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Novel therapeutics in combination with radiotherapy to improve cancer treatment: Rationale, mechanisms of action and clinical perspective

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ABSTRACT

Our increased understanding of the molecular processes underlying cellular sensitivity to ionizing radiation has led to the identification of novel targets for intervention. New agents have become available for combined use to overcome radioresistance and enhance the clinical efficacy of radiotherapy. This rational selection of potential radiosensitizers contrasts with the empirical approach that has dominated the field of chemo-radiotherapy over the last decades. It allows the identification of those patients who will benefit most from a specific combination by exploiting new predictive biomarkers of response. In this review we present several approaches of targeted radiosensitization and discuss the available *in vitro* and *in vivo* results that support their translation into clinical trials. We focus on EGFR-inhibiting, anti-angiogenic, apoptosis-modulating and PARP-interfering strategies.

1. Introduction

The combination of radiotherapy and chemotherapy is an appealing approach that has led to improved treatment results in patients with advanced solid tumors. In particular, the concurrent application of both modalities has proven effectivity and resulted in lower recurrence rates and improved survival in several tumor types, such as head and neck (Calais et al., 1999), lung (Schaake-Koning et al., 1992), and cervix cancer (Rose et al., 1999). The scheduling of radiotherapy and chemotherapy appears to be critical. In head and neck and cervical cancer, less or even no improvement in the treatment results was achieved when chemotherapy was given before or after radiotherapy (Pignon et al., 2000; Thomas, 1999). A major advantage of the combined treatment is the possibility to improve surgical results and obtain a higher organ-preservation rate, such as in patients with advanced head and neck (Hillman et al., 1998) or anal cancer (Bartelink et al., 1997). More recently it has been shown that even in notoriously therapy-resistant tumors such as gastric cancer and glioblastoma, the addition of chemotherapy to radiation improves survival (Macdonald et al., 2001; Stupp et al., 2009).

These benefits clearly outweigh the often-observed increased acute toxicity of combined radiotherapy and chemotherapy. Despite these encouraging treatment results, however, further improvement is necessary given the high number of loco-regional recurrences for which curative salvage treatment is not possible. This can be achieved by several strategies.

First, improved response prediction: new assays, especially genomic-wide micro-arrays, biomarkers and functional imaging provide us with much more knowledge of the tumor and its expected response to therapy. Insight into the deregulation of genes and proteins related to response to treatment provided by these assays may guide the application of new treatment schedules and the introduction of novel drugs in cancer treatment. This research will facilitate the selection of the most suitable treatment for each individual patient and allows for early adaptation of the initiated treatment. The avoidance of exposing patients to ineffective and unnecessary treatments and associated toxicity will also be a positive spin-off from such predictive assays.

Second, further escalation of the radiation and/or chemotherapy dose: this has become possible with the introduction of high precision radiation delivery techniques such as Intensity Modulated RadioTherapy (IMRT) and Image Guided RadioTherapy (IGRT), and with the improved supportive measures for drug-induced systemic toxicity.

This review will focus on a third strategy: the identification of a new generation of biological response modifiers for combined use with radiotherapy on the basis of their mechanism of action, as well

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as their capacity to influence pathways leading to cell death after irradiation. It is beyond the scope of this review to cover the entire area of ongoing research on potential radiosensitizers. Instead, we will concentrate on combination strategies with proven clinical efficacy and on approaches that are highly promising for combined use with radiotherapy based on their underlying mode of actions.

2. EGFR-inhibiting agents

2.1. Mechanism of action and rationale for radiosensitization

The epidermal growth factor receptor (EGFR) is a transmembrane receptor, mediating a variety of cellular processes. Upon ligand binding to the extracellular domain it auto-phosphorylates after dimerization. EGFR ligands such as EGF and the transforming growth factor alpha (TGFα) activate EGFR signaling ultimately affecting transcription, proliferation and cell cycle transitions. As a result the EGFR signaling pathway is involved in a variety of cellular responses modifying cell growth and survival. EGFR binding signals mainly via activation of PI3K/AKT/mTOR and the Ras/MAPK pathways (LoPiccolo et al., 2008). In addition, nuclear translocation of EGFR may regulate gene expression and DNA repair.

EGFR represents a promising therapeutic target since it is commonly overexpressed in human tumors. In particular, head and neck squamous cell carcinomas (HNSCC), which are frequently treated with radiotherapy or chemo-radiation often display high levels of positive EGFR staining by immunohistochemistry. Aberrant expression by the mutant form of EGFR, EGFRvIII, that lacks the extracellular domain results in constitutively active EGFR signaling. Most importantly, EGFR dependent signaling in these tumors drives proliferation, metastasis and modulates survival after radiation and other DNA damaging agents. An inverse correlation between EGFR expression and response to radiation underlines the role of EGFR in radiation response and suggests that targeting EGFR could improve radiation response (Milas et al., 2004).

