2. SCIENTIFIC BACKGROUND FOR INHIBITION OF EGFR FOR RADIOSENSITIZATION.

2.1. Rationale.

The use of fractionated radiotherapy for a number of carcinomas, prominently head and neck squamous cell carcinomas (HNSCC), is associated with tumor cell responses that counteract the effects of radiation and may result in relative clinical radioresistance. Most recently, evidence for such responses has also been generated lung carcinomas and malignant gliomas. The underlying mechanism, derived from clinical tumor control analyses, represents accelerated repopulation resulting in reduced tumor control rates with protraction of radiation treatment courses [1]. These analyses suggest that repopulation starts approximately 20 - 28 days after initiation of radiotherapy, but may begin earlier according to alternative evaluations of the same data [2,3]. The molecular mechanisms of accelerated repopulation remained unknown until we demonstrated that ionizing radiation in the therapeutic dose range can cause activation of proliferation promoting receptor Tyr kinases, prominently members of the ERBB family in many human carcinoma and malignant glioma cells. The radiation-induced broad activation of ERBB receptors, ERBB1 – 4, results in carcinoma cell proliferation, one component of a complex cytoprotective response that also includes enhanced resistance to apoptosis [4] and increased repair capacity as result of broad transcriptional activation [5].

2.2. ERBB RTK-mediated radioresistance.

First evidence for the role of ERBB RTKs in cellular radiation responses was provided by the 9-fold over-expression of EGFR in MCF-7 cells after repeated radiation exposures *in vitro* [6]. The next set of studies demonstrated *in vitro* the ability of radiation to stimulate proliferation of A431 SCCs establishing conditions under which these cells were rendered non-proliferative by low concentrations of EGF [7]. The proliferation response was dependent on radiation dose between 0.25 and 5 Gy with the steepest dose response relationship between 1 and 2 Gy; these are radiation doses employed therapeutically, thus enforcing the radiosensitizing potential through inhibition of EGFR function.

