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Review

EGFR-targeted anti-cancer drugs in radiotherapy: Preclinical evaluation of mechanisms

Michael Baumann[a,b,c,*,1], Mechthild Krause[a,b,1], Ekkehard Dikomey[d], Klaus Dittmann[e], Wolfgang Dörr[a,b,c], Ulla Kasten-Pisula[d], H. Peter Rodemann[e]

[a]*Department of Radiation Oncology,* [b]*OncoRay Center for Radiation Research in Oncology, and* [c]*Experimental Center, Medical Faculty and University Hospital Carl Gustav Carus, University of Technology, Dresden, Germany,* [d]*Department of Radiotherapy and Radiooncology, Laboratory of Experimental Radiation Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [e]*Department of Radiation Oncology, Eberhard-Karls University, Tübingen, Germany*

Abstract

Preclinical and clinical results indicate that the EGFR can mediate radioresistance in different solid human tumours. Combination of radiotherapy and EGFR inhibitors can improve local tumour control compared to irradiation alone and has been introduced into clinical radiotherapy practice. So far several mechanisms have been identified in preclinical studies to contribute to improved local tumour control after radiation combined with EGFR inhibitors. These include direct kill of cancer stem cells by EGFR inhibitors, cellular radiosensitization through modified signal transduction, inhibition of repair of DNA damage, reduced repopulation and improved reoxygenation during fractionated radiotherapy. Effects and mechanisms may differ for different classes of EGFR inhibitors, for different tumours and for normal tissues. The mechanisms underlying this heterogeneity are currently poorly understood, and predictive assays are not available yet. Importantly, mechanisms and predictors for the combined effects of radiation with EGFR inhibitors appear to be considerably different to those for application of EGFR inhibitors alone or in combination with chemotherapy. Therefore to further evaluate the efficacy and mechanisms of EGFR-inhibition in combined treatments, radiotherapy-specific preclinical research strategies, which include in vivo experiments using local tumour control as an endpoint, as well as animal studies on normal tissue toxicity are needed.
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Introduction

Inhibitors of the epidermal growth factor receptor (EGFR) are among the most promising molecular targeting agents for combination with radiotherapy [6,13,49,68,76,83]. Beginning with the first description of the receptor in 1962 [26], extensive research into the biology of this receptor led to an increasing understanding of the molecular processes dependent on the EGFR, and to the development of drugs targeting this receptor. Overexpression of the EGFR has been shown to correlate with lower tumour control rates after irradiation in several studies [1,4,23,43,45,93] but also conflicting results have been reported [19,20,70]. Stressors including irradiation can lead to autocrine secretion of the EGFR-ligand TGFα and thereby to an activation of the receptor [105,107]. Although EGFR inhibitors in themselves are not curative in solid tumours, their combination with radiotherapy might improve local tumour control due to interactions between both treatments (reviewed in [6]).

Recently the results of the first randomized clinical phase III trial on simultaneous fractionated irradiation and EGFR inhibition by the monoclonal antibody (mAb) cetuximab (Erbitux®) have been published. In this trial, application of cetuximab during primary radiotherapy of patients with head and neck squamous cell carcinoma (HNSCC) led to an improvement of local tumour control and survival compared to radiotherapy alone [13]. Clearly, this trial is a milestone for the clinical use of EGFR inhibitors and for the principles of molecular targeting in radiation oncology. However, in most radiotherapy departments worldwide, standard treatment for inoperable HNSCC is radiochemotherapy, which also improves local tumour control compared to radiotherapy alone [92]. Addition of cetuximab to cisplatin-based radiochemotherpy in HNSCC patients led to promising overall survival rates of 76% and local tumour control rates of 71% after 3 years in a phase II trial. However, this trial

[1] These two authors share first authorship, alphabetical order.

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was closed because of significant adverse events including two deaths (one due to pneumonia, one unknown reason) [91]. Retrospectively, this toxicity was not clearly attributable to the application of cetuximab. Therefore, combination of radiochemotherapy and cetuximab is further explored in clinical trials. Likely due to overlapping or counteracting effects, combination of chemotherapy and EGFR inhibition without radiotherapy led to very heterogeneous results in clinical trials on other tumour entities. For example, monotherapy with EGFR-tyrosine kinase inhibitors (TKI; gefitinib or erlotinib) in non-small-cell lung cancer (NSCLC) improved response rates and survival [109]. In contrast, addition of these drugs to platin-derivates, gemcitabine or paclitaxel did not result in improved outcome compared to chemotherapy alone [41,42,51,52]. These results imply the caveat that it cannot be taken as granted, that addition of EGFR-inhibitors to combined chemoradiotherapy will necessarily further improve treatment outcome. For example, preliminary results of a phase I/II trial on neoadjuvant radiochemotherapy with Capecitabine/Oxaliplatin combined with cetuximab in patients with rectal carcinoma [29,100] showed complete tumour response rates of only 9% in the pathohistological specimens, which was less than in historical data on radiochemotherapy without cetuximab [99]. Comparably low complete response rates of 5% have been shown for neoadjuvant radiochemotherapy in rectal cancer with Capacitabine plus cetuximab [73].

