

## Commentary

# Expanding the therapeutic repertoire of epidermal growth factor receptor blockade: radiosensitization

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### Abstract

Expression of epidermal growth factor receptor (EGFR) has been associated with radioresistance in cancer. Moreover, tumour cell recovery after irradiation paradoxically occurs, in part, as a result of activation of EGFR signalling by such treatment. A recent article by Huang, Li, Armstrong and Harari provides strong rationale for considering the anti-EGFR agent ZD1839 ('Iressa') as a radiosensitizing strategy. With the use of several *in vitro* and xenograft models of human squamous cell head and neck carcinoma, ZD1839 was shown to markedly improve radiotherapeutic response, with superior tumour inhibition and delayed tumour regrowth. Mechanisms underlying this effect included anti-proliferative and pro-apoptotic activity, with significant perturbation of tumour angiogenesis.

**Keywords:** angiogenesis, EGFR, radiotherapy, resistance, ZD1839

### Introduction

Therapy with ionizing radiation provides a key management strategy for many epithelial tumour types. Radiotherapy forms part of the multidisciplinary approach to the treatment of breast cancer, and is now of routine value after conservative surgery to reduce locoregional recurrence. However, there are significant limitations of radiotherapy in many cancers. Some are intrinsically resistant to damage by ionizing radiation. Moreover, a proportion of cells can survive irradiation, with treatment inducing their proliferation to accelerate tumour cell repopulation during radiation challenge [1]. In total, such events result in a diminished response to radiation, resistant growth and poor local control. Knowledge of the molecular mechanisms employed by tumour cells to evade the inhibitory activity of radiotherapy is essential if we are to design novel treatment strategies rationally to improve its effectiveness.

Although various intrinsic and extrinsic factors have been implicated, the role played by growth factor signal transduction in radiation resistance is particularly fascinating. Of these, perhaps the most compelling data exist for the

involvement of the epidermal growth factor receptor (EGFR/c-erbB1/HER1) pathway in determining radiosensitivity. Indeed, there has been a recent flourish of literature in this area, in part triggered by the availability of monoclonal antibodies and pharmacological inhibitors that can block EGFR. In this light, the recent article by Huang *et al.* [2] examines the anti-tumour activity of the EGFR-selective tyrosine kinase inhibitor ZD1839 ('Iressa') in combination with radiotherapy, using several experimental models of human squamous cell head and neck carcinoma. Importantly, the study demonstrates superior anti-tumour potency with ZD1839 plus radiotherapy. It sheds light on the multiple mechanisms underlying this effect, notably improved anti-proliferative and pro-apoptotic activity, as well as significant perturbation of angiogenesis, events that act cumulatively to limit cell recovery after irradiation and significantly delay tumour regrowth. Not only are such data highly supportive of a key role for EGFR in determining fundamental cancer cell biology and radioresponse, but, importantly, they confirm that anti-EGFR agents might provide considerable advantage as a radiosensitizing strategy in cancer management.

## EGFR occupies a pivotal position in cancer cell biology

EGFR is established as an important regulator of proliferation, cell survival, DNA damage repair, cell motility and invasive capacity. Furthermore, EGFR signalling promotes angiogenesis, an event essential for cancer cell viability, tumour growth and metastasis [3]. Not surprisingly, therefore, dysregulation of EGFR signalling has been closely associated with the initiation, growth and progression of the majority of human epithelial tumour types, including breast, non-small cell lung, colorectal, head and neck, ovarian, gastric and pancreatic cancer. EGFR dysregulation, commonly a consequence of receptor or ligand overexpression and hence enhanced autocrine signalling, invariably confers aggressive tumour biology in the clinic, and in some tumour types a poor prognosis [4]. In breast cancer, EGFR overexpression has been associated with reduced oestrogen receptor content, advanced clinical stage and shortened relapse-free survival [5].

## EGFR is associated with tumour cell escape from inhibition by radiotherapy

EGFR dysregulation has been implicated in therapeutic resistance to diverse agents across many cancer types. Associations have been noted between EGFR overexpression and resistance to cytotoxic agents [6] and to anti-hormonal agents in breast cancer [5]. Excitingly, an increasing body of data now indicates that this association might extend to treatment with ionizing radiation. Although most of the supportive data in this area have been derived from cancers that classically overexpress EGFR (notably including head and neck squamous cell carcinoma), there is early evidence of equivalence in breast tumour cells, in which EGFR overexpression can be more modest.