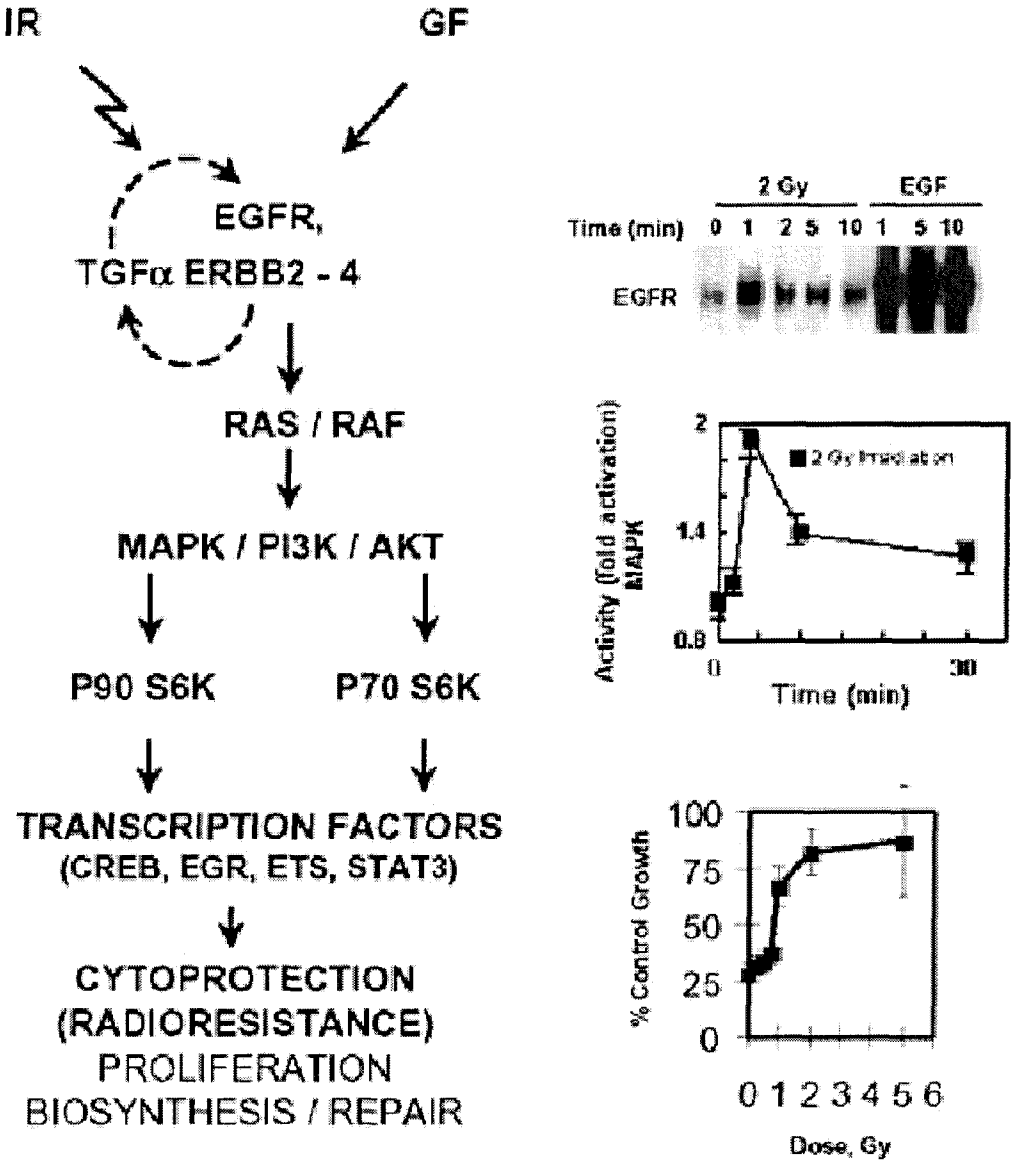

Figure 1. IR- and GF-mediated proproliferative cytoprotective response. IR-/GF-induced ERBB1 (-2, -3, -4) activation occurs within 1–10 min (panel, top right). The 21 phase of ERBB1 activation after 30 min is most likely due to release of intrinsic TGF-α. This ERBB response is modifed by RAS or RAF and results in MAPK stimulation 10–15 min after IR exposure (panel, middle right). Similar phasic responses are seen for PI3K and AKT. Transcription factors, quantitatively depending upon ERBB1/RAS/MAPK activation, initiate responses of potential relevance for cellular IR responses, such as PCNA induction by CREB and enhancement of BCL-XL expression through STAT3. As an important component of the IR-induced cytoprotectiveresponse, the proliferation response (in A431 cells) is expressed as a release from EGF-induced growth inhibition (panel, bottom right.

This proliferative response was further functionally linked to the activation of EGFR by single and repeated radiation exposures in the same dose range [8-10]. The receptor activation occurs within minutes of exposure of cells to 1 – 5 Gy and, therefore, represents one of the earliest responses of cells to radiation exposure. As the response pathway downstream of EGFR that is responsible for

transmitting the EGFR-initiated radiation signals and mediates cellular proliferation responses we identified the RAS/MAPK/P90S6K cascade [9, 11, 12].

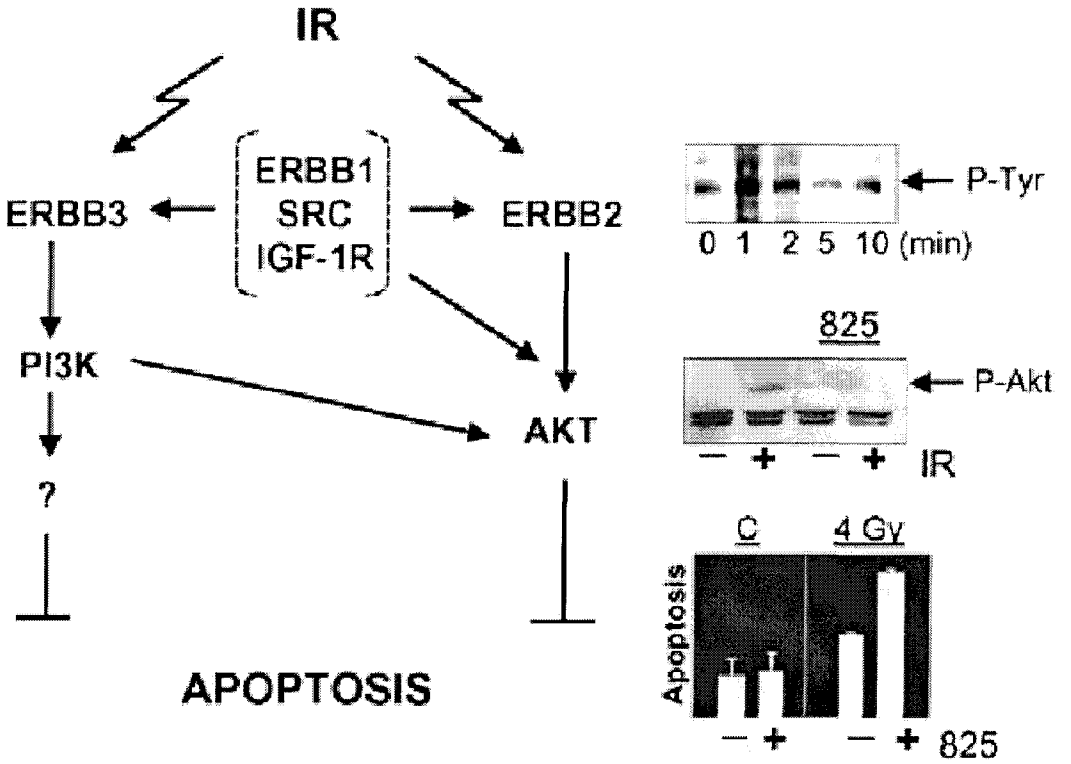

Figure 2. IR-induced antiapoptotic cytoprotective response. Simultaneous activation of ERBB2 and ERBB3 contributes to an antiapoptotic response with evidence for interactions with ERBB1, IGF-1R and SRC. ERBB3 mediates its antiapoptotic effects after binding of the P85 component of PI3K in a SRC-dependent manner. ERBB2 acts through AKT which inhibits the expression of the proapoptotic protein BAD. IR-induced ERBB2 activation(top panel) results in AKT phosphorylation which is inhibited by AG825 (middle panel). The IR-induced apoptosis is further enhanced by inhibition of ERBB2 with AG825 (bottom panel).

