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COMBINATION OF EPIDERMAL GROWTH FACTOR RECEPTOR TARGETED THERAPY WITH RADIATION THERAPY FOR MALIGNANT GLIOMAS

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1. ABSTRACT

Glioblastoma multiforme (GBM) are extremely aggressive brain tumors characterized by resistance to standard treatment modalities including surgery, radiation therapy and chemotherapy. While radiation therapy is the standard treatment after surgical resection, these tumors invariably recur and are associated with a uniformly dismal prognosis. Cytotoxic chemotherapy has failed to improve on the modest gains conferred by radiation therapy. Our understanding of the molecular events driving gliomagenesis has led to the recognition of frequent alterations in the epidermal growth factor receptor (EGFR) pathway, leading to increased aggressiveness and a poorer prognosis. Based on the importance of EGFR in the development of malignancy in multiple tumor types, several classes of novel therapeutic agents have been developed that specifically target EGFR. This review outlines the relevance of normal and aberrant EGFR signaling in the biology of gliomas, the strategies for inhibiting EGFR activity and the rationale for combining EGFR inhibitors with radiation therapy in the treatment of GBM.

2. INTRODUCTION

Glioblastoma multiforme (GBM) accounts for 25% of all primary central nervous system (CNS) tumors in adults and is associated with a uniformly dismal prognosis. Standard therapy is surgical resection followed by radiation therapy with or without adjuvant chemotherapy (1). Unfortunately, these tumors are characterized by resistance to all therapies and rapidly recur within months of treatment. Despite extensive research evaluating combinations of cytotoxic chemotherapies with radiation therapy, no substantial improvement in survival has been

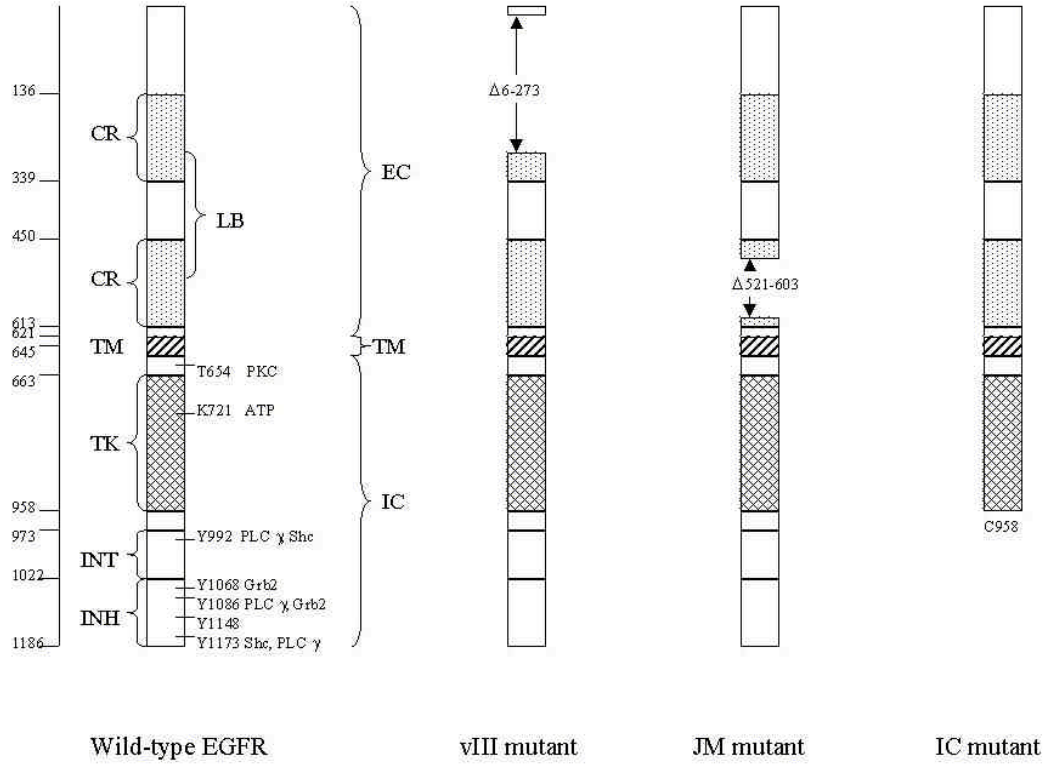

Figure 1. Structural motifs and regulatory domains of EGFR and its commonly mutated forms. Key amino acid residues important for interactions with down-stream molecules are indicated. Various domains of the EGFR protein are abbreviated as follows: CR = cysteine-rich, EC = extracellular, IC = intracellular, INH = tyrosine kinase inhibitory, INT = receptor internalization, LB = ligand binding, TK = tyrosine kinase, TM = transmembrane.