Early reports showed that activation of EGFR by exogenous EGF renders breast cancer cells radioresistant (Wollman et al., 1994). EGFR overexpressing cell lines are more radioresistant when compared to cells with normal expression levels, both in terms of clonogenic survival and apoptosis. In line with these observations, the expression of the EGFRvIII variant increased radioresistance (Weppler et al., 2007). These data emphasize the role of aberrantly expressed EGFR and activated EGFR signaling in determining radiation response (Gupta et al., 2002). Further studies gave insight into the mechanism of EGFR mediated radioresistance. EGFR tyrosine phosphorylation is stimulated by irradiation and the subsequent activated signaling via the RAS/MAPK pathway mimics ligand binding and promotes cell survival. Consequently, this results in a proliferative response after radiation and counteracts radiation induced-growth arrest and cell kill. In addition, radiation-induced nuclear translocation allows DNA-dependent protein kinase (DNA-PK) complex formation and therefore affects DNA double strand break (DSB) repair directly (Dittmann et al., 2005a). This has been proposed to confer radioresistance in cells overexpressing EGFR. Similarly, Mukherjee et al. (2009) showed that expression of the EGFRvIII variant promotes DNA DSB repair in a DNA-PK activity dependent manner.

These processes can be opposed by small molecule inhibitors of EGFR autophosphorylation or by anti-EGFR antibodies. Monoclonal antibodies specific for EGFR compete with the binding of natural ligands, thereby preventing ligand-mediated receptor tyrosine kinase phosphorylation hence reducing EGFR signaling activation. C225 (cetuximab, Erbitux) is such an IgG1 monoclonal antibody that has a strong affinity to the extracellular domain of EGFR. In cellular studies it was demonstrated that the radiation-induced nuclear import of EGFR is inhibited by cetuximab (Dittmann et al., 2005b; Huang and Harari, 2000). Cytosolic sequestration of DNA-PK by cetuximab-bound EGFR is thought to impair DSB repair after radiation causing radiosensitization (Dittmann et al., 2005b; Dittmann et al., 2008; Huang and Harari, 2000). Changes in cell cycle phase distribution, particularly the reduction in radioresistant S-phase cells, have further been proposed to alter radiation response after cetuximab exposure.

Alternatively, EGFR signaling can be modified by inhibitors of the tyrosine kinase (TKI) activity responsible for the ligand induced auto-phosphorylation. Among these are the EGFR specific TKI gefitinib (Iressa) and erlotinib (Tarceva) whereas lapatinib (Tykerb) has a dual kinase specificity towards EGFR and HER-2.

Indeed, cetuximab or TKIs have been shown to increase the radiosensitivity in a wide range of cancer cell lines (Bianco et al., 2002; Colquhoun et al., 2007; Eller et al., 2005; Raben et al., 2005; Shintani et al., 2003; Stea et al., 2003; Taira et al., 2006). In HNSCC cell lines cetuximab increased cell kill when combined with cisplatin and radiation (Zhang et al., 2009).

2.2. Preclinical evaluation

Treatment of human tumor xenografts with the anti-EGFR monoclonal antibody cetuximab increased the radiation response in several studies as determined by tumor growth delay and cure rates (Feng et al., 2007; Huang et al., 1999; Krause et al., 2005b; Milas et al., 2000, 2004; Saleh et al., 1999). In addition to the *in vitro* determined cellular radiosensitization, other effects on cell proliferation are responsible for the observed tumor response. Decreased repopulation and increased reoxygenation contributed to local control after combined cetuximab and fractionated radiation in xenograft studies (Krause et al., 2005a). Similar results were observed after administration of TKI in different xenograft models (Colquhoun et al., 2007; Feng et al., 2007; Geoerger et al., 2008; Nyati et al., 2004; She et al., 2003; Williams et al., 2002). Additive effects on response were generally observed in these studies, with occasional synergistic tumor cell kill when combined with radiation. EGFR blocking agents have been shown to enhance tumor vasculature and flow perfusion and to reduce hypoxia ultimately improving radiation response (Qayum et al., 2009).

Multimodality therapies including combinations with novel targeted agents can aggravate radiation-induced normal tissue toxicities. Because preclinical models do not consistently predict these side-effects in patients, they are often revealed in clinical phase I trials. This indicates that more studies are necessary to better characterize these complex multi-agent interactions, not only at the tumor level, but also in normal tissues (Camus et al., 2004; Inoue et al., 2003; Takano et al., 2004; Wang et al., 2008).

2.3. Clinical studies and strategies for response prediction

Major clinical trials combining EGFR targeting drugs and antibodies with radio- and/or chemotherapy have been listed in recent reviews addressing the potential and future of such an approach in head and neck and rectal cancers (Bernier et al., 2009; Marquardt et al., 2009). A pivotal trial demonstrating the added value of cetuximab when combined with radiotherapy in locally advanced head and neck cancer was performed by Bonner and colleagues (Bonner et al., 2006). This trial randomized patients between (standard) radiotherapy and radiotherapy plus weekly cetuximab. With a median follow up of 54 months, the median duration of locoregional control and survival was 24.4 and 49.0 months, respectively, for patients treated with radiotherapy plus cetuximab and 14.9 and 29.3 months, respectively for those who received radiotherapy alone ($p = 0.005$ and $p = 0.03$, respectively). Importantly, with the exception of acneiform rash and infusion reactions, the incidence