Immune biomarkers of anti-EGFR monoclonal antibody therapy

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The tumor antigen (TA)-targeted monoclonal antibodies (mAb) cetuximab and panitumumab target the human epidermal growth factor receptor and have been integrated into treatment regimens for advanced squamous cell carcinoma of the head and neck (SCCHN). The therapeutic efficacy of these mAbs has been found to be enhanced when combined with radiotherapy and chemotherapy. However, clinical trials indicate that these findings are limited to fewer than 20% of treated patients. Therefore, identifying patients who are likely to benefit from these agents is crucial to improving therapeutic strategies. Interestingly, it has been noted that TA-targeted mAbs mediate their effects by contributing to cell-mediated cytotoxicity in addition to inhibition of downstream signaling pathways. Here, we describe the potential immunogenic mechanisms underlying these clinical findings, their role in the varied clinical response and identify the putative biomarkers of antitumor activity. We review potential immunological biomarkers that affect mAb therapy in SCCHN patients, the implications of these findings and how they translate to the clinical scenario, which are critical to improving patient selection and ultimately outcomes for patients undergoing therapy.

Key words: EGFR, immune biomarkers, monoclonal antibodies, head and neck cancer

introduction

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of four structurally related receptor tyrosine kinases [1]. These transmembrane proteins are composed of an extracellular domain for binding of specific ligands, and an intracellular domain with tyrosine kinase activity. Ligand binding causes dimerization of the receptor which triggers a signaling cascade that promotes cell survival and tissue invasion, while inhibiting apoptosis [2]. EGFR is frequently overexpressed in epithelial malignancies, which corresponds to a decrease in patient survival, providing a target for tumor antigen (TA)-targeted monoclonal antibody (mAb)-based immunotherapy. Cetuximab and panitumumab are two such mAbs which have been incorporated to treatment regimens for colorectal and head and neck cancers [3–11]. As single agents, these antibodies confer clinical responses in 10%–15% of patients with advanced untreated and recurrent metastatic disease [12]. Interestingly, when used in combination with chemotherapy or radiotherapy, the efficacy of these mAbs is often improved with higher response rates, prolonged duration of response and crucially, prolonged patient survival [13, 14].

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ligand binding and receptor phosphorylation as well as associated downstream signaling while concomitantly inducing receptor downregulation through dimerization and internalization [26]. The three main pathways downstream of EGFR are the MAPK, PI3K/AKT and JAK/signal transducer and activator of transcription (STAT) pathways [27–29]. However, in addition to inhibition of these mitogenic pathways, the effects of cetuximab therapy may also be mediated by antibody-dependent, cell-mediated cytotoxicity (ADCC) [30], complement-mediated cytotoxicity [15] and adaptive immunity-mediated by CD8+ cytotoxic T lymphocytes (CTL) [31].

Panitumumab is a fully human IgG2 mAb which also targets the extracellular domain of EGFR. It binds the EGFR with great affinity ($K_d = 1.33 \pm 0.29$ nmol/l) and was developed with the intention of causing fewer hypersensitivity reactions than cetuximab [32]. However, as an IgG2 isotype, it has lower immunogenicity potential, through poor Fc-gamma receptor (FcγR) binding [16]. Approved by both the FDA and EMA for use in CRC, panitumumab has not yet been approved for use in SCCHN although several clinical trials are reported or currently underway [33]. In addition to receptor downregulation and inhibition of downstream signaling of the EGFR, the clinical efficacy of panitumumab is believed to be mediated by induction of cell cycle arrest and inhibition of angiogenesis [17, 18], as well as facilitating ADCC, potentially through FcγRIIA on neutrophils [19] although to a lesser degree than cetuximab therapy.