achieved over the past 3 decades (2). Factors contributing to the poor efficacy of chemotherapies include 1) lack of specificity of chemotherapeutic agents against gliomas, 2) excessive CNS toxicity, and 3) the low penetrance of these agents through the blood-brain barrier (3-6).

During the past few years, significant advances have been made in describing the molecular biology and genetics of gliomas, and this increased understanding has facilitated the development of an impressive armamentarium of novel cytostatic (as opposed to cytotoxic) agents designed to specifically inhibit tumor proliferation and progression. Most promising among these is a spectrum of novel agents that perturb receptor tyrosine kinase signaling that is especially critical to the biology of malignant gliomas. In this review, we will highlight the unique role played by EGFR in glioma biology, the mechanisms of constitutive activation of receptor signaling and methods of inhibiting the receptor. Finally, we will review the rationale for combining these cytostatic agents with radiation therapy.

3. RELEVANCE OF EGFR IN MALIGNANT GLIOMAS

The EGFR family consists of four closely related transmembrane receptors. EGFR (erbB1/HER1) is a 1186 amino acid polypeptide that binds epidermal growth factor (EGF), transforming growth factor alpha (TGF alpha), amphiregulin, heparin-binding EGF-like growth factor, betacellulin, epiregulin and vaccinia virus growth factor (7-13). ErbB2 (HER2), erbB3 (HER3) and erbB4 (HER4) are the other members of this family. ErbB3 and ErbB4 bind various growth factors; erbB2 has no known ligand and presumably acts through heterodimer formation with other erbB family members. All family members contain an extracellular ligand-binding domain, a transmembrane domain and an intracellular tyrosine kinase domain (Figure 1). Ligand binding results in homo- and hetero-dimerization with various family members. Dimerization facilitates autophosphorylation of tyrosine residues in the carboxy-terminus of the dimer, which can then regulate down-stream signaling pathways.

EGFR activates several downstream signaling cascades (Figure 2): the Ras/mitogen activated protein (MAP) kinase pathway, phospholipase C gamma (PLC gamma), and phosphatidylinositol 3' kinase (PI3K) (reviewed in detail in (14, 15)). The autophosphorylation of the carboxy-terminal tyrosine residues provides multiple docking sites for proteins containing *src* homology (SH-2) binding domains. These regulatory proteins include the adaptor proteins Shc, growth factor receptor-bound protein-2 (Grb-2) and son of sevenless (SOS), which bind to and facilitate the conversion of inactive Ras.GDP to activated Ras.GTP. Activated Ras recruits the serine-threonine