Additional studies elucidated in more detail the role of RTKs in mediating relative cellular resistance to ionizing radiation. Ultimate proof for the role of EGFR in cellular cytoprotective radiation responses was established through inhibition of EGFR function. A critical reagent was the dominant negative EGFR-CD533 that we have established as the most effective inhibitor of EGFR function [13], overriding the activity of kinase inhibitors and anti-EGFR monoclonal antibodies (mAbs). Over-expression of EGFR-CD533 in human tumor cells, using Ad-mediated transduction as expression tool [10], resulted in significant radiosensitization by disrupting the pro-proliferative MAPK pathway [11, 14].

2.3. Radiation-induced ERBB RTK activation.

The effects of radiation on EGFR-mediated induction of cell proliferation were indistinguishable from those of physiological growth factors (GFs), such as EGF or TGF-α. However, there are important differences in the induction of

these responses. Growth factors bind to one of the ERBB receptors, EGF/TGF-a to EGFR (ERBB1) or heregulin (HRG) to ERBB3-4, and initiate activation through an orderly induction of primary homo- and secondary hetero-dimerization events [15]. In contrast, ionizing radiation induces the simultaneous activation of all ERBB RTKs that are expressed at widely varied concentrations by different carcinoma cells [16-18]. In addition, work in progress in our laboratory demonstrates that RTKs other than ERBB, such as IGF-1R, are activated in concert with non-RTKs of SRC family. Thus, the most significant difference of receptor activation by GF and radiation is that radiation effects dominantly depend on the entire ERBB receptors expression profile of a given carcinoma cells. In addition, most recent data from our laboratory indicates that radiation and GFs induce quantitatively and qualitatively different Tyr phosphorylation patters the consequences of which therapeutic interventions require further investigation [19].

As a result, the coordinated ERBB receptor activation by radiation, despite relatively weak (2- to 5-fold stimulation) signals at the receptor level, induces strong signals along multiple cytoplasmic kinase signaling pathways. Thus, radiation induces strong signals along the pro-proliferative MAPK and the anti-apoptotic PI3K pathways. Radiation also stimulates transcriptional events through CREB controlling PCNA expression/activity and promoting DNA repair [5, 20].

Importantly, the initiation of radiation-induced signals at the level of the plasma membrane receptors has been demonstrated through systematic inhibitor experiments using DN genetic mutants of EGFR and ERBB2, EGFR-CD533 and ERBB2-CD572, respectively, as well as small molecule tyrphostin Tyr kinase inhibitors with specificities for ERBB1 and 2 and IGF-1R.

The simultaneous activation of all ERBB species by radiation places particular challenges since all carcinoma or malignant glioma cells examined express at least 2 or 3 members of the ERBB family at the same time. This makes apparent the limitations on any potential therapeutic approach that inhibits the function of a single ERBB receptor for the purpose of radiosensitization. Thus, selective inhibition of a single member of the family is unlikely to be of limited effectiveness; in addition, compensatory activation of other members of the ERBB family and other RTKs (e.g., IGF-1R) are being characterized by our group and other groups [21].

Despite the simultaneous activation of ERBB RTKs expressed by a given carcinoma or malignant glioma cell line the individual ERBB receptors signal preferentially along defined signal transduction pathways. We have established the quantitative signaling of ERBB1 through the MAPK pathway and its effects on a pro-proliferative response. ERBB2, previously thought to enhance ERBB1 related signals, in concert with ERBB3 exerts selective signaling responses counteracting apoptosis through signals along the AKT pathway. This cascade is

highly interactive with activation of the PI3K pathway which is initiated by association between the P85 component of PI3K and ERBB3. In summary, the simultaneous activation of multiple ERBB receptors leads to the stimulation of cytoplasmic protein kinases with cellular cytoprotective signals that culminate in a balance between pro-proliferative and anti-apoptotic components.

ERBB signaling may be further affected by the expression of constitutively active ERBB1 mutant receptors, such as EGFRvIII. While this receptor is not activated by EGF, due to the lacking GF-binding extracellular NH_2-domain (refs), its activation upon radiation exposure of cells exceeds that of wild-type EGFR by a factor of > 2-fold including a 3- to 5-fold greater activation of MAPK [22].

In summary, cytoprotective response components include pro-proliferative, anti-apoptotic signals and enhanced repair [5], all directly or indirectly influenced by ERBB RTK function and all contributing to increased cellular radioresistance. Therefore, maximum radiosensitization through ERBB inhibition can only be realized if inhibitors exert a sustained effect that affect all ERBB receptors expressed by a given tumor cell.

2.4. Requirements for effective disruption of radiation-induced signaling by ERBB RTKs.

With respect to cellular radiosensitivity, inhibitors of ERBB1 (EGFR) have shown activity to varied degrees, likely mediated by different mechanisms. Review of the experimental approaches used to demonstrate inhibition of EGFR function it has become clear that mAb against the EGF binding site is due to very different mechanisms that some of the immediate effects exerted by DN EGFR-CD533 and tyrphostin kinase inhibitors [15].

