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INTEGRATING RADIOTHERAPY WITH EGFR ANTAGONISTS AND OTHER MOLECULAR THERAPEUTICS FOR THE TREATMENT OF HEAD AND NECK CANCER

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BACKGROUND

Cancer accounts for 23% of all deaths and is the leading cause of death for people under age 85. About 1.4 million individuals in the United States were diagnosed with cancer in 2006 and 560,000 died of the disease. The corresponding numbers for head and neck squamous cell carcinoma (HNSCC) are 11,200 and 40,000, respectively.[1]

Radiotherapy plays a crucial role in the treatment for HNSCC. However, the 5-year relapse-free survival rate for patients with locally advanced HNSCC is 30-40%, and most patients die from locoregional disease progression. Tumor repopulation, hypoxia, intrinsic radioresistance, and dose-limiting toxicities are among the chief causes of poor outcome. The efficacy of altered fractionation (AF) regimens and combinations of radiotherapy with chemotherapy has been intensively investigated. Recent meta-analyses showed that collectively AF and radiotherapy plus concurrent chemotherapy increase the 5-year survival rate by 3.4% and 8%, respectively.[2, 3] Consequently, most centers have adopted AF and concurrent radiochemotherapy (mostly cisplatin) for the non-surgical treatment of intermediate stage and locally advanced HNSCC, respectively. Unfortunately, the systemic toxicities of chemotherapy and other side effects, particularly mucositis, of the combined therapy can be severe.

Increasing knowledge of molecular radiation biology spurred the development of rational strategies for combining radiotherapy with molecular therapeutics. Laboratory and clinical investigations on epidermal growth factor receptor (EGFR) revealed its role in carcinogenesis, tumor progression, and response to therapy. Collectively, this line of research validated the concept of selective modulation of tumor response to radiotherapy by targeting a specific growth factor signaling pathway and established a new treatment option for locally advanced

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HNSCC. This article summarizes relevant findings of laboratory and clinical investigations on EGFR put into historical perspective in order to assess the direction of future investigations in radiation oncology. The number of references is capped in compliance with the journal policy. A recent review by Nyati et al.[4] is a complementary reference.

DISCOVERY AND STRUCTURE OF EPIDERMAL GROWTH FACTOR RECEPTOR

A protein promoting neurite outgrowth in a mouse tumor was characterized in 1965 as epidermal growth factor (EGF), as it stimulated epithelial cell proliferation.[5] Its receptor was identified a decade later along with the demonstration that its tyrosine-specific phosphorylation activated intracellular signal transduction.[6] The EGFR was later characterized as a 170-kDa transmembrane receptor tyrosine kinase (RTK) of the ERBB family, which is essential for normal development. Its cDNA sequence (3633 bp)[7], chromosomal location (*7p12-p22*)[8], genomic structure, and amino-acid sequence were subsequently identified.[9]

Structurally, the EGFR is composed of four extracellular domains, a hydrophobic transmembrane region, a juxta-membrane domain, an intracellular protein tyrosine kinase domain (PTK) containing the ATP-binding pocket, and a regulatory carboxyl terminal domain (Fig. 1). It is monomeric in the basal state, but binding of a ligand produces an extended and stabilized conformation, which promotes homo- and hetero-dimerization[10] and activates signal transduction.

FUNCTION OF EPIDERMAL GROWTH FACTOR RECEPTOR

EGFR activation initiates multiple layers of signaling and amplification governing biological responses. Briefly, EGFR serves as a node in interacting networks, as its carboxyl terminal domain contains numerous distinct docking sites for various molecules in response to stimuli (Fig. 1).[11] EGFR heterodimerizes with other growth factors[12] such as other ERBB receptors and IGF-1R[13] at the cell surface. Heterodimerization is facilitated by raft colocalization[14] and result in *trans*-autophosphorylation through horizontal interactions.[14, 15] In addition, non-RTKs (e.g., Src) can participate in EGFR-mediated transactivation.[16] These dynamic interactions are regulated by site- and time-specific phosphorylations of tyrosine (Fig. 1), serine, and threonine residues. These events initiate the recruitment and phosphorylation of several intracellular substrates (signaling proteins and adaptors like Grb2 and Shc), leading to phosphorylation cascades. A specific ligand triggers preferential activation of downstream signaling pathways (Fig. 1, 2). For example, EGF might preferentially activate the PLC pathway[17], whereas TGF-α might favor the Jak-STAT pathway.[18]

Four major cytoplasmic downstream signaling routes of EGFR have been characterized (Fig. 2). The Ras-Raf-MAPK pathway leads to the activation of ERK1-2, which in turn regulates the transcription of molecules mediating cell proliferation, survival, and transformation. The PI3K and downstream serine/threonine kinase, Akt, govern cell growth, proliferation, survival, and motility. The Jak/STAT pathway translocates STAT molecules to the nucleus to induce gene transcription and mediate cell division, viability, motility, invasion, adhesion, and DNA repair. The PLC-DAG-calcium/calmoduline-PKC pathway also regulates cell cycle progression and cell motility.[17]

Nuclear EGFR pathways (Fig. 2), ligand-dependent[19] and -independent[20, 21], were recently identified. EGFR possesses nuclear localization sequence signals in its juxta-membrane domain (Fig. 1)[22] for nuclear translocation as non-membrane-bound receptor through the nuclear pore complex, or through interaction with nuclear transport receptors such as importins α/β1 and exportins.[21] Although EGFR lacks putative DNA binding domains, it has

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transactivation domains on its C-terminal extremity[21] (Fig. 2) regulating synthesis of pro-mitogenic proteins.[19] In addition, EGFR interacts with nuclear DNA-PK (Fig. 2) and promotes repair of radiation-induced DNA strand-breaks[20] (discussed below in modulation of radiosensitivity). Mitochondrial pathway[23] was recently described (Fig. 2).

