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

Strahlenther Onkol 2011;187:120–6
DOI 10.1007/s00066-010-2191-5

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

Hintergrund: Derzeit gibt es beim CEA-exprimierenden Rektumkarzinom (der UICC-Stadien II und III) keine valide Diagnostik zur frühzeitigen Response-Beurteilung auf eine neoadjuvante Radiochemotherapie. Allerdings wird vermutet, daß mit einer Immuno-Positronen-Emissions-Tomographie (Immuno-PET) und einem anti-CEA-Antikörper die Anzahl viabler Tumorzellen nach erfolgter Radiatio quantifizierbar ist. Demzufolge wurde von unserer Arbeitsgruppe zur Etablierung eines Immuno-PETs ein neues Pretargeting-System evaluiert. Dieses Pretargeting-System besteht aus dem humanisierten bispezifischen trivalenten monoklonalen Antikörper (TF2), der an CEA sowie an das radioaktiv markierte Hapten-Peptid IMP-288 bindet. Wir untersuchten in vitro die Bindung und Kinetik dieses Pretargeting-Systems vor und nach Radiatio.

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Received: June 28, 2010; accepted: November 11, 2010
Published Online: January 24, 2011

Methoden: TF2 wurde mit ^{131}I und IMP-288 mit $^{111}\text{InCl}_3$ markiert. Die Inkubation (≤ 72 h) der kolorektalen Tumorzelllinien HT29, SW480 und T84 mit unterschiedlicher CEA-Expression erfolgte mit ^{131}I -TF2 und dem TF2- ^{111}In -IMP-288-Pretargeting-System. Parallelkulturen wurden mit 2–10 Gy hochenergetischer Photonen bestrahlt. Neben der Bindung der markierten Substanzen wurden die Proliferation, Apoptose und CEA-RNA-Expression der Tumorzellen untersucht.

Ergebnisse: Der Tracer-Uptake korrelierte sowohl mit der CEA-Expression als auch der Zellzahl der jeweiligen Zelllinie (Uptake/ 10^6 Zellen: 0,3% in HT29, 1,5% in SW480 und 14% in T84; $p < 0,001$). Das Pretargeting-System wurde nach 4 h und 72 h stärker gebunden als ^{131}I -TF2 in Parallelkulturen. Lediglich in einer Zelllinie (SW480) konnte eine erhöhte Apoptoserate nach Bestrahlung nachgewiesen werden. Die Radioaktivität erhöhte dosisabhängig die spezifische Aufnahme des Pretargeting-Systems (4fach in HT29 und T84 nach 10 Gy (72 h), $p < 0,001$). Diese Ergebnisse waren CEA-mRNA-unabhängig.

Schlussfolgerungen: Das neue Pretargeting-System erlaubt eine quantitative Analyse der CEA-Expression kolorektaler Tumorzellen und ist eine vielversprechende Methode zur Evaluation der Tumorzellviabilität nach Radioaktivität.

Schlüsselwörter: Bispezifischer Antikörper · Kolorektale Karzinome · Carcinoembryonales Antigen · Radioaktivität · Pretargeting

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 ^{18}F FDG (^{18}F 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 ^{68}Ga -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

iodide was performed as previously described [30]. The radiometallation of 5 µg of IMP-288 (DOTA-D-Tyr-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂), provided by Immunomedics, Inc. (Morris Plains, NJ, USA) with 37 MBq ¹¹¹InCl₃ (Tyco Healthcare, NL) was performed in 1 ml of 0.5 M acetate buffer (pH 5). Reaction mixture was heated for 20 minutes at

93 °C. Residual ¹¹¹InCl₃ was removed by a C₁₈ cartridge (C₁₈ Sep-Pak Light Waters, Ireland).