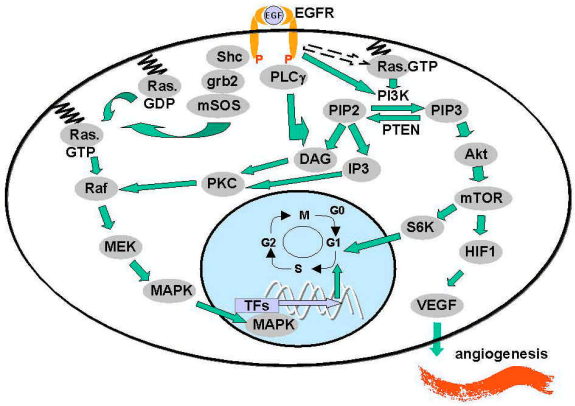

Figure 2. EGFR signaling pathway. Binding of ligand to EGFR leads to receptor dimerization, autophosphorylation and activation of downstream signaling pathways. These signals mediate cell cycle progression and angiogenesis, among other effects. EGF = epidermal growth factor, EGFR = epidermal growth factor receptor, Shc = src homology domain consensus, grb2 = growth factor receptor-bound protein 2, mSOS = mammalian son of sevenless, GDP = guanosine diphosphate, GTP =guanosine triphosphate, Raf = Ras activated factor, MEK = MAP kinase kinase, MAPK = mitogen activated protein kinase, TFs = transcription factors, PI3K = phosphatidylinositol 3' kinase, PIP2 = phosphatidyl inositol 3,4 – diphosphate, PIP3 = phosphatidyl inositol 3,4,5 triphosphate, PTEN = Phosphatase and Tensin Analog (a tumor suppressor), Akt = atypical kinase, mTOR = mammalian target of rapamycin, S6K = ribosomal S6 kinase, HIF-1 = hypoxia inducing factor, VEGF = vascular endothelial growth factor, PLC gamma = phospholipase C gamma, DAG = diacyl glycerol, IP3 = inositol 3,4,5 triphosphate, PKC = protein kinase C. The "P"s represent phosphorylation in intracellular tyrosine residues of EGFR. The broken arrow represents the Ras activation pathway depicted on the left side of the figure.

kinase Raf to the membrane and stimulates phosphorylation of MAP kinase-kinase (MEK) and subsequent phosphorylation of MAP kinase. Activated MAP kinase translocates to the nucleus and activates multiple transcription factors important for cell proliferation. Likewise, PLC-gamma is activated through association with carboxy-terminal phospho-tyrosine residues. PLC-gamma catalyzes the breakdown of phosphatidyl inositol 3,4-diphosphate (PIP2), into inositol triphosphate (IP3) and diacyl glycerol (DAG). Both of these compounds activate protein kinase C (PKC), which in turn activates the MAP kinase pathway. Thus, both the Ras and the PLC-gamma cascades converge on the MAP kinase pathway to drive cell proliferation and survival.

EGFR also modulates PI3K-dependent signaling pathways indirectly through the Ras pathway and directly through interactions with the p85 catalytic domain of PI3K. PI3K catalyzes the phosphorylation of PIP2 to phosphatidyl inositol 3,4,5 triphosphate (PIP3). This lipid second messenger activates multiple downstream pathways including the phosphoinositide-dependent kinases (PDK) PDK-1 and PDK-2. PIP3 also recruits the atypical kinase (Akt, protein kinase B) to the membrane, where it is phosphorylated and activated by PDK-1 and -2. (15). Akt promotes cell survival and prevents apoptosis through phosphorylation of multiple downstream proteins including Bad, caspase 9 and forkhead transcription factor (FKHLR1). (16, 17). Akt also promotes cell-cycle progression through activation of the mammalian target of rapamycin (mTOR). mTOR regulates the translation of select mRNA transcripts, several of which are important for cell cycle progression, through regulation of eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) and ribosomal p70S6 kinase (18-20).

Through regulation of these downstream signaling pathways, EGFR controls many important cellular functions including cell growth and division, protection from apoptosis, intracellular vesicle trafficking, adhesion, motility, invasiveness and angiogenesis (15, 21-31). Consistent with the importance of these pathways in GBM biology, either overexpression of EGFR or combined overexpression of Ras and Akt constructs in neural progenitor cells induces GBM-like tumor formation in transgenic mice (32, 33).

3.1. Alterations of EGFR in gliomas

One of the earliest identified oncogene alterations in human cancer was amplification of the EGFR gene in glioblastomas (34). In fact, the EGFR gene is amplified in approximately 40% of all malignant gliomas and the majority of these tumors also contain activating mutations of EGFR (34-41). As discussed above, the constitutive activation of EGFR activity results in dysregulation of multiple key cellular functions including increased proliferation, motility and survival of transformed cells. The most common genetic alterations of EGFR seen in GBM are reviewed below.

3.2. Overexpression of EGFR

Overexpression of wild-type EGFR in glioblastomas can arise from increased transcriptional activity or more commonly through gene amplification (35, 42-44). Amplification of EGFR gene occurs in about 40% of GBMs and invariably predates subsequent gene alterations that further augment receptor signaling (35). These gene alterations include mutations that affect the extracellular, intracellular and juxta-membrane regions of EGFR.

