Novel Carcinoembryonic-Antigen-(CEA)-Specific Pretargeting System to Assess Tumor Cell Viability after Irradiation of Colorectal Cancer Cells

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Purpose: To date, no valid imaging modality exists for early response prediction to neoadjuvant radiochemotherapy in carcinoembryonic-antigen-(CEA)-expressing rectal cancers (UICC stages II and III). It is hypothesized that the uptake of an anti-CEA antibody is directly related to the number of viable tumor cells and may be quantified by immuno-positron emission tomography (immuno-PET). Therefore, we evaluated a novel pretargeting system using TF2, a humanized bispecific trivalent monoclonal antibody (mAb), directed against CEA and the IMP-288-peptide, a hapten for binding radiometals for imaging. Uptake and kinetics of the pretargeting system were investigated in vitro prior to and after irradiation.

Methods: TF2 was labeled with 131I and IMP-288 with 111InCl3. The colorectal cancer cell lines HT29, SW480, and T84 with known varying CEA expression were incubated (≤ 72 hours) with 131I-TF2 or the TF2-111In-IMP-288 pretargeting system. Parallel cultures were irradiated with 2–10 Gy high-energy photons. Tracer uptake, proliferation, apoptosis, and CEA-RNA expression of cancer cells were investigated.

Results: The uptake of tracers was dependent on CEA expression and cell count of the cell lines (uptake/10⁶ cells: 0.3% in HT29, 1.5% in SW480, and 14% in T84, p < 0.001). The TF2-111In-IMP-288 pretargeting system showed a higher uptake after 4 and 72 hours compared to 131I-TF2 in parallel cultures. Only in one cell line (SW480) an increased apoptosis after irradiation could be detected. Irradiation increased dose dependently both the specific uptake of 131I-TF2 and of the TF2-111In-IMP-288 system (4-fold in HT29 and T84 after 10 Gy (72 hours), p < 0.001). These results were CEA-mRNA independent.

Conclusions: This novel pretargeting system allows the quantitative analysis of CEA-expressing colorectal cancer cells and represents a promising tool for evaluation of tumor cell viability after irradiation.

Key words: Bispecific antibody · Colorectal cancer · Carcinoembryonic antigen · Irradiation · Pretargeting

Neues Carcinoembryonales-Antigen-(CEA-)spezifisches Pretargeting-System zur Bestimmung der Tumorzellviabilität nach Bestrahlung kolorektaler Karzinomzellen


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Introduction

Based on several trials of the German Rectal Cancer Study Group, preoperative 5-fluorouracil (5-FU)-based radiochemotherapy (RCT) is recommended for treatment of UICC stage II and III rectal cancer in Germany [32–34, 38, 42, 43]. However, individual tumor response to RCT is heterogeneous, ranging from complete regression to total resistance [35]. From the clinical point of view, early detection of individual tumor response is mandatory for risk-adapted multimodal treatment in future trials [22].

Usually response to neoadjuvant RCT is evaluated by contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI), and endorectal ultrasound [4, 11, 16, 18, 23, 36]. These methods are not able to distinguish tumor from necrotic and/or fibrotic tissue. Furthermore, the extent of RCT-induced cancer death combined with shrinkage of the initial tumor infiltration depth can not be determined [1]. Other imaging methods like 18FDG ([18F]fluoro-2-deoxy-D-glucose) positron emission tomography (FDG-PET/CT) seem to be useful in management of colorectal cancer [3, 28, 29]. But in neoadjuvantly treated rectal cancer patients, the nonspecific glucose uptake caused by the tumor microenvironment itself or by RCT-induced tumor cell damage followed by inflammatory processes are serious limitations for the use of FDG-PET/CT in response prediction [10, 14]. In this dilemma, immuno-PET offers an innovative imaging approach using direct targeting of biomarkers (e.g., carcinoembryonic antigen [CEA]; CEACAM5 as surrogate markers for tumor cell viability) before, during, and after multimodal treatment [9, 17, 19, 20, 27, 41, 45].

CEA is expressed on the tumor cell surface of more than 90% of colorectal cancers and has been a useful marker for cancer detection in molecular imaging for nearly 35 years [13]. The tumor-to-background ratio (T/BG-R) of directly radiolabeled anti-CEA monoclonal antibodies (mAb) is poor and most crucial for scintigraphic detection of cancer. In order to improve the T/BG-R, several CEA-directed pretargeting systems using bispecific mAb and a radiolabeled hapten-peptide have been described [8, 19, 40]. In these systems, the nonlabeled mAb is separated from the radioisotope, thereby, allowing time for the mAb to localize its target and clear from the blood. The isotope is attached to a small compound with rapid renal clearance from blood and tissues.

TF2, a novel humanized, recombinant bs-mAb, assembles two Fab fragments from a humanized anti-CEACAM5 mAb (hMN-14, labetuzumab) and a Fab fragment of an anti-HSG (histamine–succinyl–glycine) antibody into a unique tri-Fab structure [37]. This bs-mAb has been paired with the hapten-peptide IMP-288 that contains two copies of the hapten histamine–succinyl–glycine (HSG) to enhance local retention [24]. This peptide has been radiolabeled with several radiometals for imaging and therapy. In preclinical models T/BG-R of more than 40-fold compared to directly labeled IgG molecules have been described [15, 37].

Within the interdisciplinary research unit KFO179 (subject: “Biological basis of individual tumor response in patients with rectal cancer”), sponsored by the German Research Foundation, we are preparing to use a TF2 pretargeting system with 68Ga-labeled IMP-288 for immuno-PET/CT imaging in patients with UICC stage II/III rectal cancer before, during, and after preoperative RCT. In this clinical study, the efficacy of immuno-PET/CT imaging for response prediction to RCT will be determined. As a first step of this challenge using a novel pretargeting system, we hypothesize that CEA expression is a quantitative biomarker for early detection of viable tumor cells. Thus, in vitro studies using the colorectal cancer cell lines HT29, SW480, and T84 with known varying CEA expression were performed to assess the following:

- What are the kinetics of TF2 and IMP-288 binding to colorectal cancer cell lines?
- Is the uptake of the bs-mAb and IMP-288 related to CEA expression?
- Does irradiation influence bs-mAb uptake?
- Are changes in uptake associated with apoptosis or with changes in CEA-RNA expression?

Materials and Methods

Radiolabeling of TF2 and IMP-288

Radiolabeling of 0.5 mg TF2 (10 mg/ml PBS, IBC Pharmaceuticals, Inc., Morris Plains, NJ, USA) with 50 MBq sodium