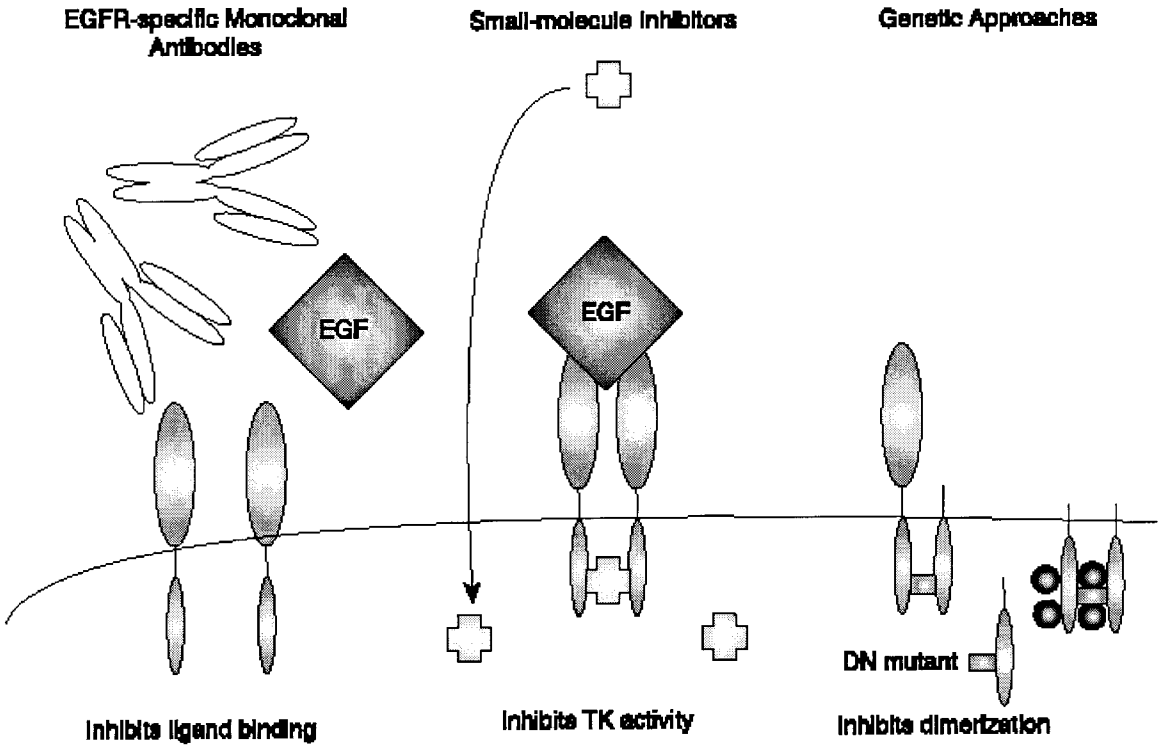

Figure 3. Approaches to disrupting EGFR signaling. At least three approaches are currently studied to target the EGFR. EGFR-specific monoclonal antibodies compete with ligand and behave as receptor antagonists. EGF-specific antibodies are also being used to direct the delivery of radionuclides, toxins, and immunomodulatory molecules. Small molecule inhibitors interact with the intracellular domain of EGFR and block the TK (tyrosine kinase) activity. Genetic approaches include antisense oligonucleotides to inhibit EGFR expression and overexpression of DN mutants that form heterodimers with wt EGFR or constitutively activated EGFR.

Anti-EGFR mAb (C225). Most studies with C225 mAb, directed against the Cys-rich EGF-binding domain of EGFR, demonstrate effects on radiation-induced activation of EGFR only after prolonged exposure of cells to the mAb with effects not exceeding additive activity. Based upon the exposure times required, the C225 mAb may actually not interfere with radiation-induced activation of EGFR but change the intracellular distribution of the receptor and the activity linked to its association with different intracellular compartments. Since C225 has only demonstrated an additive effect with respect to radiosensitization its activity as a true radiosensitizing agent requires additional investigation. Even the effects on MAPK are most likely due to an effective decrease in the ERBB1 receptor expression. The inhibitory effect of C225 on mutant EGFRvIII, an EGFR receptor species prominently activated by ionizing radiation, has not been directly demonstrated and thus remains controversial. Overall, the lack of studies establishing the effect of C225 on immediate radiation responses leave uncertainty regarding the mechanisms of action since early radiation responses have been demonstrated to affect the fate of cells with respect to survival and apoptosis.

RTK protein kinase inhibitors (tyrphostins). Small molecule ERBB Tyr kinase inhibitors appear to interfere with the physiological activation of receptors by preventing homo- and possibly heterodimerization, the latter not clearly demonstrated in experimental systems for these kinase inhibitors (claims of pan-ERBB activity of certain tyrphostins, e.g., CI1033, are not supported by currently available published information). Considering the primary and secondary interactions of ERBB receptors and their simultaneous activation after irradiation of cells, proof of such broad inhibitory activity would require activation studies in controlled expression systems, e.g., CHO cells expressing ERBB receptors with and without ERBB1. In addition to effects on ERBB1 receptors, effects on downstream kinases, such as MAPK, PI3K, AKT and JNK would have to be demonstrated since they transmit and regulate the pro-proliferative and apoptotic components of the cytoprotective response initiated by ERBB RTKs after irradiation.