3.3. Common EGFR mutations

Of the different extracellular deletion mutants, the vIII mutant is the most common, occurring in about 75% of tumors overexpressing EGFR. This variant lacks codons 6-273 (exons 2-7) in the extracellular domain adjacent to the ligand binding domain. This deletion confers ligand-independent constitutive tyrosine kinase activity (45-48). This constitutively active mutant receptor is not down-regulated, internalizing at a level as low as unstimulated wt-EGFR, suggesting that the altered conformation of the mutant receptor does not result in exposure of receptor sequence motifs required for endocytosis and lysosomal sorting (48). Even in the

absence of ligand-mediated dimerization, downstream signaling from this monomeric receptor is intact with constitutive activation of Shc/Grb2-mediated stimulation of Ras activity, and activation of PI3K (49-51). Based on the biology of EGFR signaling, it is not surprising that this mutation drives proliferation, promotes cell transformation and enhances cell motility (45-47, 52-54). In addition, expression of the vIII mutant induces chemoresistance through up-regulation of bcl-X_L and down-regulation of caspase-3-like proteases and enhances *in vivo* tumorigenicity (55, 56).

The most common intracellular domain mutation of EGFR results in a protein truncated at amino acid 958 (35). This imparts elevated and sustained ligand-dependent signaling due to deletion of a negative regulatory domain and decreased internalization of the receptor. Some ligand-independent activity may also be conferred upon the receptor, since this mutant dimerizes in the absence of growth factors (C. D. James, unpublished results). The common juxta-membrane deletion mutant EGFR results in receptors that lack amino acids 521-603. Little is known about the consequences of this mutation, other than its impairment of ligand binding and receptor dimerization (C. D. James, unpublished results). Although biochemical characterization of these EGFR mutants is ongoing, it appears that all these mutations result in increased signaling through downstream pathways and contribute to the transformed phenotype of GBM.

In addition to the mutant forms of EGFR described above, overexpression of EGF (or TGF-alpha) stimulates wild-type EGFR in GBM cells, thereby promoting increased proliferation, invasiveness and motility and angiogenesis (57-66). The concurrent expression of EGFR and its ligands EGF and TGF-alpha on glioma cell surfaces is suggestive of an autocrine or paracrine stimulatory loop, that essentially achieves the same effect as EGFR overexpression or mutation (44, 67, 68). Such an effect is similar to the early embryologic expression of TGF-alpha and EGFR noted in areas of gliogenesis and restriction of EGFR expression to proliferating zones of the postnatal brain (69-72). In addition to these parallels between EGFR expression of gliomas and glial progenitor cells, there is a unique semblance between the prominence of the growth factor signaling pathways in gliomas and the role of growth factors in the developing nervous system.

3.4. Correlation with gliogenesis

During gliogenesis, differentiation along specific neural lineages is tightly regulated by specific hierarchies of growth factors sequentially inducing their corresponding growth factor receptors. Early in the second week of embryologic development, glial growth factor 2 (GGF2) acts on neural crest cells to direct their glial, rather than neuronal commitment, and fibroblast growth factor (FGF) regulates the production of radial glial cells (73, 74). Together, these growth factors promote the formation of a lattice-like scaffolding that supports and directs the migration of neurons, and sustains the proliferation of pluripotent stem cells (75-77). The pluripotent stem cells later become less responsive to FGF and more responsive to EGF, which directs glial differentiation. EGFR expression by cells in the germinal zone has been postulated to account for the genesis, differentiation, migration and survival of many cell populations, possibly including precursor cells (69). Glial-restricted stem cells migrate to the subventricular zone where they evolve into bipotent oligodendrocyte-type-2 astrocytes (O2A), and the unipotent type-1 astrocytes (T1A) that preferentially differentiate into unipotent oligodendrocytes and unipotent astrocytes, respectively.

Distinct subsets of growth factors are responsible for promoting the growth and differentiation of oligodendroglial and astrocytic cell lineages. The major growth factor stimulating growth and division of the oligodendrocytic lineage of cells is platelet derived growth factor (PDGF), and to a lesser extent, FGF (78-81). Extended self-renewal and inhibition of differentiation can be accomplished *in vitro* by continuous stimulation with PDGF and FGF (82). A number of growth factors and neurotransmitters acting through multiple signaling cascades promote the proliferation and/or the differentiation of the type-1 astrocytic lineage cells. Prominent among these are EGF, FGF, transforming growth factor-beta (TGF-beta) and its sub-family of bone morphogenetic proteins (BMPs), insulin-like growth factor-1 (IGF-1), ciliary neurotrophic factor and leukemia inhibitory factor (83-90). As discussed previously, these growth factor signaling pathways play a prominent role not only in normal development of the nervous system, but also in the transformation of glial cells into malignant tumors. Therefore, EGFR is a key molecule in both gliogenesis and gliomagenesis, and may be an attractive target for the development of novel targeted therapeutics.