Attenuation of EGFR signaling is through dephosphorylation of key residues and removal by endocytosis. Following clathrin-mediated endocytosis, EGFR is sorted into early endosomes and directed to multi-vesicular bodies and late endosomes for degradation or recycling.[14, 24] Multiubiquitination of EGFR mediated by Cbl is essential for internalization and routing for lysosomal degradation.[14] Deficiencies in this control mechanism can result in enhanced recycling and signal amplification.

EGFR IN CANCER

EGFR is highly expressed in most carcinomas. EGFR mRNA and protein are expressed abundantly in 90% of HNSCCs and less frequently in the adjacent dysplastic lesions or in histologically normal surrounding mucosa[25], which imply that EGFR amplification plays a role in early carcinogenesis. Transcriptional targets of nuclear EGFR (Fig. 2)[21] are involved in tumor progression.

The main mechanism of EGFR upregulation is transcriptional activation, secondary to autocrine production of TGF-α.[26] TGF-α is closely related to EGF including binding to EGFR and thereby initiating signal transduction. It can be secreted by macrophages, T cells, and keratinocytes in response to tissue injury. High EGFR expression is often associated with poor prognosis and resistance to cytotoxic agents, including ionizing radiation (discussed below). High nuclear EGFR level has also been correlated with poor outcome in HNSCC.[27]

Gain of function may also occur through mutations. Activating mutations in the kinase domain found in non–small-cell lung cancer (NSCLC) appear to be rare in HNSCC. Deletion of exons 2-7 of the extracellular domain yields a constitutively active truncated EGFRvIII.[28] It is prevalent in glioblastomas and to lesser extent in HNSCC.[29] EGFRvIII and the kinase domain mutants activate survival pathways such as Akt.[30] Cross-talk with other ERBB receptors can also lead to aberrant activation.

EGFR IN RADIOTHERAPY

A. Preclinical Studies

EGFR and tumor clonogen repopulation—Repopulation of tumor clonogens during treatment is one mechanism of resistance to radiotherapy[31] (Fig. 3A). Schmidt-Ullrich et al. found that cancer cells surviving irradiation acquired a phenotype with upregulated EGFR and TGF-α.[32] They further showed *in vitro* that therapeutic dose range of radiation increased EGFR tyrosine phosphorylation[26], which was linked to critical components of mitogenic signaling pathways.[33] This adaptive response produced radioresistance and was interpreted as an underlying mechanism for accelerated repopulation.

Doses of 1-5 Gy induced a 2-to 5-fold increase in tyrosine phosphorylation within 5-10 min, as opposed to >5-fold rise induced by ligands in physiologic concentrations[26, 33] This first phase of activation, falling to baseline within 10 min, was associated with stimulation of major signaling pathways with selective functional linkage to different ERBB receptors.[33] MAPK, for example, peaked between 5-15 min and was linked to EGFR activation with additional contributions by Raf.[26] The second phase starts after 30 min and triggers pro-proliferative responses and activation of transcription factors.[34]

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Effect of EGFR on cellular radiation sensitivity—The first clue that EGFR expression might affect cellular radiation sensitivity *in vivo* emerged from a study on murine models by Akimoto and colleagues.[35] They found that single-dose irradiation induced EGFR autophosphorylation and downstream signaling only in high EGFR-expressing tumors. This phenomenon was associated with relative radioresistance. Since clonogen repopulation plays no role in determining *in vivo* tumor response to single-dose irradiation[36], these results suggest that EGFR contributes to determining intrinsic radiosensitivity. The data of a complementary correlative study[37] using specimens of patients with HNSCC (see below) are consistent with this finding.

A follow-up study [38] revealed evidence for a causal relationship between EGFR expression and radioresistance. Transfection of a full-length human EGFR vector into a low EGFR-expressing murine carcinoma cell line resulted in an EGFR level-dependent increase in radioresistance measured by clonogenic cell survival assays. It also demonstrated that exposing EGFR transfectants to the anti-EGFR antibody cetuximab reduces the expression of EGFR and downstream Akt and MAPK and reverses the cellular radioresistance (Fig. 3B).

A subsequent experiment elucidated a mechanism by which EGFR affects radiation sensitivity. [20] This thorough study showed that in contrast to cytoplasmic signaling induced by EGF, radiation triggeres nuclear EGFR translocation. This process is accompanied by a nuclear influx of proteins Ku70/80 and phosphatase 1, an increase in nuclear DNA-PK activity, and formation of DNA end-binding protein complexes containing DNA-PK, which plays a dominant role in repairing radiation-induced DNA double strand-breaks through a non-homologous end-joining mechanism. EGFR blockade by cetuximab abolishes nuclear EGFR import, diminishes radiation-induced activation of DNA-PK, inhibits DNA repair, and enhances cellular radiation sensitivity.[20]

EGFR inhibitors to enhance tumor response to radiotherapy—The concept that blockade of EGFR signaling might have antitumor activity was introduced in 1980s. Mendelsohn *et al.* provided the first pre-clinical demonstration of an antiproliferative effect of monoclonal antibodies (MAbs) directed at the EGF-binding site.[39] Since then, numerous EGFR inhibitors were studied.

The potential modulation of radiation response with EGFR inhibitors attracted attention in the mid 1990s. Several investigators showed that EGFR antagonists inhibits radiation-induced EGFR phosphorylation and tumor cell proliferation.[26, 40] Initial studies with cetuximab showed enhanced tumor response to single-dose and fractionated radiation in cell lines and in xenograft models using apoptosis[41, 42], regrowth delay[43] and tumor control[44] as endpoints. Further studies showed that the magnitude of the radiation enhancement varies among EGFR antagonists.[45] These findings sparked an interest in elucidating the mechanisms of radiation sensitization *in vivo*.

The available data suggest that cetuximab can potentially increase radiosensitivity through several processes. Briefly, these include (1) binding to domain III[46] (Fig.1) and sterical blockade of domain I, thus preventing ligand-binding and ligand-independent (i.e. radiation-induced) activation[34]; (2) preventing EGFR from adopting the conformation needed for dimerization; (3) preventing EGFR from inducing autocrine ligand production; (4) inhibiting EGFR nuclear translocation and thus impairing EGFR-mediated DNA repair[20]; (5) inducing an antibody-dependent cellular toxicity [46]; and (6) downregulating the expression of several pro-angiogenic factors, including VEGF, bFGF, IL8, and thereby promotes endothelial cell apoptosis and vasculature collapse.[47] It should also be realized, however, that reassortment of cells in a relatively radioresistant G1 phase of the cell cycle might be a drawback associated with the use of EGFR antagonists for the treatment of slowly repopulation cells.