In clinical head and neck cancer, associations have been reported between EGFR overexpression and radioresistance, increased rate of recurrence after radiotherapy, poor local control and reduced patient survival [7,8]. Similarly, increased EGFR expression has been directly associated with radioresistance in several model systems, with an inverse correlation reported between magnitude of EGFR expression and radiocurability using a cohort of murine tumours, including mammary cancer, *in vivo* [9]. In breast cancer *in vitro*, priming of EGFR signalling with EGF before irradiation acts to increase radioresistance in a time- and dose-dependent manner [10].

Intriguingly, studies *in vitro*, notably those examining the EGFR-positive MDA-MB-231 breast cancer cell line, have demonstrated that clinical doses of radiation therapy activate EGFR signalling [11–14]. Such activation of EGFR by radiation has been equated with tumour cell proliferative and cytoprotective effects, explaining not only why some EGFR-expressing tumour cells survive inhibition but also the phenomenon of promotion of tumour cell repopulation

during a course of radiotherapy. It has been suggested that radiation-induced EGFR activation (and hence evasion of inhibition and radioresistance) may be particularly obvious in EGFR-overexpressing cells [9]. Both single and repeated irradiation have been observed to drive EGFR autophosphorylation, with upregulated EGFR expression also reported. Priming of expression and proteolytic cleavage of the key EGFR ligand transforming growth factor- $\alpha$  by irradiation might contribute to this EGFR activation [12]. There is subsequently phosphorylation of the key downstream signalling elements Ras, extracellular regulated (ERK1/2) mitogen-activated protein kinase, p90 ribosomal S6 kinase and AKT [11,12], with a profile of nuclear transcription factors ultimately stimulated [14].

## EGFR inhibitory strategies improve radioresponse in tumour types including breast cancer

An obvious therapeutic consequence of these data is that EGFR blockade might cause radiosensitization. Among the EGFR-inhibitory strategies, furthest advanced in clinical study is the EGFR-selective tyrosine kinase inhibitor ZD1839, which exhibits an acceptable tolerability profile and promising anti-tumour activity in both experimental models and cancer patients [15]. Moreover, supra-additive effects have been observed with this agent in combination with cytotoxics in many tumour models including breast cancer, in which improved response has also been reported when ZD1839 is combined with anti-hormonal procedures [16]. Clinical studies are continuing in both these areas.

However, the article by Huang *et al.* is among the first to demonstrate successfully the modulation of radioresponse in human tumour cell lines and xenografts by using ZD1839. The study has employed several human squamous carcinoma cell lines established from biopsies of head and neck cancer patients with varying EGFR expression. In all the derived cells *in vitro*, ZD1839 exerted significant anti-proliferative activity, inducing arrest in the radiosensitive G1 cell cycle phase (and depletion of the radioresistant S phase fraction), as well as the enhancement of radiation-induced apoptosis. Clonogenic survival assays revealed that reduced cell survival after radiation was associated with ZD1839. Comparable data demonstrating cooperative anti-proliferative and pro-apoptotic activity of ZD1839 and irradiation have similarly been obtained within experimental non-small cell lung carcinoma, ovarian, colon and breast cancer [17,18].

Huang *et al.* have subsequently monitored tumour growth in athymic nude mice, revealing that ZD1839 also augments tumour response to radiation *in vivo*. Combined treatment with radiation and ZD1839 exhibited improved tumour growth inhibition and substantially delayed tumour regrowth in the xenografts examined in comparison with radiation or ZD1839 alone, with an additive (SCC-6

tumour) or synergistic (SCC-1 tumour) growth inhibitory effect. Substantial effects of radiotherapy plus ZD1839 *in vivo*, notably long-term tumour regression, have been confirmed in the handful of additional studies reported for a range of cancer cell xenografts, including mammary MDA-MB-468 [17,19]. Huang *et al.* have shed light on the underlying mechanism of the potency of radiotherapy plus ZD1839 *in vivo*. In addition to the anti-proliferative and pro-apoptotic effects, a series of elegant experiments revealed that ZD1839 inhibits the early angiogenic process by targeting microvascular endothelial cells, decreasing their cell-cell interaction and disrupting endotube formation, as well as diminishing tumour xenograft-induced neovascularisation of Matrigel plugs. Significant anti-angiogenic effects might result from the ability of ZD1839 to inhibit EGFR-regulated angiogenic growth factor production by tumour cells, such as vascular endothelial growth factor, basic fibroblast growth factor and interleukin-8 [17], and this agent might also directly block EGFR expressed on endothelial cells.