4. STRATEGIES FOR TARGETING EGFR

Based on the importance of EGFR signaling in the pathogenesis of many tumors, intensive research efforts have been focused on developing therapeutic strategies targeting aberrant EGFR activity (42, 43, 91). Dysregulation of EGFR and its downstream signaling pathways significantly contributes to the aggressive phenotype of malignant gliomas. This suggests that EGFR-targeted therapies may be especially useful in the treatment of malignant gliomas. Currently, two different classes of anti-EGFR inhibitors have been developed for clinical use: EGFR-specific monoclonal antibodies and small molecule kinase inhibitors.

4.1. Monoclonal antibodies

Several monoclonal antibodies have been developed that bind to the extracellular domain of EGFR. The most prominent of these antibodies currently in clinical trials is C225 (cetuximab or IMC-225, Im Clone Systems Inc.). This monoclonal antibody binds to the EGFR ligand-binding domain with an affinity similar to natural ligands and competitively inhibits EGF and TGF-alpha binding. This blocks ligand-mediated dimerization and subsequent kinase activation (92). As predicted from EGFR biology, treatment with C225 in various model systems decreases

tumor proliferation, inhibits tumor-induced angiogenesis, and reduces tumor cell invasion and metastasis (93-96). Other antibody-based EGFR-specific therapies include antibodies targeting the EGFRvIII extracellular domain deletion mutant (recognizing the EGFRvIII epitope alone or both the EGFRvIII and the wt-EGFR epitope), bi-specific antibodies which bind both EGFR and cytotoxic lymphocytes, and EGFR-specific antibodies conjugated to either immunotoxins or therapeutic radioisotopes (97-102).

4.2. Tyrosine kinase inhibitors

A variety of small molecule inhibitors of EGFR kinase activity have been developed and are currently being tested in clinical trials. These compounds are typically ATP-mimics that inhibit kinase activity through displacement of ATP from the catalytic pocket of the enzyme. ZD1839 (Iressa, Astra Zeneca Pharmaceuticals), OSI-774 (CP358,774 or Tarceva, OSI Pharmaceuticals), CI-1033 (Pfizer Inc.), EKB-569 (Wyeth Pharma), PKI-116 (Novartis Pharmaceuticals Corporation) and AG1478 (Calbiochem) are all examples of kinase inhibitors that selectively target the EGFR family of receptor tyrosine kinases (103-107). ZD1839 and OSI-774 are quinazolines and are both competitive kinase inhibitors specific for EGFR. Likewise, the pyrrolopyrimidine, PKI-116, is a competitive EGFR-specific inhibitor. CI-1033 and EKB-569 are brominated quinazoline derivatives that irreversibly bind and inhibit all HER family members (pan-erbB inhibitors). The cellular effects of these inhibitors closely mimic the biologic effects noted with C225. However, it will be interesting to see how the varying specificities and binding properties of these different drugs translate into differential biologic activities in animal models and ultimately, in the clinic.

While both antibody and small molecule strategies effectively inhibit EGFR signaling in model systems, each approach has potential limitations in the clinical setting in glioma patients. Intravenous administration of antibodies is required to prevent degradation in the stomach, and antibodies may be less likely to achieve good tissue penetration. Repetitive therapy with monoclonal antibodies also places patients at risk for developing human-anti-mouse antibodies, which would preclude subsequent antibody therapy. In contrast, small molecule tyrosine kinase inhibitors are orally administered, readily cross the blood-brain barrier and do not elicit any immune reaction. Moreover, small molecule inhibitors of kinase activity should inhibit both wild-type and mutant forms of the receptor, while EGFR-specific antibodies may lack efficacy against mutated EGFR molecules (108), (C.D. James, unpublished results). However, concomitant administration of small molecule inhibitors with common anticonvulsants can be problematic because both classes of drugs are metabolized by the same hepatic cytochrome p450 enzyme system. Thus, maintaining therapeutic drug levels can be a challenge because of the inter- and intra-patient variability in drug metabolism. In addition, drug transporters such as the multi-drug resistance protein can decrease the effective concentrations achievable in cells (109). There is data to suggest that they maintain similar potency against truncated receptors as against wild-type receptors.

