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Molecular Pathways

Clinical Cancer Research

Integration of Epidermal Growth Factor Receptor Inhibitors with Preoperative Chemoradiation

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Abstract

In many different cancer cell types, the epidermal growth factor receptor (EGFR) pathway becomes hyperactivated because of overproduction of the ligand, overproduction of the receptor, or constitutive activation of the receptor. The overproduction of EGFR and its ligands correlates with poor prognosis in several solid tumors such as lung, colon, and ovary. These observations led to the development of EGFR inhibitors for anticancer treatment. In the last few years, promising results have been obtained in several tumor types, with EGFR inhibitors given as monotherapy or in combined treatments. In particular, cetuximab in combination with curative-intent radiotherapy in head and neck cancer increases median survival over radiation alone. Similarly, the same approach might benefit patients with locally advanced rectal cancer. Unfortunately, the first clinical studies combining chemoradiation with cetuximab in rectal cancer gave disappointing results. Translational research suggested that the low response rate observed might have been due to the strong antiproliferative effect of cetuximab that may have compromised the activity of chemotherapeutics that target proliferating cells. This result indicates the need for more translational research to unravel how the molecular mechanisms might be manipulated to optimize the combined treatment regimen and to identify biomarkers that can select those patients who will derive most benefit.

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Background

Epidermal growth factor receptor pathway. Epidermal growth factor receptor (EGFR), also known as Her-1 or c-erbB-1, is a member of the c-erB family of transmembrane receptor tyrosine kinases (HER1/Erb-B1, HER2/Erb-B2, HER3/Erb-B3, HER4/Erb-B4). Ligand binding causes homodimerization or heterodimerization of EGFR with other ErbB family members, leading to receptor-linked tyrosine kinase activation (1). Although EGFR and ErbB-4 both have a ligand binding domain and tyrosine kinase activity, ErbB-2 is a ligandless receptor and ErbB-3 lacks tyrosine kinase activity and these receptors can thus only act as heterodimers (2). Several ligands have been identified for EGFR, including the EGFR-specific ligands EGF, transforming growth factor α (TGF-α), amphiregulin and betacellulin, and some ligands that are able to bind several ErbB receptors [epiregulin, heparin-binding EGF-like growth factor (HB-EGF), neuregulin 1-4; ref. 1]. The ErbB signaling network is highly complex because of the different possible dimeric receptor combinations, multiple associated ligands, and multiple downstream pathways that can be activated. Signaling diversity is a composite of the characteristics of the specific receptors and the individual ligands. Two important signaling pathways activated by the ErBb family dimers are the phosphatidylinositol-3-kinase (PI3K) pathway, which promotes tumor cell survival by activating a cascade of anti-apoptotic and prosurvival signals, and the mitogen-activated protein kinase (MAPK) pathway, which controls cell-cycle progression and proliferation (Fig. 1; refs. 3, 4).

Deregulation of the EGFR pathway. Deregulation of EGFR signaling is a hallmark of many cancers, including colorectal cancers (CRC). In general, the EGFR pathway can be hyperactivated by overproduction of ligand, overproduction of receptor, or constitutive activation of receptors (4). In several cancers, overexpression of EGFR is accompanied by enhanced ligand production that leads to continuous autocrine stimulation (3). Although EGFR is overexpressed in up to 82% of CRCs (5) and is associated with tumor development and progression, its role is not entirely clear. EGFR amplification is correlated with but does not reliably predict EGFR overexpression (6), and EGFR mutations that occur regularly in other cancer types such as lung cancer are rare in CRC (7, 6).

Interactions between the EGFR pathway and irradiation. The four cornerstones determining the response to irradiation

