGF (12:1 versus 6:1).[29] When evaluated for potential Auger electron radiotherapy effects, ^{111}In-DTPA-EGF actively taken up by EGFR+ MDA-MB-468 breast cancer cells were shown to internalize with significant nuclear translocation. Furthermore, uptake of the radiotracer resulted in slowing of cell growth and a dose-dependent decrease in surviving fraction.[30] ^{111}In-DTPA-EGF has also been prepared as a kit under good manufacturing practices. Radiotracers were labeled from the kit with high efficiency and showed a receptor binding affinity constant of 0.9-1.1 x 10^7 L/mol and maximum number of binding sites of 1.1-2.2 x 10^6 per cell.[31] Recently, the effect of gefitinib on internalization, nuclear translocation, and cytotoxicity of ^{111}In-DTPA-EGF was evaluated. In MDA-MB-468 breast cancer cells, gefitinib increased internalization and nuclear localization of ^{111}In-DTPA-EGF and decreased the surviving fraction, suggesting a potential benefit of combining tyrosine kinase inhibitors with radiolabeled EGF therapy.[32]

A potential limitation of radiolabeled EGF tracers is that present techniques may result in a mixture of heterogeneous products. The human EGF contains 5 tyrosine residues that can undergo direct radioiodination, and one N-terminal and two lysine side chain amino-groups that provide sites for indirect conjugation with ^{123}I, ^{111}In, or ^{99m}Tc (Fig. 2). Thus, present techniques give a mixture of molecules with different numbers and sites of radiolabeling that can consequently affect tracer kinetics and biodistribution. Another potential problem is that some tumors have mutated EGFR with deleted extracellular domain and ligand-independent receptor activation that may not be targeted by EGF based probes.[33-34]

Future Prospects

The EGFR has become a most attractive target for molecular imaging, and nuclear medicine is expected to play a central role in providing a practical noninvasive technique to monitor this important biologic target. Despite the many years since the introduction of the first EGFR targeted radiotracer, an imaging probe with satisfactory in

vivo stability, pharmacokinetics, target affinity and specificity, and imaging properties has yet to be developed. Tyrosine kinase inhibitors make attractive molecular probes that are certainly useful as tools to investigate the in vivo kinetics of these promising new drugs. However, poor stability and cell penetrability are among several difficulties that need to be resolved before their use can be regarded practical. Anti-EGFR antibodies were the first to be introduced as radiotracers, but they suffer from the usual limitations of large-sized probes including poor tissue permeation and delayed blood clearance. These tracers, however, are valuable tools for understanding the in vivo pharmacokinetics of therapeutic antibodies. The utility of smaller antibody fragments appears promising but will need to be validated by further experiments. EGF peptides are an outstanding candidate for EGFR imaging because they have superior binding affinity, their small size leads to better intratumoral permeation and faster background clearance, and cell membrane penetration is not required to target the extracellular domain of EGFR. At present, the major limitation of this class of probes is that tumor uptake level is insufficient, partly due to excessively rapid blood clearance rates. Modifications of the tracer to attain optimal in vivo kinetics and enhanced tumor uptake may allow the practical use of EGF radiotracers for EGFR imaging.

The future success of EGFR targeting molecular imaging will depend on whether the techniques provide measurements that correlate with actual EGFR concentration and can help predict the response to EGFR targeted therapeutics. This in turn will depend on newer radiotracers that allow high affinity and highly specific tumor imaging with improved in vivo stability and pharmacokinetic properties. The success of these endeavors is expected to help expand the clinical application of nuclear imaging techniques for such areas as early cancer detection, individualized treatment stratification and dose optimization, treatment monitoring, and acceleration of new drug development for cancer therapy.

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