DN EGFR-CD533. The third genetic approach of inhibiting ERBB RTKs through over-expression of DN EGFR-CD533, lacking the entire cytoplasmic, COOH-terminal domain has been shown to be effective disrupting the function of ERBB1 and other ERBB RTKs. The mechanism rests on the formation of non-functional heterodimers involving EGFR-CD533. This hetero-dimerization includes interaction with wild-type EGFR, ERBB2, -3, and –4. However, even this approach may be leaky in certain carcinoma cells that express relatively high levels of ERBB3 leading to the compensatory increase in Tyr phosphorylation upon over-expression of EGFR-CD533. This mechanism, mediated by SRC, will furnish a strong anti-apoptotic response as one component of cytoprotection mediated by ERBB RTKs. However, the continuous expression of EGFR-CD533 for > 4 weeks and the clearly demonstrated pan-ERBB inhibitory effects predict that EGFR-CD533, possibly other similar DN ERBB receptors, exert the most powerful inhibition of radiation-induced receptor activation and their downstream effectors.

2.5. ERBB RTK inhibition and compensatory responses within the RTK network.

Our comprehensive studies on the mechanisms of radiation-induced ERBB RTK activation have identified important response features that have significant bearing on the therapeutic translation of ERBB inhibitors for tumor cell radiosensitization. The most important feature to be considered is the finding that all ERBB receptors of a given cell are simultaneously activated, thus creating the need for broad receptor inhibition. While during the early phase of development of EGFR inhibitors target specificity was considered desirable our recent studies refute that contention. In contrast, the studies with DN EGFR-CD533 demonstrate the importance of having an EGFR inhibitor with pan-ERBB inhibitory function. In addition, the simultaneous activation of all ERBB receptors by radiation and the significant variation of ERBB expression profiles by different

tumor cells requires that, for any inhibitor with defined ERBB RTK specificity, the ERBB receptor expression profile be known.

The expression of DN ERBB receptors may also be more effective since this DN receptor is expressed continuously by at high levels, thus exerting a potentially much more pronounced inhibitory effect than tyrphostin kinase inhibitors with fluctuating drug levels or mAb that, in addition to sterically inhibiting EGF binding, have profound effects on turn-over enhancing in internalization and decreasing the surface expression of EGFR. Such effects are not sufficiently understood except for recent studies that suggest potential differences in receptor signaling function depending upon their location in the plasma membrane *vs.* intracellular membranes. An additional benefit of EGFR-CD533 may be, by inference from studies of the stability of heterodimeric complexes, that EGFR-CD533 homo- and hetero-dimeric complexes may be more stable both at the cell surface and in intracellular membranes. Thus, further enhancing the inhibitory effects [23-25].

2.6. Extent of radiosensitization by DN EGFR-CD533.

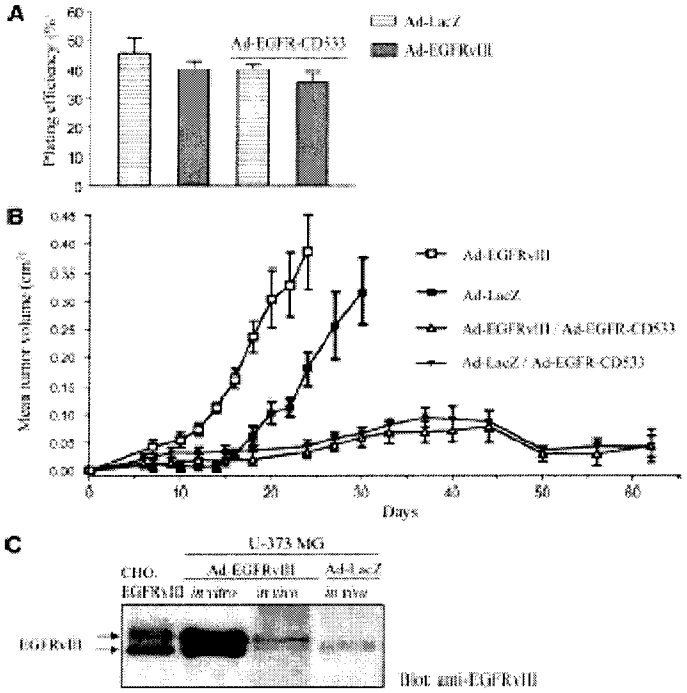

Figure 4. U-373 malignant glioma (MG) xenograft growth in nudemice of cells infected *in vitro* with Ad-EGFR-CD533 or Ad-EGFRvIII and a combination of the two relative to Ad-LacZ control. *A*, colon formation efficiency of U-373 cells transduced *in vitro* with Ad-LacZ, Ad-EGFRvIII, Ad-LacZ _ Ad-EGFR-CD533, or Ad-EGFRvIII _Ad-EGFR-CD533 before inoculation. Transduced U-373 cells were plated for colony formation assays at the time that same batch of cells was inoculated into nude mice. *B*, tumorigenic activity expressing EGFRvIII compared with coexpression of EGFRvIII with EGFR-CD533. *Error bars* represent mean SE for five animals per group. *C*, Western blot analyses of Ad-mediated expression of EGFRvIII in established U-373 xenograft tumors at the same time at which growth curves under *B* were terminated. U-373, cells transduced with Ad-EGFRvIII *in vitro* (*in vitro lane*) and lysate of CHO.EGFRvIII cells served as positive controls for EGFRvIII.