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B. Correlative Biomarker Studies and Clinical Trials

A correlative biomarker analysis using specimens of patients with locally advanced HNSCC enrolled into a phase III trial showed that high EGFR expression was a strong, independent predictor of local-regional control (LRC) and overall survival after radiotherapy alone.[37] Concurrently, Roberts *et al.* demonstrated in a pilot study[48] the safety of combining cetuximab with radiotherapy and recommended a regimen consisting of one loading dose before radiotherapy, followed by seven weekly doses concurrent with radiotherapy.

Preclinical studies, biomarker analysis, and the pilot trial provided the impetus in completing a phase III study testing the efficacy of combining cetuximab with radiotherapy.[49] This pivotal trial demonstrated that the addition of eight doses of cetuximab to radiotherapy improved LRC significantly (3-year rate: 47% vs 34%, p=0.005) without increasing radiation side effects, including mucositis, dysphagia, pain, etc. There were also significant increases in median survival time (from 29 to 49 months) and 3-year survival rate (from 45% to 55%), as shown in Fig. 3C. Consequently, cetuximab in combination with radiotherapy was approved as a frontline treatment for locally advanced HNSCC.

Collectively, coordinated studies established the proof-of-principle that modulating a perturbed signaling pathway can lead to a selective tumor radiosensitization and thereby truly improve the therapeutic index. Such clear improvement in the therapeutic index has not been accomplished by combining radiation with traditional chemotherapy.

Building on this success, rational combinations of radiotherapy, with or without chemotherapy, with other novel agents are being explored. In patients with locally advanced HNSCC, for example, the nonsurgical standard of care has changed from radiotherapy alone to concurrent radiochemotherapy after the phase III cetuximab trial had been launched. Therefore, trials have been commenced to assess the feasibility of combining cetuximab with radio chemotherapy. A phase II trial showed that the combination of radiotherapy-cetuximab with cisplatin resulted in 3-year overall survival of 76%, despite the occurrence of two early deaths (pneumonia and unknown cause).[50] This regimen is now being evaluated in a phase III trial. The combination of radiotherapy-cetuximab with gemcitabine in HNSCC yielded a complete response rate of 77% and no major toxicities (Table 1).

It is also important to realize that cetuximab does not benefit 85-90% of patients with locally advanced HNSCC. Over 50% of patients receiving the combination still developed local-regional relapse. It is thus critical to develop assays to identify such “resistant” tumors to personalize therapy. It is also vital to understand the biological basis of resistance to EGFR antagonists to develop alternative strategies.

PREDICTIVE BIOMARKERS FOR TUMOR RESPONSE TO ANTI-EGFR THERAPY

Emerging data indicate that EGFR expression, mainly measured by immunohistochemistry (IHC), is an independent predictor of HNSCC response to conventionally fractionated[37] or accelerated[51, 52] radiotherapy or radiotherapy plus chemotherapy. However, the predictive power varies among the series. The search for predictive biomarkers for response to EGFR antagonists has been disappointing. Counterintuitively, pre-treatment tumor EGFR expression was not found to predict response to EGFR antagonists.[53, 54] Some patients with negative EGFR colorectal cancer even benefited from cetuximab.[55]

Noteworthy is that the assay methodology varied widely. A standard IHC assay generally deems a cell positive when >30,000 EGFR receptors are present. Unfortunately, the number and density of receptors required to mediate a given biologic effect is not known. This

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deficiency could partially account for the discrepancies between studies. In addition, quantitative-EGF binding experiments showed the presence of high- and low-affinity forms of EGFR on the cell surface[56], whose proportions, roles, and dynamics are still largely unknown. Moreover, infiltrating inflammatory cells can also express EGFR and thereby further confounding the finding. So it is crucial to standardize the assays (fixative, storage time, scoring method) and perform better organized correlative analyses to resolve this important topic. Combining IHC assay with FISH might also yield better predictive power for the response to EGFR inhibitors, as shown in NSCLC.[57]

There appears to be a dose–response relationship between the incidence and severity of skin rash and a clinical benefit in some tumors[53, 58] but data in HNSCC are contradictory.[54, 59] Of note is that the recording and reporting of rash have not been standardized. Even if the association is further confirmed, rash might be useful only for titrating the dose of EGFR inhibitors in individual patients, e.g., escalating the dose until a rash appears, but not for identifying patients who might benefit most from the therapy.

OVERCOMING RESISTANCE TO EGFR INHIBITORS

Why EGFR antagonists do not affect the growth or radiation sensitivity of most HNSCC is unclear. A number of hypotheses and research directions for overcoming resistance are briefly summarized. First, cetuximab or TKIs given as single agents in the current dose regimens might not effectively suppress EGFR-mediated signaling. Therefore, the potential benefits of other dose regimens, other antibodies, alternative TKIs, antisense nucleotides, or various combinations of these agents have been investigated. A recent preclinical study showed that three additional doses of cetuximab given after concurrent radiation-cetuximab improved LRC compared to concurrent radiation-cetuximab alone.[60] Other interesting antibodies are hR3 (longer half-life than cetuximab) and panitumumab (human MAb with higher affinity for EGFR). Radiotherapy plus hR3 yielded 3-year overall survival and 2-year disease-free survival rates of 67% and 65%, respectively, in patients with locally advanced HNSCC [61]. A phase III trial showed a benefit of panitumumab compared to best supportive care in patients with metastatic colorectal cancers that have failed chemotherapy.[58]

Two types of TKIs are now available. Type 1, such as erlotinib and gefitinib, targets the kinase ATP binding site in its active conformation. The combination of erlotinib and gefitinib with various radiochemotherapy regimens are being evaluated (Table 1). CI-1033 is another TKI that binds irreversibly to all ERBB kinases (a pan-ERBB inhibitor), and EGFRvIII, resulting in a prolonged suppression of downstream signaling. In HNSCC cell lines, CI-1033 blocked cell growth, downregulated specific genes co-regulating *in vivo* neoplastic behavior, and sensitized cells to radiotherapy. Type II TKIs, such as lapatinib, have an additional binding site immediately adjacent to the ATP docking site. Its longer half-life correlates with a prolonged down-regulation of receptor tyrosine phosphorylation in tumor cells relative to erlotinib and gefitinib.[62] It is being tested in phase II trials (Table 1).