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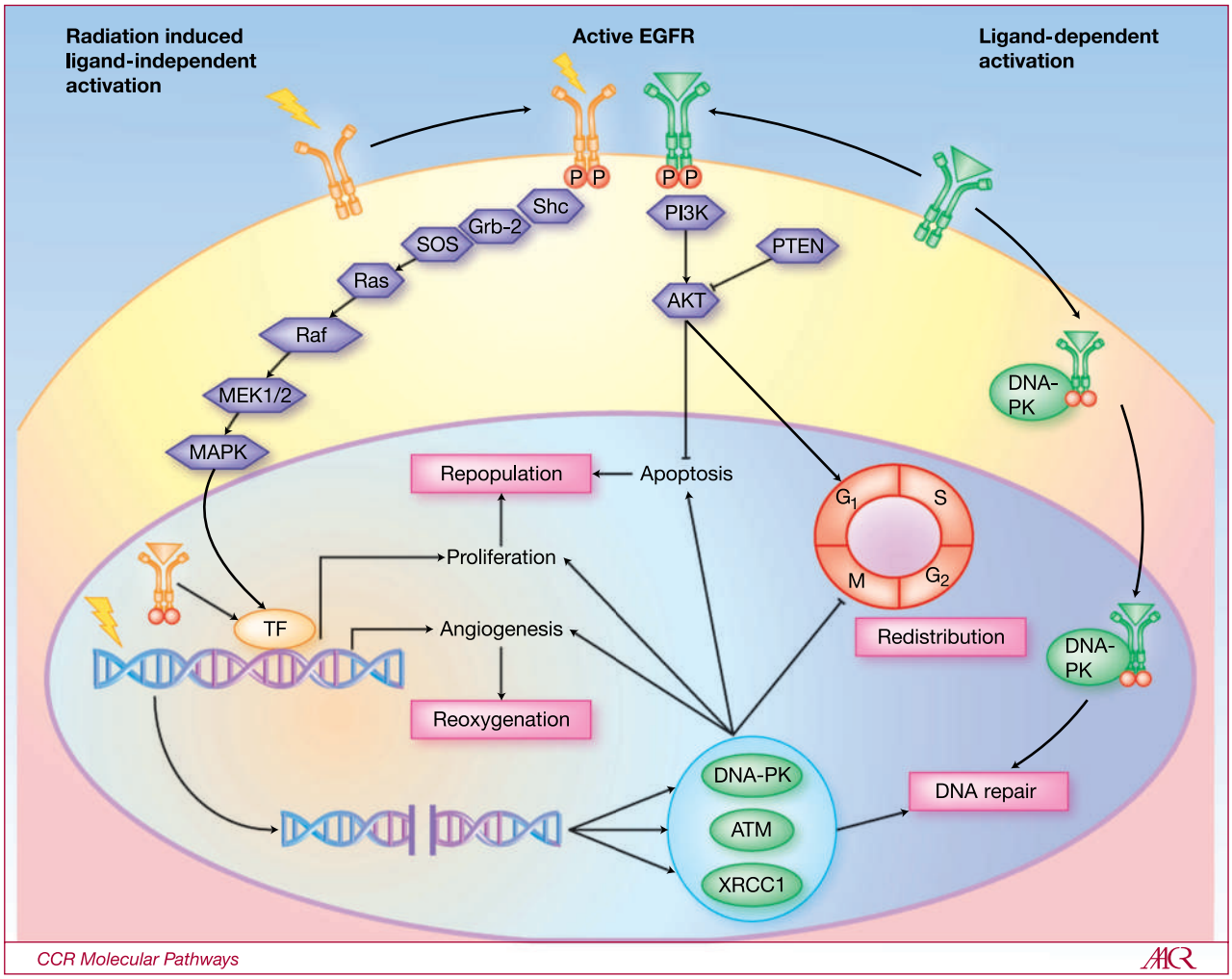

Fig. 1. The EGFR pathway is activated by binding of ligands to the receptor or ligand-independent activation by, e.g., radiation. After phosphorylation of the receptor, two main signaling pathways are activated. The MAPK pathway leads to transcription of growth factors with a pro-proliferative and pro-angiogenic effect. The AKT pathway leads to evasion of apoptosis and stimulates cell cycle progression. Next to the cytoplasmic pathways activated by EGFR, EGFR can also play a direct role in the nucleus by translocation of the DNA repair protein DNA-PK from the cytoplasm to the nucleus and direct activation of transcription factors. Double-stranded breaks caused by irradiation lead to activation of the DNA damage sensors (DNA-PK, ATM, XRCC1, etc.), which activate the effector pathways leading to DNA repair, cell cycle arrest (redistribution), angiogenesis (reoxygenation), apoptosis, and proliferation (repopulation). Inhibition of the EGFR pathway by EGFR inhibitors leads to inhibition of translocation of DNA-PK (less DNA repair), blocking of the cell cycle, inhibition of the anti-apoptotic AKT pathway, and inhibition of the MAPK pathway, leading to less proliferation and angiogenesis. Inhibition of these pathways prevents proper recovery of the cells after irradiation, leading to cell death and thus better tumor response.

are the 4 R's: repair of sublethal DNA damage, redistribution in the cell cycle, repopulation of tumor cells, and reoxygenation of the tumor cells. In recent years, it has become clear that the EGFR pathway seems to be involved in all of these aspects (Fig. 1). Therefore, inhibition of the EGFR pathway could be an ideal way to increase response of the tumors to radiotherapy.