The radiosensitization mediated by EGFR-CD533 is based on the perturbance of interactions between members of the ERBB and other RTKs. This perturbance occurs at the protein level through the formation of non-functional heterodimers between resident wild-type ERBB receptors and EGFR-CD533 ([9, 13, 26]). Because of the prolonged high-level expression of EGFR-CD533 the effect on tumor cells is profound and results in conversion of cells, even A431 cells expressing 10^7 EGFR molecules per cell, into a EGFR dominant negative phenotype. The activity of EGFR-CD533 has been tested by two independent experimental approaches; one is the Tet-inducible expression of the transgene in mammary carcinoma cells stably transfected with EGFR-CD533 under control of a Tet-on promoter, the other is the transduction of cell after infection with the adenoviral (Ad) expression vector, Ad-EGFR-CD533. Both yield similar high expression levels of the transgene *in vitro* and result in similar significant radiosensitization due to disruption of ERBB RTK-mediated cytoprotective radiation responses [27].

Single radiation dose response analyses, quantified by clonogenic survival, demonstrate radiosensitization by the expression of EGFR-CD533 in different human carcinoma and malignant glioma cells with enhancement ratios (ERs) ranging from 1.3 to 1.7 [14, 18]. These radiosensitizing activities were preserved when cells were exposed to repeated radiation exposures *in vitro*, again yielding ERs near 1.5 [14].

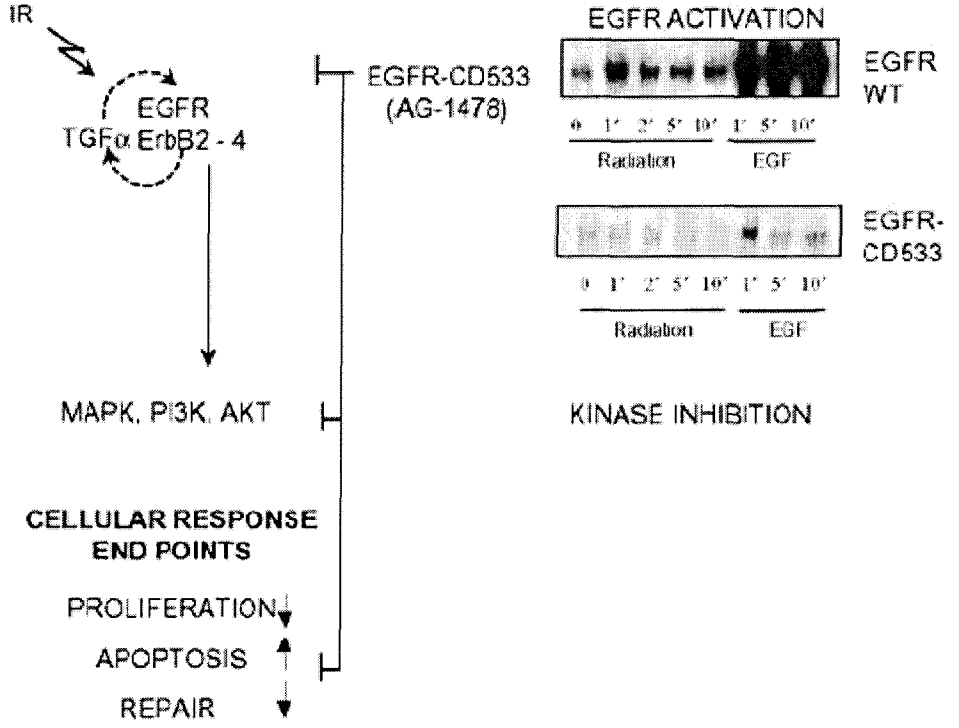

Figure 5. The inhibition of ERBB1 by incubation of cells with the Tyr kinase inhibitor AG1478 or overexpression of EGFR-CD533 completely blocks IR- and GF-induced ERBB1 Tyr phosphorylation. The overexpression of EGFR-CD533 completely blocks the

IR-induced activation of downstream cytoplasmic protein kinases (AG1478 is partially active in this system). As a result, the IR-induced cytoprotective response is disrupted as manifested by elimination of the IR-induced proliferation, increased rates of apoptosis and reduced DNA repair

The radiosensitizing activity of EGFR-CD533 were also tested *in vivo* using two assay systems, *ex vivo* clonogenic survival and tumor growth delay; the former quantifies the extent of tumor cell radiosensitization *in vivo* while the latter examines the effects of radiation treatments on the entire tumor including potential interactions between tumor and host tissues. The radiosensitization was studied in tumors generated from stable transfected MDA-EGFR-CD533 cells after treatment of mice with doxycycline (Tet) and after infusion of Ad-EGFR-CD533 through six-track positive pressure infusions on two consecutive days [28]. Tumor cell radiosensitization *in vivo* yielded an ER of 1.85 for tumor infused with Ad-EGFR-CD533 vs. MDA-EGFR-CD533 tumors in which EGFR-CD533 was expressed in all tumor upon infusion of mice with Tet (ER1.80) [28].