Dual-agent targeting of the EGFR pathway (gefitinib or erlotinib plus cetuximab) or multi-target TKIs showed more pronounced inhibition of cell proliferation and tumor growth in preclinical models.[45] Preliminary clinical studies (colorectal, HNSCC, and NSCLC) also showed that cetuximab plus gefitinib had a superior pharmacodynamic signal inhibition and greater clinical activity than either agent alone.

Second, EGFR mutations may result in aberrant signaling and poorer response to EGFR antagonists or radiotherapy. EGFRvIII was detected in a number of tumors and differed from wild-type EGFR (EGFRwt) in its preferential activation of downstream signaling pathways. [30] In a series of 33 patients, EFGRvIII and EGFRwt were simultaneously expressed in 42% of HNSCCs.[29] Transfection of EGFRvIII into HNSCC cell lines decreased cisplatin-induced

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apoptosis and cetuximab-induced growth inhibition. This observation formed the basis for investigating EGFRvIII-specific monoclonal antibody. Other somatic mutations of the kinase domain occur in 1% and 7% of Caucasian and Asian patients with HNSCC, respectively.[63] Their biologic impact is largely unknown owing to the low incidence.

Third, constitutive activation of signaling pathways downstream of EGFR by upregulation of other ERBB receptors or RTKs can promote survival (see review by Kalyankrishna and Grandis[18]). For example, a high level of activated Akt can occur downstream of EGFR inhibition through alternative upstream-activated Src, Ras, mutated PTEN, or amplification of the PI3K catalytic subunit. The STAT3 and STAT5 pathways can be constitutively active in HNSCC. Overexpression of six major ERBB family ligands can activate ERBB receptors and IGF-1R, resulting in resistance to EGFR inhibition. Upregulation of IGF-1R, for instance, resulted in sustained signaling through the PI3K pathway, leading to antiapoptotic and proinvasion effects and resistance to a TKI in a glioblastoma model.[13] Activation of EGFR-independent pathways, such as G protein-coupled receptors (GPCRs)[64], may promote survival and resistance to EGFR inhibitors. Of note is that ionizing radiation can activate all ERBB receptors[12], IGF-1R[13], and some metalloproteases and integrins.[18]

Fourth, HNSCCs commonly express high level of VEGF, which supports tumor growth by stimulating angiogenesis. EGFR signaling also stimulates VEGF expression by tumor cells. One mechanism of acquired resistance to EGFR inhibitors is the selection of tumor cell subpopulations with increased angiogenic potential[65], suggesting that VEGF might be upregulated by alternative pathways. Therefore, many preclinical studies address the efficacy of targeting EGFR and angiogenic pathways simultaneously. VEGF-A is a key regulator of tumor-induced endothelial cell proliferation and vascular permeability. Adding an anti-VEGFR antibody DC101 to cetuximab, for example, significantly reduced tumor vascularity, inhibited tumor growth, and increased apoptosis in both tumor and endothelial cells.[66] ZD6474 (vandetanib) is a small molecule TKI with specificity towards VEGFR and EGFR. Preclinical studies of ZD6474 demonstrated radiation sensitization of various xenografts[67] and reduction of microvascular density in tumors resistant to cetuximab or gefitinib. These results provided a rationale for the clinical evaluation of ZD6474 with taxanes or cetuximab. Preliminary data from a phase II trial testing sorafenib, a potent inhibitor of the Raf-1, B-Raf, VEGFR-2-3, and PDGFR-B pathways, in metastatic or recurrent HNSCC were recently reported.

COMBINING RADIOTHERAPY WITH OTHER MOLECULAR THERAPEUTICS

Numerous strategies are emerging based on better understanding of tumor biology. A comprehensive overview is beyond the scope of this article, but some strategies are briefly summarized to illustrate the need for extensive commitment and the scientific-practical obstacles to the development of novel therapeutic strategies.

Sensitizing tumors to radiotherapy by targeting the resistant **hypoxic tumor cells** has been attempted for many decades. The clinical results of oxygen mimetic agents (nitroimidazole compounds) have been disappointing with the exception of nimorazol in a Danish trial.[68] The availability of tirapazamine (TPZ), a bioreductive cytotoxic agent that is toxic to hypoxic cells, has renewed interest in this field. Phase III trials testing its combination with radiation and cisplatin in the treatment of HNSCC were launched based on encouraging results of a phase II study.[69] The first efficacy analysis showed no overall survival benefit in favor of TPZ. This finding along with the increased treatment-associated mortality observed in the experimental arm of the second trial led to early termination of this clinical development program in HNSCC (L. Peters, personal communication, 2006). A phase III trial assessing the efficacy of ARCON, which combines accelerated radiotherapy with carbogen (inhalation of hyperoxic gas) and

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nicotinamide (a vasoactive agent) to decrease chronic and perfusion-limited hypoxia, respectively, is approaching completion of patient accrual.

Cyclooxygenase-2 (COX-2), a key enzyme for the synthesis of prostaglandins (PGs), is another target. COX-2 prevents cell damage by ionizing radiation.[70] COX-2 and PG overexpression are linked to carcinogenesis, tumor growth, facilitation of metastatic spread, and decreased immunosurveillance.[70] In addition, macrophages and other inflammatory cells that infiltrate the tumor can produce COX-2 and thereby contributed to increased tumor radioresistance. PGs also enhance bFGF-induced angiogenesis through induction of VEGF.[70] Celecoxib, a COX-2 inhibitor, enhances cellular sensitivity to radiation *in vitro*[70] through inhibition of DNA repair processes and vasculature collapse. Unfortunately, the increased cardiovascular toxicity associated with long-term use of COX-2 inhibitors as chemoprevention in individuals with colorectal adenoma has prematurely dampened the interest, mostly from the industrial sector, in the drug's development as a cancer therapy since 2005. It is worth stressing that the benefit-risk ratio might still be favorable in cancer patients, which is being addressed in an ongoing clinical trial (Table 1).