Cell Cultures

All human cell lines used were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and subcultured in 25 cm² culture flasks and 7 ml cell culture medium according to their recommendations. The cell line SW480 is known to produce 0.7 ng CEA/ml/10⁶ cells in 10 days, T84 to produce 600 ng CEA/ml/10⁶ cells in 10 days (ATCC), and HT29 was reported to produce 2.5 ng CEA/ml/10⁶ cells in 24 hours [6].

Table 1. Primers: Carcinoembryonic antigen (CEA) and reference genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH), hypoxanthine phosphoribosyltransferase 1 (HPRT1), ubiquitin C (UBC).

Table 1. Primer: Carcinoembrionales Antigen (CEA) und Referenzgene Glycerinaldehyd-3-phosphat-Dehydrogenase (GAPDH), Hypoxanthin-Phosphoribosyl-Transferase 1 (HPRT1), Ubiquitin C (UBC).

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Source
CEA	CCCAGACTCGTCTTACCTTTCG	TTGGCGATAAAGAGAAGTGTGT	^a
GAPDH	CCCTTCATTGACCTCACTACAT	ACGATACCAAAGTTGTCATGGAT	[2]
HPRT1	TGACACTGGCAAAACAATGCA	GGTCTTTTACCAGCAAGCT	[2]
UBC	CGGTGAACGCCGATGATTAT	ATCTGCATTGTCAAGTGACGA	[5]

The indicated primer sequences were taken from the following sources: ^a PrimerBank (<http://pga.mgh.harvard.edu/primerbank/index.html>), [2], [5].

Figure 1. Mean uptake/10⁶ cells of ¹³¹I-TF2 (TF2) and the TF2-¹¹¹In-IMP-288 pretargeting system (TF2-IMP288). The individual uptake of ¹³¹I-TF2 and TF2-¹¹¹In-IMP-288 depended significantly on the individual CEA expression (in SW480: 0.7 ng CEA/10⁶ cells/10 days, in HT29: 25 ng CEA/10⁶ cells/10 days, in T84 600 ng CEA/10⁶ cells/10 days, [n = 9 each, *p < 0.001]). After 4 hours and after 72 hours, the uptake of the pretargeted ¹¹¹In-IMP-288 was significantly higher than the uptake of the directly labeled antibody ¹³¹I-TF2 (**p < 0.05). Error bars = standard deviation (SD).

Abbildung 1. Mittlerer Uptake/10⁶ Zellen von ¹³¹I-TF2 (TF2) und des ¹¹¹In-IMP-288-TF2-Pretargeting-Systems (TF2-IMP288). Die Bindung von ¹³¹I-TF2 und TF2-¹¹¹In-IMP-288 war signifikant von der jeweiligen CEA-Expression abhängig (bei SW480: 0,7 ng CEA/10⁶ Zellen/10 d; bei HT29: 25 ng CEA/10⁶ Zellen/10 d; bei T84 600 ng CEA/10⁶ Zellen/10 d; [jeweils n = 9, *p < 0.001]). Nach 4 h und nach 72 h war die Aufnahme TF2-¹¹¹In-IMP-288-Pretargeting-Systems signifikant höher als die des direkt markierten Antikörpers ¹³¹I-TF2 (**p < 0,05). Fehlerbalken = Standardabweichung (SD).

Uptake Measurements and Cell Count

To assess the capacity of cells for the labeled compounds, 1 µg/ml medium of ¹³¹I-TF2 or 20 ng/ml medium ¹¹¹In-IMP-288 was simultaneously applied to three parallel cell cultures for each interval (4, 24, 48, and 72 hours, respectively) to achieve a final activity of ¹³¹I-TF2 at t₀ = 100 kBq/ml medium or ¹¹¹In-IMP-288 at t₀ = 70 kBq/ml medium. Before measuring tracer uptake, culture medium was discarded, and the cells were washed twice with phosphate buffered saline (PBS) buffer pH 7.4 [30].