The radiosensitization by growth delay yielded ERs between 2 and 4 for U373 malignant glioma and A431 SCC tumors (xenografts in nude mice). Additional *in vivo* experiments demonstrate the effectiveness of EGFR-CD533 in disrupting the function of EGFR and EGFRvIII; evidence for the latter is shown in tumor growth experiments from U373 cells that have been transduced with Ad-EGFRvIII, Ad-EGFR-CD533 or both prior to injection into nude mice. While strong tumor growth promoting property of EGFRvIII was demonstrated by accelerated growth of U373 cell transduced with EGFRvIII.

We next tested the effectiveness of EGFR-CD533 in its ability to inhibit EGFRvIII under conditions of experimental gene therapy using U373 xenograft tumors were infused with double combinations of Ad vectors, Ad-EGFRvIII/Ad-LacZ, Ad-EGFRvIII/Ad-EGFR-CD533 and Ad-EGFR-CD533/Ad-EGFRvIII. Importantly, EGFR-CD533 could override the strong cytoprotective effects of EGFRvIII *in vivo* since tumors expressing high levels of EGFR-CD533 and EGFRvIII demonstrated marked radiosensitization relative to the appropriate controls (Lam, unpublished). In these *in vivo* studies we also demonstrated that the glioma/carcinoma cells transduced with Ad vectors expressed the transgene for extended periods of time with significant expression levels even 4 weeks after transduction. This prolonged expression is ideal for combined use with radiotherapy that is typically delivered over a 4 – 6 week time period.

2.7. Recent clinical experiences

Of the EGFR inhibitors, both C225 and AG1839 ("Iressa") are currently in clinical trials and have generated variable outcomes. Both agents have demonstrated clinical activity as single agents but results of combined use of EGFR inhibitors with standard cytotoxic agents and radiation are ongoing and have not always produced favorable results (negative results for NSCLC with standard chemotherapy) or have not yet been reported (e.g., Imclone Phase III of accelerated radiotherapy combined with chemotherapy plus/minus C225 mAb).

There is also yet unpublished data that the systemic application of both C225 and "Iressa" in combination with other forms of cancer therapy may produce toxicities not identified during early Phase I/II trials. It is certainly conceivable that doses (with systemic effectiveness) of systemically administered anti-EGFR inhibitors produce un-acceptable toxicities when combined with standard cytotoxic agents, thus requiring the local administration of ERBB RTK inhibitors for maximum therapeutic effects.

2.8. Summary

The expression of dominant negative EGFR-CD533 in human carcinoma and malignant glioma cells has been established as a highly effective means of inhibiting the cytoprotective responses mediated by EGFR and other ERBB RTKs after irradiation of cells. Importantly, relative to other inhibitors of EGFR, EGFR-CED533 has pan-ERBB inhibitory activity, acts over weeks in transduced cells and, therefore, deserves to be developed as a third genetic approach of anti-EGFR therapy. While the delivery of Ad-EGFR-CD533 does not represent a problem in experimental tumor xenografts, the Ad vector delivery may prove more challenging in the clinical situation. For this reason and because of the well-documented dependence of malignant glioma cells on over-expressed wild-type and constitutively-active mutant EGFRvIII molecules we have chosen glioblastoma multiforme as the tumor to be treated combining Ad-EGFR-CD533 delivery with highly conformal or intensity-modulated radiotherapy.

1. Withers, H.R., J.M. Taylor, and B. Maciejewski, *The hazard of accelerated tumor clonogen repopulation during radiotherapy.* Acta Oncol, 1988. **27**(2): p. 131-46.
2. Thames, H.D., et al., *Fractionation parameters for human tissues and tumors.* Int J Radiat Biol, 1989. **56**(5): p. 701-10.
3. Bentzen, SM, and Thames HD. *Clinical evidence for tumor clonogen regeneration: interpretations of the data.* Radiother Oncol, 1991. **22**:p. 161-166.
4. Contessa, J., et al., *Compensatory ErbB3/c-Src Signaling Mediates Cell Survival in Breast Carcinoma Cells.* Cancer Research, 2004.
5. Amorino, G.P., et al., *Dominant-negative cAMP-responsive element-binding protein inhibits proliferating cell nuclear antigen and DNA repair, leading to increased cellular radiosensitivity.* J Biol Chem, 2003. **278**(32): p. 29394-9.
6. Schmidt-Ullrich, R.K., et al., *Altered expression of epidermal growth factor receptor and estrogen receptor in MCF-7 cells after single and repeated radiation exposures.* Int J Radiat Oncol Biol Phys, 1994. **29**(4): p. 813-9.
7. Kavanagh, B.D., et al., *Radiation-induced enhanced proliferation of human squamous cancer cells in vitro: a release from inhibition by epidermal growth factor.* Clin Cancer Res, 1995. **1**(12): p. 1557-62.
8. Schmidt-Ullrich, R.K., et al., *Radiation-induced autophosphorylation of epidermal growth factor receptor in human malignant mammary and squamous epithelial cells.* Radiat Res, 1996. **145**(1): p. 81-5.

