

REVIEW

Epidermal growth factor receptor imaging in human head and neck cancer xenografts

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ABSTRACT

Molecular imaging of specific biomarkers can have prognostic, predictive or monitoring value in head and neck squamous cell carcinoma (HNSCC). The epidermal growth factor receptor (EGFR) is involved in various radiation resistance mechanisms as it steers the pathways related to DNA damage repair, proliferation, hypoxia and apoptosis. Radiolabeled F(ab')₂ fragments of the EGFR antibody cetuximab can be applied for non-invasive imaging of this receptor. Preclinical studies have shown that radioresistant tumors had a higher tracer uptake after irradiation, probably due to upregulation of membranous EGFR, thereby increasing target availability possibly as a compensation mechanism. Tumors with increased EGFR availability were also more responsive to the EGFR inhibitor cetuximab. Potentially, radionuclide imaging of the EGFR can be applied for monitoring treatment regimens in clinical practice.

Improvements have been implemented in cancer treatment over the last decennia. These include the introduction of hypoxia modification and targeting the epithelial growth factor receptor (EGFR) in combination with radiotherapy [1–5]. By adaptations based on the characteristics of the tumor of the patient, treatment outcome can be improved and overall treatment-related side effects can be reduced. The heterogeneity of the patient population drives the search for tumor-specific biomarkers. This heterogeneity, as well as the observation that specific characteristics of the microenvironment are maintained when human tumors are transplanted in immune compromised mice, makes these relevant models for testing new therapies [6].

In this review we will discuss recent developments of the application of radiolabeled EGFR antibodies for quantifying EGFR expression in human head and neck cancer xenografts.

Imaging biomarkers for head and neck squamous cell carcinoma treatment

Molecular imaging of specific biomarkers can have prognostic, predictive or monitoring value in head and neck squamous cell carcinoma (HNSCC) [7].

A promising approach to evaluate therapy outcome is the development of imaging tracers for early response monitoring. As tumor glucose consumption, proliferation and hypoxia are often enhanced as compared to normal (adjacent) tissues, tracers targeting these features were tested as imaging biomarkers. By conducting multiple positron emission tomography (PET) scans, prior to and early after onset of treatment, molecular imaging can reveal patient-specific information on (therapy-induced) changes in the tumor microenvironment [7]. A biomarker of interest is the EGFR as it steers the pathways related to DNA damage repair, proliferation, hypoxia and apoptosis in HNSCC [8–11]. The chimeric antibody cetuximab binds with high affinity to the EGFR and has been used in treating HNSCC, though it remains unclear which patients will benefit from this therapy [5]. Imaging with tracers produced by direct labeling of the target drugs themselves provides information on the pharmacodynamic and pharmacokinetic characteristics of the drugs [12,13]. Cetuximab has been labeled with several radionuclides and has been investigated preclinically in several HNSCC models [14–16]. We have labeled cetuximab with ¹¹¹In for SPECT-imaging, showing

good tumor uptake and optimal tumor-to-background images at 3–7 days after injection in mice with subcutaneous human HNSCC FaDu tumors [17]. We developed ^{111}In -labeled F(ab')_2 fragments of cetuximab, which in preclinical models exhibit rapid clearance from the blood stream and better tumor penetration relative to the whole IgG [18–20]. After intravenous injection ^{111}In -cetuximab- F(ab')_2 specifically targets systemically accessible EGFR with an optimum tumor-to-background ratio at 24 hours after tracer injection. Furthermore, the tracer could distinguish between several HNSCC xenografts with different EGFR expression profiles and in three of four models this correlated with sensitivity to EGFR-inhibition with cetuximab [9,21]. Tracers based on antibody fragments have considerable renal uptake which can be reduced by co-injection of basic amino acids, (i.e. arginine and lysine) or the gelatine-based plasma expander gelofusine [22–24]. In addition, when using anti-EGFR based tracers, the liver can act as a basin, which could limit tracer uptake in the tumor. Administering higher protein doses could counteract this effect, thus careful dosing of the tracer is crucial [25].

Cetuximab sensitivity is not solely determined by the amount of EGFR available at the tumor cell surface. For example, the EGFR can be bypassed via signaling through ErbB family members, activating the same downstream pathways. Many ErbB family members are also frequently overexpressed in HNSCC, albeit usually to a lesser degree than EGFR [26,27]. Other transmembrane receptors like insulin-like growth factor receptor (IGF1R) and hepatocyte growth factor receptor (c-MET) are also coupled to the same pathways, and are two well documented sources of treatment resistance in HNSCC [9]. A preclinical study reported an additive effect of blocking ERK phosphorylation (with the anti-IGF-1R antibody cixutumumab) together with cetuximab in HNSCC xenografts [28]. Combination of inhibitors could potentially lead to more effective treatment of HNSCC. In addition to other membrane receptor proteins negating EGFR inhibition, the downstream pathways P13-K/AKT, RAS/ERK and STAT can be independently constitutively activated [29–32]. This would omit the need for EGFR activation and result in continued tumor cell survival. For example, constitutive activation of STAT3 enables tumor growth without the requirement of growth factors [33]. In vitro studies have shown that blocking of STAT3 can induce apoptosis, inhibit cell proliferation and suppress angiogenesis [34]. However, STAT3 inhibition will also affect normal tissues and so far few translational studies have been reported.

