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EGFR expression levels affect the mode of action of EGFR-targeting monoclonal antibodies

Stefanie Derer, Stefan Lohse and Thomas Valerius*

Division of Stem Cell Transplantation and Immunotherapy; 2nd Department of Medicine; Christian-Albrechts-University and University Hospital Schleswig-Holstein; Kiel, Germany

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Epidermal growth factor receptor (EGFR) expression levels appear to modulate the efficacy of EGFR-targeting monoclonal antibodies. More specifically, we observed that high EGFR densities negatively affect the effects of EGFR-specific antibodies on EGFR phosphorylation yet exacerbate Fc-mediated tumor-cell killing. These results suggest that the predominant mode of action of EGFR-targeting antibodies depend on EGFR expression levels.

In 2004, the first monoclonal antibody targeting the epidermal growth factor receptor (EGFR), cetuximab (a chimeric IgG1 also known as Erbitux®, from Merck-Serono S.A.), was approved by the U.S. Food and Drug Administration (FDA) for the therapy of metastatic colorectal carcinoma (mCRC). Two years later, the U.S. FDA extended the approved indications of cetuximab to squamous cell cancer of the head and neck (SCCHN). In addition to cetuximab, a second EGFR-targeting monoclonal antibody, panitumumab (a human IgG2 also known as Vectibix®, from Amgen Inc.), has been approved for the therapy of mCRC. However, although both these EGFR-targeting antibodies modestly prolong the survival rates of patients, complete remissions are rarely achieved. Therefore, several approaches are under investigation to identify biomarkers that may allow for the prediction of the clinical efficacy of EGFR-targeting anticancer strategies and hence for a more precise selection of patients that may truly benefit from these therapeutics.[1]

Many studies have linked the clinical response of cancer patients to EGFR-targeting antibodies with the expression level of EGFR ligands, mutations in the downstream mediators of the EGFR signaling cascade as well as with polymorphisms of EGFR or the Fc γ receptors (FcγRs) IIa (131H/R) and IIIa (158V/F). Oncogenic mutations in the gene coding for the GTPase *v-K$_i$-ras2* Kirsten rat sarcoma viral oncogene homolog (*KRAS*) have been unravelled as the most prevalent factors underpinning the resistance of mCRC patients to EGFR-targeting antibodies. Accordingly, patients bearing *KRAS*-mutated CRC are currently excluded from EGFR-directed antibody-based therapies.[1]

Interestingly, a recent retrospective analysis of a randomized, multicenter, Phase III study of cetuximab in combination with cisplatin/vinorelbine (CV) vs. CV alone in the first-line treatment of patients with advanced non-small cell lung cancer (NSCLC) (FLEX study) has demonstrated a positive correlation between the clinical response to cetuximab and EGFR expression levels, as determined by quantitative immunohistochemistry.[2] These observations have led to a controversial discussion about the impact of EGFR expression levels on the efficacy of EGFR-targeting antibodies, in the setting of NSCLC as well as in other clinically-relevant scenarios such as CRC and SCCHN.[3] In this context, our group initiated a systematic analysis to unravel how different expression levels of EGFR at the cell surface affect the mode of action of EGFR-specific antibodies (**Fig. 1A**). Importantly, a positive correlation was observed between EGFR expression levels and the Fc-dependent antineoplastic effects of EGFR-targeting antibodies, including antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Conversely, the Fab-dependent effects of EGFR-specific antibodies such as the inhibition of ligand-induced receptor phosphorylation negatively correlated with EGFR expression levels.[4] Based on these data, it may be hypothesized that the Fc-dependent anticancer activity of EGFR-targeting antibodies is predominant in tumors expressing high EGFR levels, provided that neoplastic lesions are accessible for effector cells and/or the complement system, whereas Fab-mediated effect may be most relevant in tumors that express reduced EGFR levels. As oncogenic *KRAS* signaling downregulates the expression of EGFR on the cell surface, oncogenic *KRAS* mutations not only interfere with the cancer cell-intrinsic effects of EGFR-targeting antibodies but also are expected to limit their Fc-dependent antineoplastic effects.[5] Furthermore, data from the Human Protein Atlas (www.proteinatlas.org/ENSG00000146648/normal) and own unpublished observations indicate that EGFR is expressed to lower levels in the normal colon than in the lung. Taken together, these observations may explain why *KRAS* mutations are associated with resistance to EGFR-targeting antibodies in CRC but not in NSCLC patients.