9. Schmidt-Ullrich, R.K., et al., *Radiation-induced proliferation of the human A431 squamous carcinoma cells is dependent on EGFR tyrosine phosphorylation.* Oncogene, 1997. **15**(10): p. 1191-7.
10. Contessa, J.N., et al., *The inducible expression of dominant-negative epidermal growth factor receptor-CD533 results in radiosensitization of human mammary carcinoma cells.* Clin Cancer Res, 1999. **5**(2): p. 405-11.
11. Reardon, D.B., et al., *Dominant negative EGFR-CD533 and inhibition of MAPK modify JNK1 activation and enhance radiation toxicity of human mammary carcinoma cells.* Oncogene, 1999. **18**(33): p. 4756-66.
12. Schmidt-Ullrich, R., *Local tumor control and survival: clinical evidence and tumor biologic basis.* Surg Oncol Clin N Am, 2000. **9**(3): p. 401-14, vii.
13. Redemann, N., et al., *Anti-oncogenic activity of signalling-defective epidermal growth factor receptor mutants.* Mol Cell Biol, 1992. **12**(2): p. 491-8.
14. Lammering, G., et al., *Radiosensitization of malignant glioma cells through overexpression of dominant-negative epidermal growth factor receptor.* Clin Cancer Res, 2001. **7**(3): p. 682-90.
15. Schmidt-Ullrich, R., et al., *Receptor tyrosine kinases and cellular responses to ionizing radiation.* Oncogene Reviews, 2003.
16. Bowers, G., et al., *The relative role of ErbB1-4 receptor tyrosine kinases in radiation signal transduction responses of human carcinoma cells.* Oncogene, 2001. **20**(11): p. 1388-97.
17. Lammering, G., et al., *Adenovirus-mediated overexpression of dominant negative epidermal growth factor receptor-CD533 as a gene therapeutic approach radiosensitizes human carcinoma and malignant glioma cells.* Int J Radiat Oncol Biol Phys, 2001. **51**(3): p. 775-84.
18. Lammering, G., et al., *EGFR and its mutant EGFRvIII as modulators of tumor cell radiosensitivity (abstract).* Int J Radiat Oncol Biol Phys, 2001. **51**(3, suppl 1): p. 103.
19. Schmidt-Ullrich, R., et al. *Genetic Inhibition of erb-B receptors reveals anti-apoptotic erb-B3/c-Src signaling*. in *AACR*. 2003. Washington, DC.
20. Amorino, G.P., et al., *Epidermal growth factor receptor dependence of radiation-induced transcription factor activation in human breast carcinoma cells.* Mol Biol Cell, 2002. **13**(7): p. 2233-44.
21. Chakravarti, A., J.S. Loeffler, and N.J. Dyson, *Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling.* Cancer Res, 2002. **62**(1): p. 200-7.
22. Lammering, G., et al., *EGFRvIII-mediated radioresistance through a strong cytoprotective response.* Oncogene, 2003. **22**(36): p. 5545-53.
23. Yarden, Y. and M.X. Sliwkowski, *Untangling the ErbB signalling network.* Nat Rev Mol Cell Biol, 2001. **2**(2): p. 127-37.
24. Wang, Y., et al., *Endosomal signaling of epidermal growth factor receptor stimulates signal transduction pathways leading to cell survival.* Mol Cell Biol, 2002. **22**(20): p. 7279-90.

25. Burke, P., K. Schooler, and H.S. Wiley, *Regulation of epidermal growth factor receptor signaling by endocytosis and intracellular trafficking.* Mol Biol Cell, 2001. **12**(6): p. 1897-910.
26. Kashles, O., et al., *A dominant negative mutation suppresses the function of normal epidermal growth factor receptors by heterodimerization.* Mol Cell Biol, 1991. **11**(3): p. 1454-63.
27. Schmidt-Ullrich, R., et al., *ERBB receptor tyrosine kinases and cellular radiation responses.* Oncogene, 2003. **22**(37): p. 5855-5865.
28. Lammering, G., et al., *Epidermal growth factor receptor as a genetic therapy target for carcinoma cell radiosensitization.* J Natl Cancer Inst, 2001. **93**(12): p. 921-9.
29. Todd, D.G., et al., *Ionizing radiation stimulates existing signal transduction pathways involving the activation of epidermal growth factor receptor and ERBB-3, and changes of intracellular calcium in A431 human squamous carcinoma cells.* J Recept Signal Transduct Res, 1999. **19**(6): p. 885-908.
30. Schmidt-Ullrich, R.K., et al., *Molecular mechanisms of radiation-induced accelerated repopulation.* Radiat Oncol Investig, 1999. **7**(6): p. 321-30.

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