Studies employing alternative EGFR inhibition strategies in irradiated tumour cell xenografts as well as *in vitro* exhibit equivalent effects to the recent ZD1839 studies from Huang *et al.* Thus, superior anti-tumour activity versus radiotherapy alone has been reported extensively by this research group when radiotherapy is combined with the anti-EGFR antibody IMC-C225 [20]. Comparable data have also been obtained in various tumour cell lines, including breast cancer, using the EGFR-selective tyrosine kinase inhibitor AG1478 [11], EGFR dominant-negative constructs [21,22] and the irreversible pan-erbB inhibitor CI-1033 [23]. Again, enhanced radiosensitivity seems to result from the induction of apoptosis, the accumulation of cells in radiosensitive cell cycle phases, the downregulation of angiogenic factors and the inhibition of damage repair after irradiation by the redistribution of DNA repair enzyme [20].

## Conclusion

It is clear from the above preclinical studies, exemplified by this new article from Huang *et al.*, that there is increasing rationale for considering anti-EGFR agents such as ZD1839 as a radiosensitizing strategy in cancer management. Indeed, data from a novel clinical investigation by Robert *et al.* [24] are highly encouraging, in which all 16 locally advanced head and neck cancer patients treated with IMC-C225 plus radiotherapy exhibited an objective response, with no additional toxicity. The results of continuing clinical trials examining ZD1839 (or IMC-C225) in combination with radiotherapy in multiple human malignancies are eagerly awaited.

However, it is important to note that in the study by Huang *et al.* tumour regrowth was delayed rather than prevented by the use of ZD1839 in combination with radiation, and that pro-apoptotic activity, although clearly enhanced over

radiation alone, was nevertheless relatively modest. Thus, although there is obviously considerable promise for this new strategy, our optimism should perhaps be cautious. Moreover, breast cancer cell studies have revealed additional complexities that might potentially influence the outcome of EGFR-targeted inhibitory strategies in combination with radiotherapy if employed in this disease. For example, there is increasing evidence that receptors and signalling pathways other than EGFR might be primed by irradiation [12,14]. Multiple erbB tyrosine kinase receptor family members have been observed to be activated indiscriminately by the irradiation of mammary cells, with HER2 being able to influence EGFR activation in response to radiation [13]. Moreover, radiation has been shown to be able to prime several key nuclear transcription factors (such as CCAAT-enhancer-binding protein and Stat1) in breast cancer cells in addition to those that are regulated by EGFR [14]. If we are to employ rationally and gain maximal benefit from inhibitors such as ZD1839 in breast cancer, therefore, it is essential that we more fully elucidate the increasingly complex interrelation between radiation, recruited signal transduction pathways and cellular outcome.

Finally, a key contribution to any success of this combination strategy will be to determine appropriate dosages and sequence of the treatment, and to define accurately which patients to treat. The latter could prove particularly problematic because EGFR overexpression is evident in only about 40% of breast cancer patients [4,5,25] and elevated expression of the receptor in tumour cells does not always associate with activity of the pathway or a growth inhibitory response to anti-EGFR agents [26]. In turn, experiences in several tumour types with ZD1839 plus chemotherapy demonstrate that anti-tumour responses might not require EGFR overexpression [27]. Indeed, in the study by Huang *et al.*, ZD1839 anti-proliferative responses were most extensive in SCC-13Y tumours that exhibited the lowest EGFR expression, whereas in various tumour cells *in vitro*, including breast cancer, it is HER2 overexpression, rather than EGFR level, that seems to associate with increased ZD1839 sensitivity [28]. Clearly there remains much to be learned about the action of ZD1839 in clinical breast cancer and its role, if any, in influencing radiosensitivity.

## Competing interests

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