While EGFR-targeted therapies have promising efficacy in several epithelial malignancies, the redundancy of mitogenic signaling pathways in gliomas may render a subset of these tumors resistant to EGFR inhibition. PDGF, heregulin/neuregulin, bFGF, insulin-like growth factor (IGF), TGF-beta and VEGF have all been implicated in the biology of malignant gliomas (reviewed in detail in (110)). In one example, resistance to EGFR-targeted therapy was mediated through up-regulation of IGF receptor-I (IGFR-I) (111). IGFR-I-mediated stimulation of PI3K circumvented the inhibition of a parallel EGFR pathway and promoted invasion and prevented apoptosis despite EGFR inhibition. While these parallel pathways may be important for cell survival under changing environmental conditions, the redundancy of these signaling pathways may limit the efficacy of monotherapy. One avenue for future investigations will involve targeting multiple pathways in an attempt to overcome this problem.

5. COMBINED RADIATION AND EGFR INHIBITOR THERAPY: MECHANISMS OF ACTION

Radiation therapy is the standard of care for glioblastoma multiforme following stereotactic biopsy or surgical resection. While radiation doubles the median survival of patients with glioblastoma, these tumors invariably recur, frequently within or just outside the irradiated field (112). Clinically, EGFR overexpression correlates with relative resistance to radiation therapy in GBM and in other cancers (113-119). Moreover, several laboratories have demonstrated that ionizing radiation exposure induces EGFR autophosphorylation, and this radiation-inducible activation of EGFR may contribute to radiation resistance of tumors in animal models (120-128). Based on these observations, the combination of EGFR inhibitors with radiation therapy has been evaluated by multiple laboratories.

The majority of pre-clinical studies evaluating the combination of EGFR inhibition with radiation have been performed with the EGFR-inactivating C225 antibody. In clonogenic *in vitro* survival assays, C225 only modestly enhances the lethal effects of ionizing radiation, suggesting that EGFR blockade should have only limited effects on the efficacy of radiotherapy (129-132). Interestingly, in marked contrast to these *in vitro* results, concurrent administration of C225 and radiation in human tumor xenograft models demonstrates a profound enhancement of efficacy for combination therapy compared to either therapy alone (129, 131, 133-135). In glioma model systems, the combination of C225 and radiation significantly enhanced the survival of mice bearing established intracranial glioblastoma xenografts (136). Similar enhancement of radiation induced cell killing has been seen with a neutralizing monoclonal antibody against TGF-alpha, tyrosine kinase inhibitors and dominant-negative EGFR constructs (137-139). The marked dichotomy between these *in vitro* and *in vivo* results suggests that EGFR modulates key processes important for survival following radiation that are unique to solid tumors grown in animal models and presumably in spontaneously occurring human tumors.

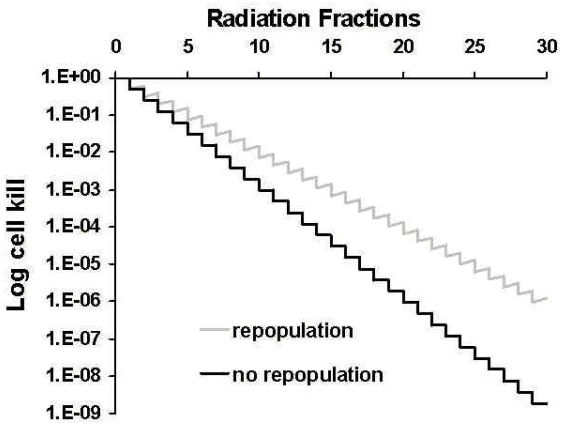

Figure 3. Theoretical effect of tumor repopulation on tumor control probability. In each curve, 50% cell killing was assumed for each dose of radiation. Continued cell proliferation during a course of fractionated radiation therapy results in partial tumor repopulation between fractions of radiation (dark line). In theory pharmacologic inhibition of tumor repopulation (light line) could significantly improve the efficacy of therapy.