*Correspondence to: Thomas Valerius; Email: t.valerius@med2.uni-kiel.de
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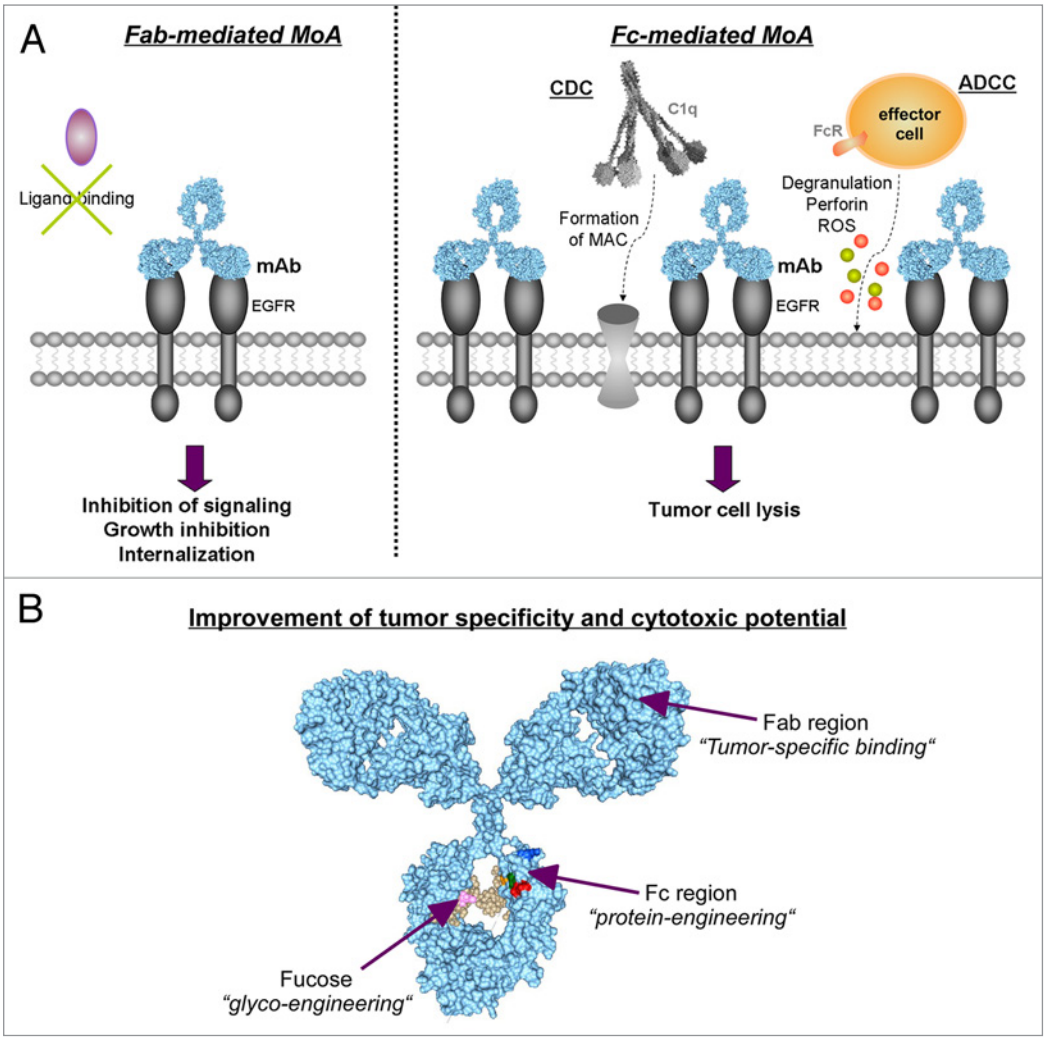