The mechanism of action that has been studied most extensively is the role of EGFR in the repair of sublethal damage after irradiation. There are several ways in which EGFR and/or its downstream signaling effectors may affect elements of the repair process. First, EGFR itself can affect the intracellular distribution of DNA repair proteins by translocation of the DNA-dependent protein kinase (DNA-PK) from the cytoplasm to the nucleus initiating DNA repair (8). Moreover, the signaling pathways of EGFR (PI3K/AKT, Ras/Raf/MEK/ERK) can affect the transcription of DNA repair genes (*Rad51*, *ATM*, *XRCC1*, etc.) and directly or indirectly control the phosphorylation status of key repair proteins (DNA-PK, ATM, etc.; ref. 9). EGFR inhibitors can interfere with these processes by inhibition of the downstream signaling pathways and a redistribution of DNA-PK with a reduction in the level of DNA-PK in the nucleus.

EGFR inhibitors may also cause a redistribution of the cell cycle by blocking the cells in the G1 phase. In addition to the G1 block, after combined treatment, the cell cycle may be prolonged by a radiation-induced G2 block, both resulting in a decrease of the S-phase fraction, which might

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contribute to a decrease of repopulation (10, 11). This combined effect of radiation and EGFR inhibition on two distinct cell cycle checkpoints may prove formidable for the cancer cells to withstand and thus increase the tumor response.

Repopulation of tumor clonogens during treatment is another mechanism of resistance to radiotherapy. It has been shown that cancer cells surviving irradiation acquire a phenotype with upregulated EGFR and TGF-α (12), and thus more activation of the downstream pathways with a pro-proliferative effect (MAPK) and an anti-apoptotic effect (PI3K). This adaptive response produces radioresistance and is a mechanism of accelerated repopulation during radiation treatment. EGFR inhibitors are able to inhibit this adaptive response, making the tumor more susceptible to irradiation damage. Moreover, irradiation can also activate the EGF receptor via a ligand-independent mechanism that also leads to activation of the proliferation-stimulating pathways (13). Further, this activation can be counteracted by the use of EGFR inhibitors.

The influence of EGFR inhibition on reoxygenation of the tumor cells is less straightforward. It is well established that EGFR inhibitors can downregulate angiogenic processes through inhibition of vascular endothelial growth factor. Anti-angiogenic effects, however, can theoretically both improve or impair tumor oxygenation and thereby modulate the effects of radiotherapy in opposite directions. Preclinical results suggest, however, an improvement of the reoxygenation by EGFR inhibition, possibly due to the more rapid regression of the tumors, which affects the tumor perfusion, oxygen, and nutrient supply. Another speculation is that the chronically hypoxic cells, which express more EGFR, are more sensitive to EGFR inhibition, thereby leading to a lower percentage of hypoxic cells in the tumor (14).

Clinical-Translational Advances

Predictive markers in colorectal cancer. The major challenge with all targeted therapies is to identify good predictive markers to select patients for this treatment, because not all patients will respond. Although it was hoped that EGFR expression in the tumor would correlate with response to therapy, recent studies showed that its detection, at least by immunohistochemistry, does not (15–17).

Most of patients with metastasized CRC in whom tumor shrinkage is achieved after treatment with monoclonal antibodies targeting EGFR exhibit increased EGFR copy number, and a nonincreased EGFR copy number detected by fluorescence *in* situ hybridization (FISH) is a predictive factor of resistance (18, 19). However, only a small fraction of patients with increased EGFR gene copy number respond, and the clinical utility of this biomarker is questionable. In addition, standardization of the FISH assay is mandatory to increase reproducibility. In rectal cancer, EGFR gene copy number as a predictive factor has not yet been investigated.