The efficacy of combination therapy has been variously ascribed to marked enhancement of radiation-induced cell killing, inhibition of tumor proliferation, cell cycle redistribution, and inhibition of angiogenesis (130, 131, 133-135). However, the radiobiological mechanisms responsible for the apparent synergistic interaction between radiation and EGFR blockade *in vivo* are not entirely clear. In general, the response of a solid tumor to a fractionated course of radiation therapy is governed by four major factors: 1) intrinsic radiosensitivity, 2) continued tumor proliferation, 3) hypoxia, and 4) distribution of cells within the cell cycle. All of these factors may be important in the mechanism of EGFR-mediated radiosensitization and will be discussed below.

5.1. Intrinsic Radiosensitivity

The ability of individual cells to recover from an acute radiation exposure is referred to as intrinsic radiosensitivity (140). EGFR inhibitor therapy may directly impact on radiosensitivity through inhibition of pro-survival cell signaling pathways. Two of these key pathways are the Ras pathway and the PI3K pathway. Hyper-activation of the Ras signaling pathway through expression of oncogenic forms of Ras results in increased radioresistance in multiple tumor cell types (141-144). Moreover, disruption of downstream Ras signaling, either through antisense constructs targeting Raf expression or small molecule inhibitors of Ras activation, lead to increased radiation sensitivity (reviewed in (145)). Similarly, inhibition of PI3K activity with the small molecule inhibitor LY294002 increases the radiosensitivity of tumor cells (142). The key downstream targets in these pathways responsible for modulating radiosensitivity remain to be elucidated, although presumably these pathways function by preventing the activation of radiation-inducible cell-death programs. Therefore, EGFR-inhibitor therapy may downregulate the pro-survival signals propagated through these two pathways and result in increased cell death following radiation.

EGFR inhibitor therapy also may disrupt DNA repair. In a recent study, incubation of cells with the anti-EGFR C225 antibody promoted the association of the DNA-dependent protein kinase (DNA-PK) with EGFR and sequestration of this key DNA repair enzyme in the cytoplasm of cells (146). DNA-PK, in conjunction with its Ku70 and Ku80 binding-partners, binds to and directs the repair of DNA double strand breaks. Therefore, redistribution of DNA-PK from the nucleus to the cytosol in response to C225 therapy may result in decreased repair of potentially lethal radiation-induced DNA double strand breaks (133). It will be interesting to see if these effects on DNA-PK sub-cellular localization can be recapitulated with small molecule EGFR inhibitors.

5.2. Tumor cell proliferation

Radiation therapy is typically delivered daily to patients in 30 to 35 fractions delivered over a course of six to seven weeks. Unfortunately, tumor cell proliferation does not stop with the first dose of radiation, and continued proliferation of surviving tumor clonogens between radiation doses, otherwise known as tumor cell repopulation, increases the total number of tumor cells that must be killed in order to sterilize a tumor (Figure 3) (147). Evidence from clinical studies suggests a 10-15% loss in local control for every week of treatment prolongation beyond the usual six or seven weeks, which supports the idea that tumor proliferation during a course of radiation therapy can have a significant adverse impact on tumor control (148-151). Moreover, tumor proliferation rates actually may increase following the initiation of radiation therapy in a phenomenon known as accelerated repopulation (152). Especially for epithelial malignancies, the idea that tissues would respond to radiation-induced injury by accelerated proliferation is in keeping with studies of radiation-induced injury of epithelial tissues like skin and oral mucosa (153, 154).

EGFR-inhibitor therapy may target both standard repopulation and accelerated repopulation. In theory, any cytostatic agent combined with radiation therapy has the potential to inhibit tumor clonogen repopulation and improve the overall efficacy of therapy (Figure 3) (155). EGFR signaling is important for driving tumor cell proliferation, and, not surprisingly, EGFR inhibition significantly inhibits cellular proliferation *in vitro* and *in vivo* (129-135). Moreover, clinically relevant doses of radiation in tissue culture models stimulates autophosphorylation of EGFR, and this radiation-inducible activation of the receptor could be linked to accelerated tumor repopulation (120-123, 156). Inhibitors of EGFR kinase activity should block receptor autophosphorylation and potentially inhibit accelerated repopulation.