The concept of targeting **angiogenesis** in cancer has been pioneered by Folkman since 1970s. Agents targeting the VEGF-VEGFR signaling axis used to overcome tumor resistance to EGFR antagonists are presented above. In general, radiation oncologists have been skeptical about combining anti-angiogenic agents with radiotherapy because of concerns of inducing tumor hypoxia and thus diminish the response to radiotherapy. Recent preclinical data, however, suggest that some anti-angiogenic agents may induce a transient normalization window with increased blood flow and tumor oxygenation.[71] In addition, emerging data show that bevacizumab (antibody against VEGF-A) added to chemotherapy increases the response rate and survival in a number of cancers. This feature made it interesting for combining bevacizumab with radiotherapy and chemotherapy for the treatment of cancers in which distant metastasis is the main pattern of relapse (e.g., nasopharyngeal carcinoma). Clinical trials testing the combination of bevacizumab with radiochemotherapy are ongoing in rectal cancers[72] and HNSCC (Table 1). Agents targeting vascular endothelium, including combretastatin A-4, are also being tested.

Some ongoing trials combining radiotherapy with EGFR-inhibitors, anti-angiogenic agents, or other targeted therapies, as presented above, or at the 2006 annual ASCO meeting are summarized in Table 1.

CONCLUSION

Advances in the understanding of tumor biology have opened a new strategy for developing novel cancer therapy. Research on the EGFR signaling pathway exemplifies this quest. Discoveries of the contribution of the EGFR signaling pathways to several key cellular regulatory processes, their perturbation in epithelial neoplasms, and the improvement in tumor response to therapy led to the conception and completion of an integrated research program producing a new frontline therapy modality for locally advanced HNSCC. Although this pivotal phase III trial yielded the proof-of-principle that selective tumor sensitization to radiotherapy can be accomplished by modulating a perturbed signaling pathway, the clinical benefit resulting from this strategy was rather modest, and many questions remain to be addressed. For example, why the magnitude of EGFR expression does not correlate with tumor response to EGFR antagonists is poorly understood and even counter-intuitive. Further investigations are underway to identify biomarkers that predict the response to EGFR inhibitors and isolate the mechanisms that underlie the lack of cetuximab-mediated sensitization in the majority of HNSCC to radiotherapy. Lessons learned from the work on EGFR will contribute to the development of strategies to augment tumor response by modulating other signaling

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pathways individually or in combination, which will bring us closer to the implementation of personalized cancer therapy.

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GLOSSARY

Akt
: also known as protein kinase B (PKB). There are three isoforms Akt1, Akt2, Akt3

ATP
: adenosine triphosphate

bFGF
: basic fibroblast growth factor

B-Raf
: v-raf murine sarcoma viral oncogene homolog B1

Cbl
: Casitas B-lineage lymphoma proto-oncogene (ubiquitin ligase; also called c-Cbl)

DAG
: diacylglycerol

DNA-PK
: DNA-dependent protein kinase

EGFRvIII
: truncated constitutively active variant of EGFR

ERBB
: family of receptor tyrosine kinase receptors including EGFR/HER1, HER2/ERBB2, HER3/ERBB3, HER4/ERBB4

FISH
: fluorescence in situ hybridization

HB-EGF
: heparin-binding EGF

IGF-1R
: Insulin Growth Factor 1 Receptor

IL8
: interleukin 8

Jak
: Janus kinase

MAPK
: mitogen-activated protein kinase

PDGFR-B
: platelet-derived growth factor B

PI3K

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phosphatidylinositol 3-serine/threonine kinase

PKC
serine/threonine kinase protein kinase-C

PLC
phospholipase c (PLC usually stands for PLC-gamma)

PTEN
phosphatase and tensin homolog

Raf
v-raf-1 murine leukemia viral oncogene homolog

Raft
membrane microdomains rich in sphingolipids and cholesterol. Caveolae are sometimes considered as a caveolin-positive subset of lipid rafts

Ras
rat sarcoma viral oncogene homolog

SH2-domain
Src homology 2 protein domain

Shc
SH2 containing transforming protein (protein adaptor)

Src
sarcoma viral oncogene homolog (also called c-Src)

STAT
signal transducer and activator of transcription

TGF-α
transforming growth factor α

TKI
low-molecular-weight ATP-competitive inhibitor of the receptor’s tyrosine kinase

VEGF
vascular endothelial growth factor

VEGFR
vascular endothelial growth factor receptor

Y
tyrosine residue (amino acid)