Irradiation and Apoptosis

Apoptosis was determined by a Caspase-Glo™ 3/7 Assay Systems (Promega) in an ElisaReader (GENIOS PLUS, Tecan Deutschland GmbH, Crailsheim, Germany). Cell cultures were irradiated 24 hours and 48 hours before measurement with single fractions of 2 Gy and 4 Gy. Sham irradiated cultures were incubated with 200 µl PBS, 100 kBq/ml ¹³¹I-TF2 or 1 µg/ml TF2 and 70 kBq/ml ¹¹¹In-IMP-288. Two hours before measurement, cells were released by actase/EDTA and equal cell counts were seeded into a 96-well plate. The luminescence referenced against untreated cells was determined at 30 minutes, 2 hours, 4 hours, and 24 hours after assay start (n = 6).

RNA Analysis

Total RNA of 10⁶ cells per sample (T84 and SW480, 0–10 Gy) was iso-

lated (RNeasy mini kit, Qiagen, Hilden, Germany). The amount of RNA was quantified spectrophotometrically. Reverse transcription to complementary DNA was effected

for 1 hour with 1 µg total RNA using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and 20 units per sample recombinant RNase inhibitor (USB, Cleveland,

OH, USA). Transcript numbers of target and reference genes were quantified relatively by real-time PCR using HotStart-IT SYBR Green qPCR-Master Mix (USB, Cleveland, OH, USA) in a real-time PCR machine (HT7900, Applied Biosystems, Foster City, CA, USA). PCR conditions were 45 cycles with annealing at 60 °C for 20 s and elongation at 72 °C for 40 s for each primer pair. Specific primer pairs were synthesized by MWG-Biotech AG (Ebersberg, Germany). Efficacy of primers was tested by serial dilution (factor 625) and specificity by melting curve and agarose gel electrophoresis. Data of CEA were normalized to weighted mean expression of GAPDH, HPRT1, and UBC serving as reference genes. Primer sequences are given in Table 1.

Statistical Analysis

Statistical analyses (Statistical Analysis Software: SPSS 15.0 for Windows) were performed using nonparametric tests (Wilcoxon, Mann-Whitney). Medians (M), means (m), and standard deviations (SD) were calculated. Differences were considered significant at $p < 0.05$. Figures display the means and standard deviations as well as the statistical results of particular experiments.

Specific Tracer Uptake and Influence on Proliferation

Cell cultures received either 1 µg/ml ^{131}I -TF2 (100 kBq/ml) or 1 µg/ml ^{131}I -TF2 and 50 µg/ml cold antibody. Specific uptake was expressed as the percent uptake by subtracting ^{131}I -TF2 uptake in the presence of excess cold TF2. Antibody uptake was determined through 72 hours ($n = 9$ intervals each). To determine the influence of ^{131}I -TF2 and TF2 on proliferation, control cultures were supplemented either with 200 µl phosphate buffered saline (PBS) or with excess (50 µg/ml) cold antibody.

Cell cultures received 20 ng/ml ^{111}In -IMP-288 (70 kBq/ml) or 20 ng/ml

Figure 2. Mean uptake/ 10^6 cells of HT29 and T84 cells irradiated with 0–10 Gy high energy photons. The uptake of ^{131}I -TF2 increased significantly after irradiation ($n = 9$, $p < 0.05$). The increase was significantly dose dependent in both cell lines 48 hours and 72 hours after irradiation ($n = 9$, $p < 0.05$). Error bars = standard deviation (SD).

Abbildung 2. Mittlerer Uptake/ 10^6 Zellen von HT29- und T84-Zellen, die mit 0–10 Gy hochenergetischer Photonen bestrahlt wurden. Die Bindung von ^{131}I -TF2 stieg nach Bestrahlung signifikant an ($n = 9$, $p < 0.05$). 48 h und 72 h nach Bestrahlung war diese Zunahme in beiden Zelllinien signifikant dosisabhängig ($n = 9$, $p < 0.05$). Fehlerbalken = Standardabweichung.