**Fc-gamma receptor polymorphisms and clinical response**

Although anti-EGFR mAbs inhibit EGFR tyrosine phosphorylation and subsequent downstream signaling, evidence suggests that triggering of ADCC mediated by mAb-activated NK cells has a significant role to play in their effectiveness [34]. Cetuximab treatment of SCCHN cells *in vitro* does not result in apoptosis or lysis of tumor cells [20] this only occurs in the presence of lymphocytes, supporting an immune-mediated mechanism contributing to the antitumor effect of anti-EGFR mAb therapy, ADCC is a lytic reaction characterized by cetuximab and panitumumab coating EGFR on the surface of tumor cells and binding to FcγRs expressed on immune effector cells, activating them and resulting in lysis of the antibody-coated target cells (Figure 1). Cetuximab-mediated ADCC reactions can be enhanced by cytokines, including interleukin 12 (IL-12), interleukin 2 and other NK cell-activating cytokines thus indicating the importance of NK cell function in effective ADCC reactions. Studies in both breast cancer and SCCHN indicate that cytokine activation of NK cells results in enhanced NK cell lytic activity against cetuximab-coated cells as well as increased antitumor effects mediated by these activated NK cells [35, 36].

Three classes of FcγR encoded by eight genes have been identified in humans, FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16a). Some FcγR display allelic polymorphisms which generate allotypes that have been reported to be functionally relevant in the ADCC mechanism [21]. A clinically relevant example of this is seen in the gene encoding FcγRIIIa, where a single-nucleotide substitution at position 158 results in the substitution of phenylalanine (F) by valine (V) in the IgG-binding domain, which results in varying patient response to cetuximab treatment [16]. Unlike in CRC where better clinical response to cetuximab therapy has been shown to correlate with the FcγRIIIa VV or FF genotypes [16, 22, 37], *in vitro* studies in SCCHN appear to indicate a correlation with the FcγRIIIa VV genotype and more potent ADCC [21, 38]. Clinical data are not yet available from cetuximab clinical trials but a retrospective cohort showed a non-significant association with the V-encoding allele. Interestingly, this phenomenon is also reported in patients treated with the

![Figure 1. Antibody-dependent, cell-mediated cytotoxicity (ADCC). ADCC mediated by tumor antigen (TA)-targeted monoclonal antibodies (mAbs) in the tumor microenvironment. TA-positive tumor cells are exposed to TA-targeted mAbs which leads to opsonization through binding of cetuximab and panitumumab to the TA epitopes expressed on tumor targets. NK cell recognition of opsonized tumor cells is mediated via Fc gamma receptor (FcγR) IIIC/CD16 while FcγRIIA/CD32 mediates recognition. This effect is diminished by regulatory T cells (Treg) which exert a suppressive function on effector immune cells through secretion of interleukin 10, transforming growth factor-β and adenosine into the tumor microenvironment.](image)
Anti-EGFR mAb therapy is associated with proinflammatory side-effects, the most common of which is the development of a characteristic skin rash [41, 42] suggesting that EGFR signaling is involved in the control of immunoregulatory genes. Interestingly, the skin rash may correlate with improved response to therapy and better clinical outcome for cetuximab-treated patients [43]. Overexpression of EGFR associated with SCCHN has been shown to repress expression of the human leukocyte antigen (HLA) class I and antigen-processing machinery (APM) components [44–47], a phenomenon associated with tumor cell escapes from CTL recognition and lysis [48]. The process of tagging endogenous proteins for degradation and processing before formation of the HLA class I–peptide complexes to be presented on the cell surface involves the cooperation of multiple components within the APM of antigen-presenting cells. These HLA class I–peptide complexes are then recognized by T cells expressing the corresponding T-cell receptors. The resulting impaired tumor antigen (TA) processing and presentation leads to poor recognition of tumor cells by CTL and the development of an immune escape phenotype that correlates with poor clinical outcome [48–51]. Although the exact molecular mechanisms for this remain under investigation [52], these effects appear to be ameliorated by anti-EGFR antibody therapy [53]. Cetuximab has been shown to upregulate HLA class I antigens on the level of gene transcription and correlates with increased cutaneous expression of HLA class I and II proteins in patients who developed skin rash upon cetuximab therapy [44]. The cytokines and molecular signals that regulate the finely tuned APM are often disrupted in SCCHN [49, 54]. One such molecular signal is the transcription factor STAT1. The activated form of STAT1, phospho-STAT1 (pSTAT1) and has been shown to be necessary for APM expression and subsequent T-cell recognition [55, 56]. The characteristic low basal pSTAT1 level which is seen in SCCHN has been attributed to Src homology-2 domain-containing phosphatase (SHP2), a known negative regulator of the JAK-STAT1 signal transduction pathway, which is frequently overexpressed in multiple malignancies [52, 57, 58].