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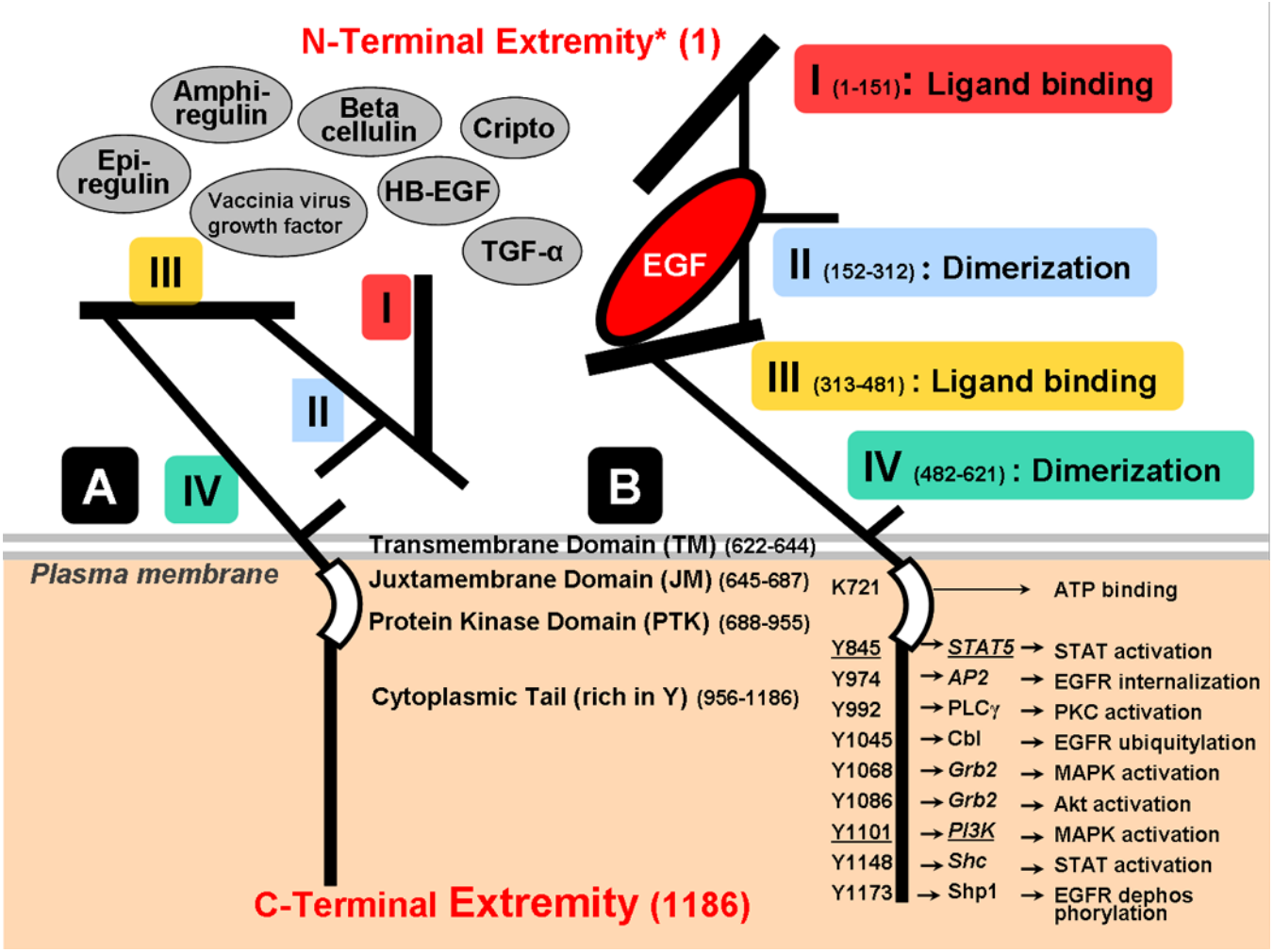

Figure 1. Schematic illustration of EGFR structure in its tethered (A) and untethered (B) form

There are 4 extracellular domains, collectively called ectodomain, of which domains I and III (also referred to as L1 and L2) are involved in ligand binding and the cystein rich domains II and IV (CR1 and CR2) in dimerization. The remainder of the structure consists of a hydrophobic transmembrane domain (TM), a juxta-membrane domain (JM), an intracellular protein tyrosine kinase domain (PTK), including the ATP binding pocket with receptor kinase activity (RTK), and a regulatory carboxyl terminal domain (reviewed by Jorissen *et al.*[73]). The eight ligands (12 ligands for all ERBB receptors together) presently known are EGF, TGF-α, HB-EGF, amphiregulin, betacellulin, epiregulin, vaccinia virus growth factor and *cripto*. Binding of a ligand (e.g., EGF in Panel B) to Domains I and III alters and stabilizes the spatial configuration promoting homo- and hetero-dimerization[10] and subsequent activation (also see Fig. 2).

Tyrosines of the EGFR cytoplasmic tail (e.g., Src activation sites underlined) represent docking sites for adaptors[15] or for downstream proteins. Phosphorylation of the EGFR C-terminus, by autophosphorylation or transphosphorylation by other kinases such as Src[16] and Jak-2, provides specific docking sites for specific interaction domains of intracellular signal transducers and adaptors, leading to their colocalization and to the assembly of multicomponent signaling “particles.” Signaling proteins that associate directly with some EGFR tyrosines are illustrated in panel B.