Failure of EGFR overexpression to correlate with outcome and to predict response to EGFR inhibitors can be attributed to several molecular mechanisms. One is constitutive activation within one or more of several signaling pathways downstream of the receptor. One of the main signaling pathways triggered by EGFR activation is the MAPK cascade initiated by KRAS. K-*ras* mutations, occurring in about 30% of all CRCs, have been found by several groups to correlate with the response to the anti-EGFR monoclonal antibodies cetuximab and panitumumab in patients with metastatic CRC (20–25), with clinical benefit restricted to tumors with wild-type *KRAS*. However, for patients with advanced rectal cancer receiving cetuximab combined with (chemo) radiation, no correlation was found between *KRAS* status and pathological response although the number of patients was low and precluded any definite conclusions (26). This result may be because colon tumors are more dependent on the KRAS pathway than rectal tumors (27), in which alternative escape mechanisms may predominate, or the addition of EGFR inhibition to radiation and/or chemotherapy may make these mutations of less importance.

BRAF is the principal downstream effector of KRAS, and mutations in this gene have recently been shown to play a role in the resistance to EGFR-targeted monoclonal antibodies in metastatic CRC (28). In this patient group, 14% of the patients with wild-type *KRAS* showed a mutation in *BRAF*, which suggests that a combined assessment of *BRAF* and *KRAS* mutations might allow a better selection of patients likely to respond to this treatment.

The other main downstream signaling pathway of EGFR involves PI3K/PTEN/AKT, which is highly mutated in many cancers. Several groups have assessed the usefulness of mutations in this pathway in metastatic CRC to predict response to monoclonal anti-EGFR antibodies with conflicting results (29–32). In rectal cancer specifically, the occurrence and predictive value of these mutations has not been assessed as yet.

In addition to constitutive activation of downstream pathways, the effects of EGFR-targeted monoclonal antibodies can also be bypassed by the activation of other family members such as HER-2 or HER-3 or other receptor tyrosine kinases such as insulin-like growth factor I receptor (IGF-1R). Several reports showed a higher expression and signaling of both HER-2 and HER-3 after EGFR inhibition (33, 34). It has recently been suggested that this result might be attributed to a decreased degradation rate for EGFR, leading to higher EGFR expression and enhanced heterodimerization of EGFR with HER2 and HER3 (34). Similarly, increased signaling via the VEGFR-1 and IGF-1R pathway was shown to be correlated with resistance to anti-EGFR therapy in several cancer types *in vitro* and *in vivo* (35–37).

The expression of EGFR ligands (TGF-α, EGF, amphiregulin, epiregulin, etc.) might also play a role in the response to EGFR inhibition. Overexpression of EGFR and its ligands, in particular TGF-α, correlates with poor prognosis in several solid tumors such as lung, colon, and ovary (38).

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Further, Khambata-Ford and colleagues showed that mRNA expression of epiregulin and amphiregulin in metastatic CRC correlated with disease-free survival in patients treated with cetuximab monotherapy (39), and in genome-wide cDNA micro-array analyses in non–small cell lung carcinoma, several EGFR ligands (TGF-α and amphiregulin) were identified as important genes correlating with response to treatment (40). In rectal cancer, the expression of high TGF-α after a loading dose of cetuximab correlated with T downstaging after treatment with cetuximab and (chemo) radiation (26). However, larger studies are needed to confirm these data, and other biomarkers need to be investigated in this patient group.

Rectal cancer experience. CRCs are the second leading cause of cancer-related deaths in the Western world (41), and rectal cancer accounts for about 30% of CRCs. The development of rectal tumors is equally common in men and women and mostly occurs after the age of 60 years. The development of rectal cancer is, in accordance with colon cancer, caused by an accumulation of genetic and epigenetic alterations that can be broadly categorized as two types: tumors characterized by multiple mutations due to chromosomal instability and tumors with a failure of mismatch repair resulting in the microsatellite instability-high phenotype (42). Locally advanced rectal tumors, which are tumors reaching to and beyond the endopelvic fascia, are not curatively resectable using total mesorectal excision alone. Therefore, radiotherapy alone or in combination with chemotherapy is used prior to surgery to reduce the tumor size. Extensive studies on the use of (chemo) radiotherapy for these tumors showed that preoperative radiotherapy decreases local recurrence rates by 50 to 60% as compared with surgery alone (43–49), and the addition of chemotherapy to radiotherapy has been shown to further increase local control (50–54). On the basis of these results, the current standard treatment of locally advanced rectal cancer consists of preoperative chemoradiation followed by total mesorectal excision surgery.