While overexpression of EGFR has not been shown to be correlated with SHP2 increase in SCCHN, in other malignancies, such as lung cancer, activating mutations of EGFR have been linked to activation of SHP2 [59]. Studies are currently underway in SCCHN to investigate the role that EGFR signaling downregulation by anti-EGFR mAb may have in inhibiting SHP2 and subsequent increase in pSTAT1, which has been shown to restore antitumor immunity in the tumor microenvironment.

modulation of the human leukocyte antigen class I and antigen-processing machinery expression by EGFR-specific mAb

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signal transducer and activator of transcription 3 as a mechanism for SCCHN immune escape

EGFR overexpression in SCCHN cells induces the constitutive activation of STAT3, a known oncogenic transcription factor which results in tumor growth [60–63], and immunosuppression [52, 55]. Other receptors from the ErbB/HER family can also be ligated by their tumor-overexpressed ligands causing EGFR-independent STAT3 activation in an autocrine or paracrine fashion. Notably, the interleukin 6 (IL-6) receptor (IL-6R)/CD130 signaling complex has also been shown to be a major pathway involved in EGFR-independent STAT3 activation and tumorigenesis [64–66]; indeed, it is overexpressed in SCCHN cells and its detection is correlated with poor survival in SCCHN patients [67]. Thus, the expected cetuximab-mediated growth inhibition of SCCHN via blocking EGFR-dependent survival signals may not be fully effective, since STAT3 constitutive activation takes place through alternative pathways, such as IL-6/gp130.

SCCHN cells exhibit a constitutively active STAT3 that blocks apoptosis and induces proliferation, angiogenesis and immune evasion [68, 69]. STAT3 is considered an oncogene and its inhibition leads to apoptosis in vitro and to an impaired tumor growth in xenografted mouse models [70, 71]. STAT3 plays a major role in promoting tumor immune evasion. Previous work shows that it not only inhibits production of inflammatory signals from tumor cells, but also production of immunosuppressive mediators, thus inducing a tolerant tumor microenvironment [72, 73]. Due to this situation, SCCHN cells may evade cetuximab-dependent immune effector cell tumor cytolyis, as well as EGFR signaling inhibition, through mechanisms discussed below and shown in Figure 2.

EGFR-mediated STAT3 activation induces the expression of vascular endothelial growth factor (VEGF), IL-6 and interleukin 10 (IL-10) in many tumors including melanoma [64, 67]. Increased levels of VEGF have been found to induce tumor growth, metastasis and resistance to treatment in SCCHN [74, 75]. In a recent phase II clinical trial, the addition of bevacizumab (a humanized anti-VEGF-A IgG1 mAb) with cetuximab therapy was shown to induce a 16% clinical response rate in 46 patients with recurrent or metastatic SCCHN [76], suggesting a potential role for combination therapy, though further studies are needed to validate these findings. IL-6, IL-10 and VEGF are known to activate STAT3 in tumor-associated immune cells, providing a feed-forward mechanism to ensure a STAT3-dominated tumor permissive microenvironment. Tumor-secreted transforming growth factor-β (TGF-β), VEGF and IL-10 induce T-cell tolerance through inhibition of dendritic cell (DC) differentiation and maturation [77, 78]. Moreover, blocking STAT3 in macrophages-induced restoration of T-cell responsiveness by restoring the secretion of IL-12 and CCL5. Likewise, IL-10 protects tumor cells from CTL lysis by downregulation of APM components (TAP1, TAP2) and surface HLA class I expression [79, 80]. Thus, immunosuppressive cytokines induced by EGFR-JAK2-STAT3 activation, potentially inhibited by EGFR-targeted mAb, may serve as biomarkers of response to anti-EGFR therapy.
**HPV status**