Therefore, to take full advantage of the potential of EGFR inhibitors in radiation oncology, detailed understanding of the underlying mechanisms of combined radiotherapy or radiochemotherapy and EGFR inhibition both in tumours and in normal tissues is necessary. As a sequel to a previous overview [6], the present work reviews the mechanisms of action of EGFR inhibitors and their interaction with radiobiological mechanisms that determine outcome of radiotherapy.

Cancer stem cells, clonogenic cells, and the case for radiotherapy specific preclinical research strategies

Cancer stem cells are defined as those cancer cells that have the capacity to self-renew and to cause the heterogeneous lineages of cells that comprise the tumour [25]. They have been shown to represent a distinct subpopulation of cells in many human tumours [2,27,38,82,94,98,112], while non-tumourigenic cells constitute the bulk of tumour cells [25,97]. An important implication of this concept is that cancer stem cells are possibly more resistant to treatment with drugs or irradiation [25,97].

In curative radiotherapy, success is not determined by the effect on the mass of the tumour cells, but by the effect on cancer stem cells that, when they survive, can produce a recurrence [5,10]. Recurrent tumours after radiotherapy by definition originate from at least one surviving cancer stem cell, while permanent local tumour control requires inactivation of all cancer stem cells. Thus, in vivo radiation tumour control assays determine functionally the inactivation of cancer stem cells [63,65]. In this respect inactivation of cancer stem cells in vivo is functionally the same as inactivation of clonogenic tumour cells, which are defined to posses the ability to form an expanding family of daughter cells, which in vivo translates into a recurrent tumour. In contrast, in vitro colony forming ability does not necessarily mirror cancer stem cell content and their survival in vivo [65,66,101,102]. Nevertheless, colony forming assays are standard techniques for a first assessment of the potential of new treatments combined with radiation and for mechanistic studies. To draw conclusions on the efficacy of such combined treatments for translation into clinical applications these experiments need to be complemented by in vivo studies [65].

Several investigations (reviewed in [65]) have shown that the effects of molecular drugs combined with radiotherapy, on the mass of tumour cells, which is measured by tumour regression or growth delay, do not necessarily reflect the inactivation of cancer stem cells. For example application of an EGFR-TKI either before, or during or after fractionated radiotherapy always decreased tumour volume and increased growth delay compared to radiation alone, but in none of these settings improved local tumour control [7,61,63]. Therefore, to evaluate the efficacy and mechanisms of EGFR-inhibition in combined treatments, radiotherapy-specific preclinical research strategies which include in vivo experiments using local tumour control as an endpoint are needed [5].

Biological mechanisms of action and interaction with effects of irradiation on tumours

Direct inactivation of cancer stem cells

Recurrences after high dose irradiation often occur from a few surviving clonogenic cells. Thus, additional kill of a low number of clonogenic cells by a further treatment would be expected to have additive effects on local tumour control [6,65,66]. EGFR inhibition in vitro and in vivo has been shown to increase apoptosis of tumour cells [7,12,14,17,22,44,54,74,104], however, the contribution of apoptosis to the curative effect of radiotherapy in solid tumours is controversial.

Fig. 1 summarizes in vivo data allowing to assess the potential of EGFR inhibitors when combined with radiation to directly kill cancer stem cells. Application of the mAb C225 before single dose irradiation of A431 tumours under normal blood flow led to a slight improvement of local tumour control, whereas continuation of C225 treatment after irradiation significantly enhanced this effect [80]. These data are in line with a recently published study, showing that application of C225 during and after fractionated irradiation has higher effects on local tumour control compared to simultaneous treatment or irradiation alone [77]. These observations seem to contradict previous in-vitro data, which show no direct inactivation of clonogenic cells by C225 in the same tumour cell line [104]. Nevertheless, in light of the general limitations of in-vitro assays, independent cytotoxic effects of C225 on clonogenic A431 cells are the most likely reason for the improvement of local tumour control in both experiments. Also in FaDu tumours, application of C225 before single dose irradiation under homogeneous hypoxia significantly improved local tumour control compared to irradiation