For the clinic, PET scanning is preferred over SPECT scanning because of the better resolution

[35]. Cetuximab has been labeled with Zirconium-89 (^{89}Zr). The relatively long half-life of the radionuclide (78.4 hour) matches with the pharmacokinetics of the IgG, and ^{89}Zr -cetuximab is considered a potential useful tracer for the clinic [15,16,36]. Cetuximab- F(ab')_2 labeled with the PET radionuclide copper-64 (^{64}Cu β^+ 656 keV, half-life = 12.7 hour), is in good concordance with the half-life of the radionuclide and the kinetics of the F(ab')_2 fragments in vivo. In HNSCC models UT-SCC-8 and UT-SCC-45, ^{64}Cu -cetuximab- F(ab')_2 visualized the heterogeneously expressed EGFR and showed tumor-specific uptake, potentially making it a more effective tracer for monitoring EGFR-inhibition therapy than ^{89}Zr -cetuximab. It should be noted that anti-EGFR antibodies like cetuximab do not cross-react with murine EGFR. Therefore, the interaction of anti-EGFR antibody-based tracers with EGFR expressed in normal tissues like the liver and skin cannot be studied in HNSCC xenograft models [37].

Monitoring of HNSCC treatment

The effect of radiotherapy on EGFR expression in two HNSCC xenograft models was visualized by ^{111}In -cetuximab- F(ab')_2 SPECT. Radioresistant tumors had a higher tracer uptake after irradiation, probably due to upregulation of membranous EGFR, thereby increasing target availability possibly as a compensation mechanism [38]. Tumors with increased EGFR availability were also more responsive to the EGFR inhibitor cetuximab. Recently, similar findings were reported as a single dose of irradiation significantly increased cell-surface expression of EGFR in HNSCC [39]. The hypothesis that an increase in membranous EGFR would result in an increase in cetuximab targeting, and thus inhibit downstream cell survival signaling, should be further investigated clinically. We investigated whether tumor perfusion and vessel density could have affected the radiation-induced (increase in) tumor uptake of ^{111}In -cetuximab- F(ab')_2 [38]. Tumor perfusion and vessel density were evaluated and differed between the HNSCC models. In one of the models it appeared that there was an increase in vessel density and tumor perfusion after irradiation. This could result in an increase of tracer uptake in the tumor, also mediated by the enhanced permeability and retention (EPR) effect. However, autoradiography correlated strongly with EGFR distribution as determined immunohistochemically indicating that the tracer reaches the EGFR irrespective of the (change in) tumor perfusion.

Further investigation of EGFR-inhibition treatment monitoring in addition to radiotherapy is clinically relevant. We investigated ^{111}In -cetuximab-

F(ab')₂ tumor uptake in HNSCC xenografts treated with radiotherapy and/or cetuximab. This enabled us to visualize changes in EGFR-expression in response to EGFR inhibition with cetuximab and EGFR inhibition in combination with radiotherapy [40]. Surprisingly, in both HNSCC models, an increase of tracer uptake in the tumor correlated to non-response [40]. However, the mechanism is yet unclear as higher ¹¹¹In-cetuximab-F(ab')₂ uptake was not in all cases accompanied by an increase in membranous EGFR expression as determined after immunohistochemical staining. However, findings from immunohistochemical staining have some limits, as it is a static evaluation, inconsiderate of cellular dynamics and tumor heterogeneity. This restricts the value of EGFR staining from single biopsies and supports longitudinal EGFR monitoring with EGFR-targeting tracers.

Future prospects

A phase II clinical trial aimed to investigate the predictive value of ⁸⁹Zr-cetuximab in determining treatment outcome of chemoradiation with cetuximab in HNSCC patients, but has been discontinued due to logistic complications [41]. After the positive results of combining cetuximab with radiotherapy in HNSCC the combination of radiotherapy with panitumumab, a human monoclonal antibody that targets EGFR, showed no benefit [5,42]. More studies are needed to elucidate the role of anti-EGFR-based tracers in the management of HNSCC. Other EGFR-targeting antibodies could also have a role in predicting or monitoring HNSCC treatment. For example, panitumumab, a human anti-EGFR monoclonal antibody that does not evoke an immunological response, is approved for the treatment of patients with EGFR-expressing metastatic colorectal cancer with disease progression after chemotherapy [43,44]. To assess which receptors might be dominant in survival signaling, it would be interesting to compare tracers targeting different ErbB receptors in relation to treatment response monitoring. For example, as cetuximab only targets ErbB1, it could be combined with a radiolabeled tracer targeting the HER2, as like the EGFR, HER2 is known to lead to activation of the P13-K/AKT pathway [45].

Conclusion

Methods to facilitate personalized therapy are widely investigated and shape the future of cancer treatment as new biomarkers are identified on genetic, molecular and metabolic levels. Translational research forms

the bridge between preclinical development and clinical application, although this is not always straightforward. Many imaging biomarkers have been characterized, but only a few have reached clinical implementation, urging for a greater continuity between concept development and applicability. Future studies should exploit the benefits of radionuclide imaging in finding predictive and monitoring agents for drug efficacy and treatment regimens in order to progress to improved personalized healthcare.

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