In the USA and Northern Europe, the incidence rate of some oropharyngeal cancers, particularly those of the tonsil and tongue base have steadily increased since the 1970s. This is especially noted in younger patients without the usual recognized risk factors for SCCHN [81–83]. Along with other etiological factors, the primary cause for this has been attributed to persistent infection with high-risk human papillomavirus (HPV) [84, 85]. Tumors that are positive for HPV have a different biology and phenotypical features than HPV-negative tumors. They tend to be poorly differentiated with scant keratinization [86]. The difference in HPV-positive and -negative cancers is noted in prognostic outcomes for the patient, with HPV-positive disease being exquisitely radiosensitive and therefore having more favorable clinical outcomes than patients with smoking-related SCCHN [87, 88]. Results of a multicenter, randomized phase III trial by the Radiation Therapy Oncology Group (RTOG) looking at concurrent radiation and cisplatin versus concurrent radiation, cisplatin and cetuximab for stage III and IV SCCHN suggested that patients with HPV-positive tumors may not benefit from addition of cetuximab therapy to cisplatin. An ongoing RTOG trial 1016 aims to evaluate the impact of EGFR-specific mAb, cetuximab versus cisplatin in the context of HPV + disease. A similar phase III SPECTRUM trial that looked at the safety and efficacy of panitumumab in recurrent or metastatic SCCHN found that Panitumumab plus cisplatin improved both overall survival (OS) and progression-free survival (PFS) in HPV-positive tumors, and showed no incremental benefit in patients with HPV-negative tumors. HPV status was classified differently in the RTOG and SPECTRUM trials, clouding the interpretation of these results. Further studies are warranted to delineate the role of HPV status as a potential immune biomarker of anti-EGFR monoclonal antibody therapy.

**regulatory T cells and tumor-associated macrophages**

EGFR-specific CTL have been identified in SCCHN patients but not in healthy donors suggesting that EGFR may function as an immunogenic TA in cancer patients [89]. During therapy with...
anti-EGFR mAbs there is an observed enhancement of EGFR-specific CTL frequencies, the generation of these CTL appears to be initiated by cetuximab-induced NK cell activation [31, 90]. Despite the elevated frequency of EGFR-specific CTL in SCCHN patients, it was noted that these CTL did not sufficiently inhibit tumor growth. One explanation for finding has been proposed to be due to is elevated levels of suppressive regulatory T-cell (Treg) populations in the circulation and tumor sites of these patients. Tregs possess a strong suppressive function, through TGF-β, IL-10 and adenosine produced by the ecto-enzymes CD39 and CD73, and are believed to result from chronically activated T cells [91]. CD25+CD4+ Treg cells constitutively express the transcription factor Foxp3 and regulate immune self-tolerance by suppressing aberrant or excessive immune responses which may be harmful to the host. Abnormalities in Treg function are seen in many autoimmune and inflammatory diseases [92, 93]. Within the tumor microenvironment, it is believed that the development of Tregs results from chronic exposure of T cells to self-antigens, including EGFR. Therefore, although CD8+ T cells are present in some SCCHN patients, their antitumor activity may be suppressed by Tregs [94] (Jie HB, in press). Anti-EGFR mAb therapy significantly increases both the frequency of EGFR-specific TIL and Tregs in both the circulation and tumor sites of patients with SCCHN. Several additional cytokine interactions are involved in generation of Tregs in SCCHN patients. TGF-β, a known immunosuppressive cytokine, is found to be present at high concentrations in plasma of cancer patients and is associated with disease progression and poor response to immunotherapy. TGF-β production is characteristic of many malignancies including melanoma, breast and colon cancer and is known to prevent proper CTL generation and function [95]; however, TGF-β secretion by SCCHN is still not well characterized. Interestingly, TGF-β is involved in the generation of Tregs that are known to inhibit CD8+ T-cell activation, interferon-γ production and proliferation [96]. Additionally, Tregs can secret TGF-β and IL-10 in a STAT3-dependent fashion further propagating the immunosuppressive signals [97, 98]. This intricate tumor cellular network also includes TAMs, which are induced by monocyte exposure to tumor-secreted IL-6, IL-10 and VEGF [99]. TAMs suppress DC maturation in an IL-10-dependent manner that in turn inhibits CD8+ T-cell proliferation and function [100, 101]. TAM-derived IL-10 can also induce differentiation of naïve T cells into Tregs [98], and thus may suppress mAb-induced cellular immunity in the tumor microenvironment.