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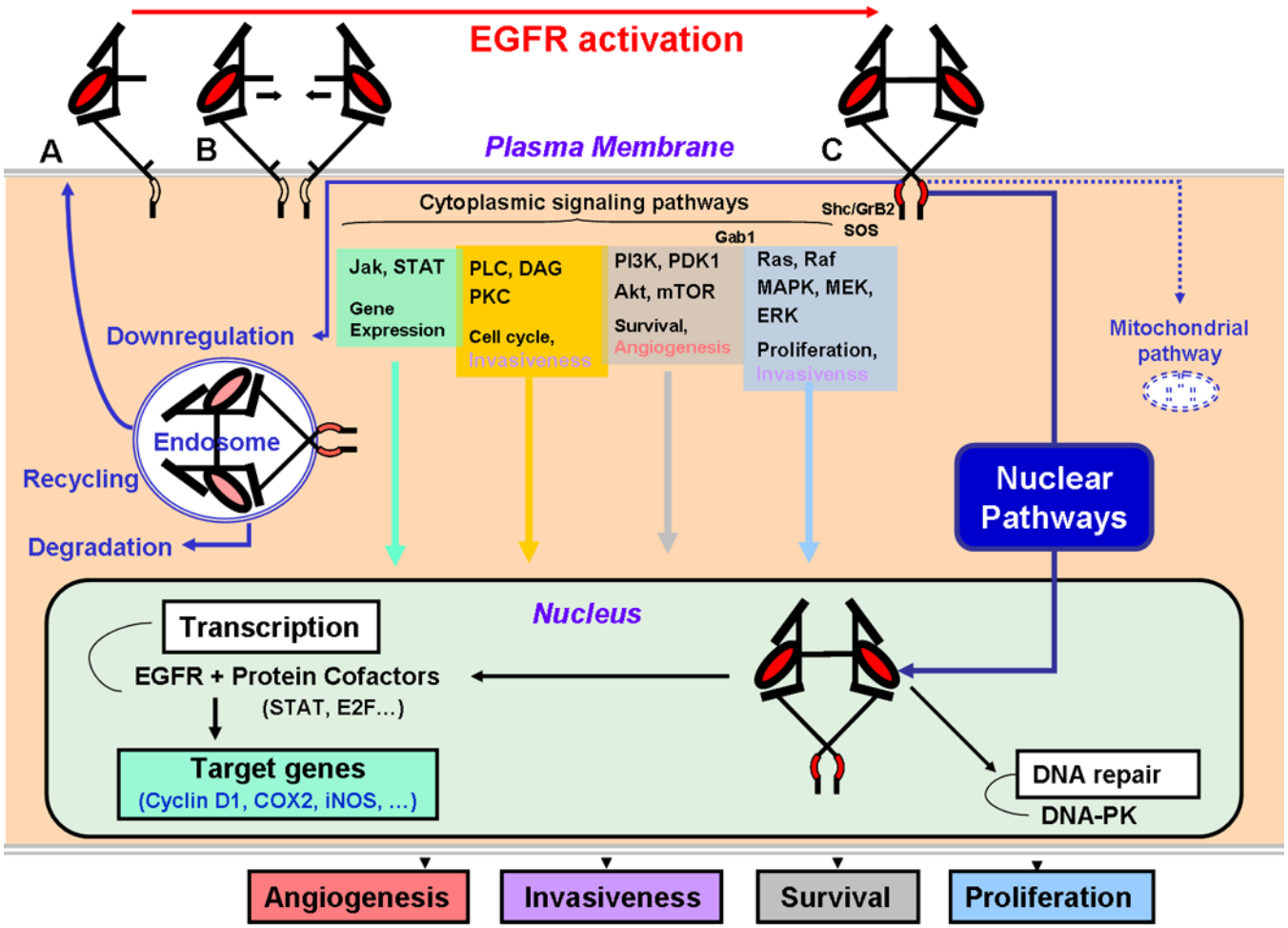

Figure 2. EGFR activation and downstream signaling

Upper panel illustrated ligand-induced conformational change followed by dimerization progressing from Panels A to C (see Jorissen *et al.* for complete review.[73]). This process triggers downstream signaling through four major cytoplasmic pathways depicted schematically. Nuclear influx of EGFR initiates interaction with transcription factors and proteins participating in DNA double-strand break repair. Endosomal degradation or recycling regulates EGFR signaling. A mitochondrial pathway has recently been described and its functions is under investigation.

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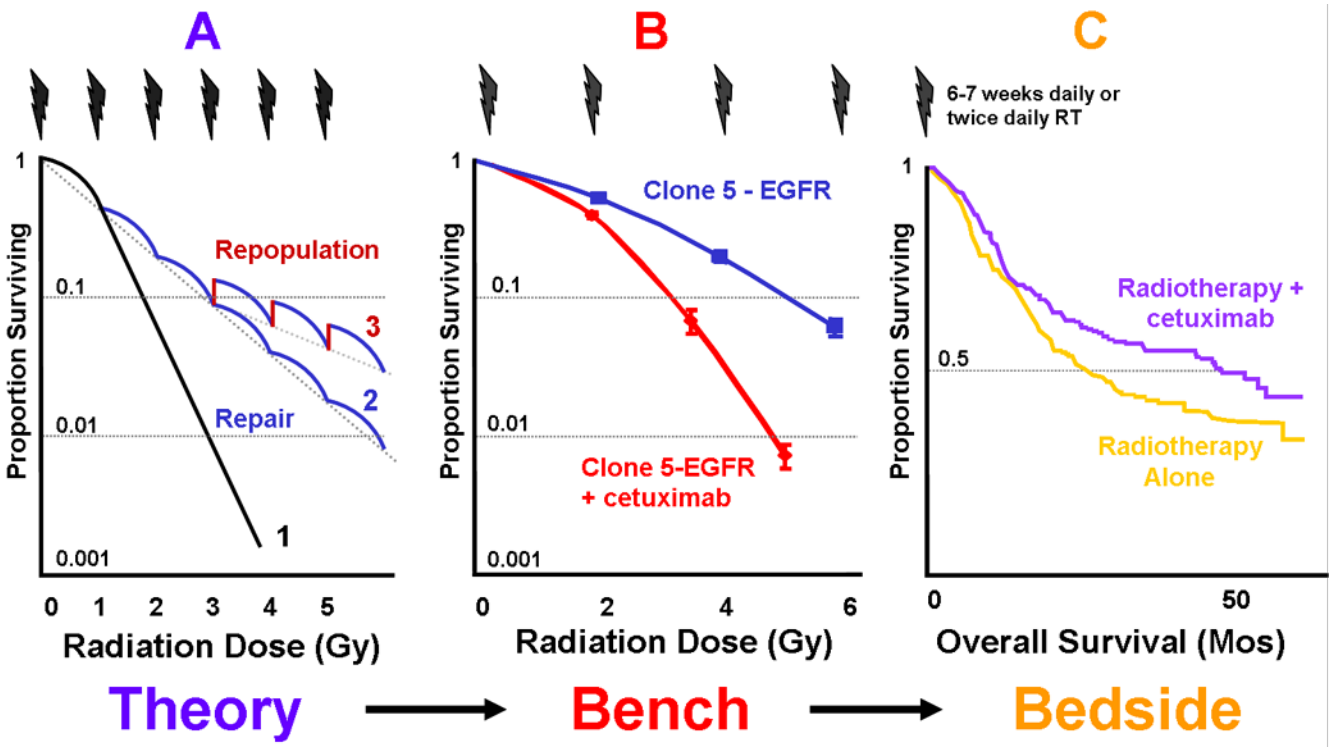

Figure 3. Integration of traditional and molecular radiology for the development of a novel combined therapy modality

Panel A illustrates the survival curve of a single dose exposure along with the effects of sublethal damage repair (from curve 1 to 2) and clonogen repopulation (from curve 2 to 3) between fractions resulting in an increase in cell survival. Panel B shows that radiation resistance resulting from transduction of EGFR can be offset by blocking the EGFR by specific antibody.[38] Panel C summarizes the results of a pivotal randomized clinical trial showing an improvement in overall survival, resulting from better local-regional control, by adding cetuximab to radiotherapy in patients with locally advanced HNSCC.[49]

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Table 1

Examples of ongoing clinical trials assessing combinations of radiotherapy with molecular therapeutics for the treatment of head-and-neck carcinomas. Preliminary results of some trials were presented at the 2006 Annual meeting of the American Society of Clinical Oncology (abstract number).