5.3. Inhibition of angiogenesis

Combination therapy with specific angiogenesis inhibitors significantly enhances the efficacy of fractionated radiation therapy in animal models (157-162). This suggests that the anti-angiogenic effects of EGFR

inhibitor therapy may contribute to the radiation "sensitizing" effects of these agents. The downstream signaling pathways controlling EGFR-mediated angiogenesis have been partially elucidated. EGFR promotes hypoxia-inducible and hypoxia-independent tumor angiogenesis (64-66, 163). EGFR-mediated angiogenesis is primarily driven through downstream activation of a PI3K – Akt – mTOR-signaling pathway (164). Activation of mTOR promotes the accumulation of the hypoxia-inducible factor-1 alpha (HIF1-alpha). Dimerized with HIF1-beta, the HIF1 complex drives transcription of multiple hypoxia-inducible genes including vascular endothelial growth factor (VEGF) reviewed in (165). Particularly in malignant gliomas, VEGF-mediated changes in vascular permeability play a key role in promoting the development of edema surrounding and associated with these tumors (166-169). Peri-tumoral edema, especially within the closed confines of the skull, may result in increased intra-tumoral pressure and decreased perfusion in areas of marginal vasculature. Overall, these factors may reduce the perfusion of marginal areas of the tumor vasculature and increase tumor hypoxia. Although speculative at this point, these observations suggest that constitutive activation of EGFR with subsequent overexpression of VEGF might actually increase areas of transient hypoxia within a tumor. Hypoxic cells are significantly more resistant to the lethal effects of ionizing radiation than normally oxygenated cells (170). Thus, it may be possible that down-regulation of VEGF through EGFR inhibitor therapy might improve tumor oxygenation and, consequently, improve the efficacy of radiation therapy.

5.4. Redistribution of cells in the cell cycle

As noted earlier, EGFR inhibitor therapy leads to G1 cell-cycle arrest. Cells in G1 phase are intermediately sensitive to radiation, as compared to more radioresistant cells in G0 and S phase (171). Therefore, drug mediated redistribution of cells from S to G1 may contribute to augmentation of radiation response in animal models (130). However, human solid tumors typically have a much smaller fraction of cells actively engaged in the cell cycle. Therefore, redistribution of cells from S phase may have only modest effects on radiosensitivity in human tumors.

6. PERSPECTIVE

This review summarizes the role of EGFR signaling in the pathogenesis and malignant behavior of gliomas. While a variety of growth factors are important for the proliferation and survival of glioma cells, EGF and TGF-α seem to be pivotal players that orchestrate their mitogenic effects through EGFR signaling pathways. Ligand-driven phosphorylation of EGFR leads to increased proliferation, motility, adhesion, invasiveness, angiogenesis and decreased apoptosis. These effects are magnified by the amplification and overexpression of wild-type or constitutively activated receptors. Based on the importance of EGFR in the biology of malignant gliomas, it may be possible that EGFR-targeted therapies will reduce the malignant behavior of these invariably fatal tumors. Moreover, combining EGFR blockade with radiation therapy may improve the efficacy of radiation therapy.

The exciting pre-clinical work has prompted multiple clinical trials evaluating EGFR inhibitors as mono-therapy and in combination with radiation. In early clinical trials, C225 combined with radiation therapy in the treatment of head and neck cancers resulted in promising clinical response rates (172). This prompted an ongoing multi-center randomized clinical trial comparing radiation alone to radiation with C225 in head and neck cancer. While combination studies of EGFR inhibitors in GBM are not as advanced, the North Central Cancer Treatment Group (NCCTG) is completing a large phase II clinical trial evaluating treatment of GBM patients with ZD1839 following the completion of radiotherapy in patients with stable disease. A subsequent trial through the Mayo Clinic and the NCCTG will evaluate concomitant therapy with radiation and the EGFR inhibitor, OSI-774, in patients with previously untreated GBM. These clinical trials represent the first generation of molecularly targeted therapeutic strategies that will hopefully enhance the efficacy of radiation in the dreaded disease.

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