newer EGFR-specific mAb

Several novel anti-EGFR antibodies have recently been brought to the market, although none are currently approved for use against SCCHN within the USA, there are currently ongoing clinical trials underway to investigate their efficacy. Zalutumumab is a fully human IgG1 mAb, which was tested as a second-line agent in a recent phase III randomized clinical trial of 286 patients with recurrent or metastatic disease refractory to platinum-based therapy. SCCHN patients who received zalutumumab with supportive care versus supportive care alone demonstrated no statistically significant improvement in OS between either group. Of note however, the PFS appeared longer in the zalutumumab-treated group compared with supportive therapy alone [102]. Nimotuzumab is a humanized IgG1 mAb currently approved for use in the management of SCCHN in countries outside the USA and Europe. A potential advantage of nimotuzumab is its lower immunogenic side-effects profile [103]. Initial phase I studies revealed an absence or mild skin toxicity and hypersensitivity reactions [104].

summary

Immune therapy continues to be an evolving area of cancer therapy, in order to better evaluate patient response to therapy, certain immune biomarkers should be identified and monitored; here, we have described the potential immune biomarkers of cetuximab and panitumumab therapy. Although the importance of FcγR polymorphisms in the ADCC reaction has been noted in the breast cancer and CRC models, it is yet to be seen whether it is a useful biomarker in the setting of SCCHN. In vitro studies indicate a correlation with the FcγRIIA VV genotype and more potent ADCC reactions; however, this finding did not appear to correlate with disease-specific survival in a large retrospective study involving cetuximab and SCCHN. Future prospective cohorts must be interrogated.

Constitutive STAT3 activation seen in SCCHN promotes tumor immune evasion, and contributes to induction of a tolerant tumor microenvironment. Additionally, it mediates expression of cytokines and growth factors known to promote tumorigenesis and correlates with poor clinical outcomes, an effect that may take place through alternative, EGFR-independent pathways. Clinical trials looking at combining EGFR blockade as well as blockade of pathways downstream of STAT3 may indicate a role for STAT3 or TGF-β modulation in successful immunotherapy. The identification of EGFR-specific CTL in SCCHN patients, which increase in frequency during treatment with TA-targeted mAbs suggests that EGFR may function as an immunogenic protein in these patients, and that cetuximab may drive NK: DC priming of cellular immunity. However, the strong suppressive function of Treg populations in the circulation of these patients may be responsible for the lack of antitumor effects seen with despite this elevated frequency of EGFR-specific CTL in SCCHN patients. The suppressive functions of Tregs are mediated primarily through TGF-β and IL-10 which can be additionally be secreted by Tregs in a STAT3-dependent fashion, further propagating the immunosuppressive signals. As the field of TA-targeted mAb-based immunotherapy continues to expand, a greater understanding of the immunogenic mechanisms that underlie their antitumor effect is necessary. Future mAb trials should monitor immune biomarkers and correlate these with patient response.

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references


Estimates of benefits and harms of prophylactic use of aspirin in the general population


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Background: Accumulating evidence supports an effect of aspirin in reducing overall cancer incidence and mortality in the general population. We reviewed current data and assessed the benefits and harms of prophylactic use of aspirin in the general population.

Methods: The effect of aspirin for site-specific cancer incidence and mortality, cardiovascular events was collated from the most recent systematic reviews. Studies identified through systematic Medline search provided data regarding harmful effects of aspirin and baseline rates of harms like gastrointestinal bleeding and peptic ulcer.