Principal Investigator or Leader	N	Phase	Tumor Stage	RT combined with	Results or Endpoints
De La Garza, ASCO 2006 (#15502)	20	II	Locally advanced	Cetuximab + gemcitabine	CR 77%, 89% compliance, 42% G-3 mucositis, 21% G-3 rash
Langer (ECOG E3303)	68	II	Locally advanced	Cetuximab + cisplatin	PFS, LC, OS
Kataria (NCT00343083)	60	II	Locally advanced	Cetuximab + paclitaxel + carboplatin	LRC, PFS, pathologic RR, toxicities
Mesia (NCT00251381)	90	II	Locally advanced	Cetuximab	LRC, toxicity
Ang (RTOG 0522; NCT0026594)	720	II	Locally advanced	Cetuximab + cisplatin (accelerated fractionation)	DFS, OS, LRC, toxicity, QOL, and correlative study
Harari (RTOG 0234; NCT00084318)	230	II-R	Locally advanced	Cetuximab + cisplatin or docetaxel (adjuvant)	DFS, LRC,OS, toxicity, correlative study
Argiris (NCT00226239)	40	II	Locally advanced	Cetuximab + cisplatin	RR, toxicities, control and survival rates
Argiris (NCT00291707)	40	I	Locally advanced	Cetuximab + pemetrexed	MTD, DLT, Toxicity, RR
Cohen, ASCO 2006 (#5506)	69	II	Locally advanced	Gefitinib + 5FU + hydroxyurea	CR 89%, RR 98%, PFS 1-year 85%
Doss, ASCO 2006 (#5543)	45	II	Locally advanced	Gefitinib + paclitaxel + carboplatin	CR 32%, 1-year PFS 68% 1-year OS 86% 1 toxic death
Hainsworth (NCT00193284)	50	II	Locally advanced	Gefitinib +/- docetaxel after induction	RR + OS + TTP + toxicity
AstraZeneca (NCT00228488)	60	II	Locally advanced or first recurrence	Gefitinib	effect on gene expression profiles
Morris (NCT00083057)	30	I	Locally advanced or first recurrence	Gefitinib + paclitaxel	MTD, RR
AstraZeneca (NCT00233636)	28	II	Locally advanced	Gefitinib	RR, TTP, OS
Wadler (NCT00195078)	29	I-II	Locally advanced	Gefitinib + cisplatin	RR, DFS, duration of response, toxicity
Adelstein (NCT00352105)	66	I-II	Locally advanced	Gefitinib + cisplatin + FU	OS, DFS, toxicity
Raben (NCT00033449)	30	I	Locally advanced	Gefitinib + cisplatin + maintenance	MTD, toxicity, RR, PFS, OS
Le (NCT00185835)	10	I	First recurrence	Gefitinib + cisplatin (reirradiation)	Toxicity, DFS, OS, RR, duration of response, correlative study
Bensadoun (GORTEC 2004-02; NCT00169221)	140	II-R	Locally advanced	Cisplatin +/- gefitinib (post-operative adjuvant)	Safety, Efficacy
AstraZeneca (NCT00229723)	224	II	Locally advanced	Gefitinib + cisplatin	LCR, PFS, OS, tolerability, correlative study
Herchenhorn ASCO 2006 (abstract 5575)		I-II	Locally advanced	Erlotinib + cisplatin	Toxicity, RR
Savvides ASCO 2006 (#5545)	23	I	Locally advanced	Erlotinib + docetaxel (+ maintenance)	CR: 83%
Brizel (NCT00140556)	30	I-II-R	Locally advanced	Erlotinib + bevacizumab + cisplatin (hyperfractionation)	RR, LRC, OS, Completion rate
Glisson (NCT00113347)	24	I	Locally advanced	Erlotinib + docetaxel	MTD, TTP, OS, swallowing function
Gillison (NCT00049166)	48	I	Locally advanced	Erlotinib +/- cisplatin (IMRT)	MTD, correlative study, PET
Klug (NCT00304278)	20	II	Locally advanced	Erlotinib intra-arterial + cisplatin	Safety, LRC
GSK Clinical trial (NCT00371566)	90	II-R	Locally advanced	Lapatinib	RR, toxicity
Harrington ASCO 2006 (#5553)	17	I	Locally advanced	Lapatinib + cisplatin	Toxicity: 2 DLT (Grade 3-4)
Seiwert ASCO 2006 (abstract 5530)	43	I	First recurrence	Bevacizumab + FU + hydroxyurea	2-Year OS: 26 months
Savvides (NCT00281840)	30	II-R	Locally advanced	Bevacizumab + docetaxel	TTP, RR, duration of response, LRC, OS, toxicity
Prellop ASCO 2006 (#5582)	28	I-II	Locally advanced or first recurrence	Celecoxib + paclitaxel + carboplatin + maintenance	G3-4 febrile neutropenia: 24%, 2-Year OS: 65%, LC: 76%

CR: complete response, DFS: disease-free survival; DLT: dose limiting toxicity; FU: fluorouracil; G: grade; IMRT: intensity modulated radiotherapy; LC: local control; LRC: local-regional control; MTD: maximally tolerated dose; OS: overall survival; PFS: progression free survival; QOL: quality of life; R: randomized; RR: response rate; TTP: time to tumor progression.