Figure 1. EGFR expression levels affect the mode of action of EGFR-targeting monoclonal antibodies. (**A**) Modes of action of EGFR-targeting antibodies in tumor therapy. Epidermal growth factor receptor (EGFR) targeting monoclonal antibodies are able to elicit distinct effector mechanisms leading to tumor cell destruction. Fab-mediated effects (left panel) include the inhibition of ligand binding, and hence of proliferation, the induction of apoptosis as well as EGFR internalization. Fc-mediated mechanisms (right panel) are triggered as the Fc region binds either the complement component C1q to induce complement-dependent cytotoxicity (CDC), or Fc receptors on effector cells to trigger antibody-dependent cell-mediated cytotoxicity (ADCC). (**B**) Structural model of human IgG1. Amino acid substitutions and fucose residues in heavy chains are highlighted. Image generated with the 3D-molecule viewer package of NTI Vector (Life Technologies). MAC, membrane attack complex. Pdb file from Clark, MR, *Chem Immunol* 1997; 65:88–110.

In order to improve the modest efficacy of EGFR-directed antibodies, many approaches have been developed (**Fig. 1B**).[1] Among these, improving the binding affinity of the Fc portion of IgG1 molecules for FcγRIIIa by glycosylation or protein engineering has been shown to successfully enhance ADCC as mediated by NK cells, irrespective of *KRAS* mutational status. In line with this notion, a glyco-engineered EGFR-targeting antibody has demonstrated some clinical efficacy in a Phase I clinical study.[6] However, enhancing the affinity of EGFR-directed antibodies for FcγRIII (CD16) limits the recruitment of polymorphonuclear leukocytes (PMNs) as effector cells. Myeloid cells—like monocytes/macrophages and PMNs—are the predominant effector cell population for the human IgG2 antibody panitumumab. The recruitment of myeloid cells can be improved by employing EGFR-targeting antibodies of the IgA isotype.[1] In order to recruit T cells as the main effectors of EGFR-directed monoclonal antibodies, EGFR- and CD3-targeting molecules, so-called bispecific T-cell-engagers (BiTes), have been generated, resulting in significant antineoplastic effects in mouse xenograft models.[7] Besides engineering approaches, combination strategies involving antibodies that target non-overlapping epitopes in the extracellular domain III of EGFR have been investigated. Thus, combinations of two non cross-blocking EGFR-directed monoclonal antibodies have been demonstrated to initiate CDC against tumor cells in vitro, while the same effect was not observed in the presence of single EGFR-directed antibodies.[1] Of note, this concept has also been successfully demonstrated to enhance tumor growth inhibition by EGFR-targeting antibodies in vitro and in vivo, and has already entered a Phase II clinical trial.[8]

Since EGFR is also expressed by non-malignant, epithelial tissues (www.proteinatlas.org/ENSG00000146648/normal), enhancing the efficacy of EGFR-targeting antibodies may be accompanied by increased toxicity. For example, high EGFR expression levels in the skin have been associated with skin rashes, the most common side-effect of EGFR-directed antibody therapy. That is why antibodies have been developed that exclusively target tumor-specific EGFR epitopes, such as the EGFR variant III (EGFRvIII). Interestingly, a tumor-specific increase in cytotoxicity was observed when EGFRvIII- and EGFR-directed antibodies were combined, which was further enhanced by Fc protein-engineering.[9] Alternatively, the selectivity of these antibodies for cancer cells can be enhanced by the masking of antigen-binding sites with peptides that are specifically cleaved off by tumor-specific proteases.[10]

To conclude, the expression level of EGFR on the surface of cancer cells may be connected to distinct modes of action of EGFR-targeting antibodies and hence may cause significant differences in their clinical efficacy. The major limitations of EGFR-directed antibody therapy may be overcome by novel promising approaches that improve the specificity of these agents for tumor cells while increasing their capacity to recruit effector cells and the complement system. This said, a full understanding of the interplay between EGFR expression levels and the clinical efficacy of therapeutic EGFR-targeting monoclonal antibodies has not yet been reached.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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