Although concurrent radiochemotherapy has produced important advances, further progress using these modalities is limited by their toxicity. Increasing knowledge of the molecular pathways that drive CRC and the availability of drugs that target these pathways has, therefore, led to trials adding them to conventional treatments in an effort to improve the therapeutic outcome. Most prominent in this regard are the EGFR inhibitors. The most common pharmacological approaches to inhibit EGFR have been the development of monoclonal antibodies (cetuximab, panitumumab, etc.) and tyrosine kinase inhibitors (gefitinib, erlotinib, lapatinib, etc.). Of the monoclonal antibodies and small-molecule inhibitors that have been developed to target EGFR in CRC, the most promising results have been obtained with the monoclonal antibodies cetuximab and panitumumab, which both improve outcome of metastatic disease either in monotherapy or in combination with chemotherapy (16, 55–58).

When combined with curative-intent radiotherapy in head and neck cancer, cetuximab increases median survival over radiation alone (59). As mentioned above, this improvement can been attributed to several mechanisms, of which the most important may be G1 arrest caused by EGFR inhibition and the inhibition of radiation-induced translocation of EGFR to the nucleus with subsequent activation of DNA-PK, undermining the ability of EGFR to activate DNA repair mechanisms (60). Irrespective of the mechanism, the potency of cetuximab in improving the outcome of radiotherapy in head and neck cancer suggested a potential role for cetuximab in the first-line treatment of patients with locally advanced rectal cancer receiving chemoradiotherapy. Surprisingly, the pathological complete response rate in these studies was only 5%, 9%, and 8% (61–63). These data contrast with the 16% pathological complete response rate observed by the group of Rödel and colleagues when they used the same regimen without cetuximab (64) and the 11% of complete responses that is obtained with standard (chemo) radiotherapy [radiation therapy + 5-fluorouracil (5=FU)] in advanced rectal cancer (50, 52). Although nonrandomized, these two trials raise the question about how to optimally combine anti-EGFR monoclonal antibodies with chemoradiation and highlight the need for a better understanding of the molecular mechanisms involved. This lack of molecular knowledge when initiating clinical trials combining (chemo) radiotherapy with EGFR inhibitors has been discussed in detail by Nyati and colleagues (60). The main discussion points were the absence of patient selection and a suboptimal sequencing of (chemo) radiotherapy with EGFR inhibitors giving rise to antagonistic rather than synergistic effects of EGFR inhibitors with chemotherapy. They suggest that EGFR inhibitors given before or concurrently with chemotherapy might counteract the effect of cell-cycle dependent chemotherapy by inducing G1 arrest, which could be overcome by giving the chemotherapy before the EGFR inhibitors. With this latter combination, the effect of chemotherapy might further be enhanced by inhibition of EGFR upregulation and inhibition of DNA repair.

We investigated these hypotheses at the molecular level using biopsies and blood samples from our trial that combined preoperative chemoradiation with cetuximab (61). The results suggested that the low pathological response rate might have been induced by the strong antiproliferative effect of cetuximab, compromising the activity of chemotherapeutics that target proliferating cells (26). Rödel and colleagues also suggested that the G1 cell cycle arrest caused by the addition of cetuximab impairs the efficacy of 5-FU derivatives, oxaliplatin or irinotecan, all of which exert their main cytotoxic and radiosensitizing action mainly in the S/G2/M phases (62). In addition, high tumor proliferation in rectal cancer cells before or after (chemo) radiotherapy has been associated with better response to radiotherapy, and so we cannot exclude that the cetuximab-induced decrease in tumor proliferation could have also impaired the efficacy of radiation therapy (65, 66).

The suggestion, therefore, is that EGFR inhibition might be more effective if it is started after, and not

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before, (chemo) radiotherapy, or if combined with radiotherapy in the absence of chemotherapy, or given after (chemo) radiation as maintenance therapy. The *in vivo* data published by Milas and colleagues showing the superior effect of cetuximab given during and after radiation as compared with cetuximab given before irradiation support this hypothesis (67).

To further assess the potential value of anti-EGFR monoclonal antibody in rectal cancer, new clinical trials combining cetuximab or panitumumab with preoperative radiation therapy in the absence of chemotherapy will be initiated in the near future. In our study, panitumumab will be continued for 3 weeks after the end of radiation therapy to maximize the chance of improving the pathological response rate. In concert with these trials, further examination of the molecular pathways that determine the efficacy of this treatment will be done in an attempt to develop predictive markers that may be specific for rectal cancer rather than for CRC in general.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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