^{111}In -IMP-288 and 200 ng/ml cold IMP-288. Parallel cultures were preincubated for 24 hours with 1 $\mu\text{g}/\text{ml}$ cold TF2 prior to ^{111}In -IMP-288 application ($n = 9$ each interval). To determine the influence of IMP-288 on proliferation, the medium of control cultures was supplemented with 200 μl PBS or with 20 ng/ml cold IMP-288. Uptake experiments after irradiation were performed as previously described [30].

Results

Radiolabeling yield was $>90\%$. The radiochemical purity of ^{131}I -TF2 decreased slowly from $97.8 (\text{m}) \pm 1.2\%$ shortly after labeling to $93.6\% \pm 1.8\%$ after 72 hours. The radiochemical purity of ^{111}In -IMP-288 decreased from $95.2\% \pm 0.2\%$ shortly after labeling to $92.1\% \pm 1.2\%$ after 72 hours, independent of temperature and solvent.

Table 2. Percentage of CEA expression of T84 cells standardized to the expression sham-irradiated controls at the given times. CEA expression levels were also normalized to the expression of housekeeping genes (GAPDH, HPRT1, and UBC).

Tabelle 2. Prozentsatz der CEA-Expression von T84-Zellen bezogen auf die Expression scheinbestrahlter Kontrollen zu demselben Zeitpunkt. Die Werte der CEA-Expression wurden zusätzlich auf die Expression der Housekeeping-Gene (GAPDH, HPRT1, UBC) bezogen.

	24 hours	48 hours	72 hours
2 Gy	68	75	100
4 Gy	95	112	96
10 Gy	70	140	172

^{131}I -TF2 and ^{111}In -IMP-288 Uptake

At each time, the uptake of ^{131}I -TF2 was specific and significant ($p < 0.01$) in T84 and HT29 cells. The uptake maximum of ^{131}I -TF2 in T84 cells ($14 \pm 4.3\%$) was observed after 24 hours or after 48 hours in HT29 cells ($1.5\% \pm 0.39\%$). After 72 hours the uptake of ^{131}I -TF2 decreased significantly ($p < 0.05$) in both cell lines. Uptake of ^{131}I -TF2 was appreciably lower in SW480 cells, with an average specific uptake maximum of 0.3% ($p < 0.05$).

At all times uptake of the ^{111}In -labeled peptide without preincubation with TF2 was $<0.1\%$ in all cell lines. Incubation with 10-fold excess of cold IMP-288 had no influence on the binding of ^{111}In -IMP-288.

The kinetics of the ^{131}I -TF2 and TF2- ^{111}In -IMP-288, respectively, pretargeting systems are shown in Figure 1. Specific binding for pretargeted ^{111}In -IMP-288 was found in all cell lines at all time points ($p < 0.05$). Specific binding of ^{111}In -IMP-288 was detected in the first interval examined (4 hours) and was significantly higher at this interval than that measured with ^{131}I -TF2 ($p < 0.05$). Uptake of the pretargeted ^{111}In -IMP-288 measured at 4 hours remained relatively fixed over 72 hours in the SW480 and HT29 cells, while it increased in the T84 cells at 24 hours, but thereafter, uptake of the ^{131}I -TF2 and TF2- ^{111}In -IMP-288 remained at a more elevated level (14%). ^{131}I -TF2 and TF2- ^{111}In -IMP-288 uptake varied significantly based on CEA expression (Figure 1). The correlation between individual cell count and TF2- ^{111}In -IMP-288 uptake was almost linear in all three cell lines (not shown). RNA analyses confirmed the reported ratio of CEA expression between

T84 and SW480. CEA expression in T84 cells reached the level of housekeeping genes (CEA in T84: 23–24 cycles, CEA in SW480: 34 cycles). Cell proliferation and viability was not affected by unlabeled or radiolabeled TF2 or IMP-288.

Irradiation

Compared to sham-irradiated cells the specific uptake of TF2- ^{111}In -IMP-288 in SW480 cells increased slightly after irradiation. This up to 1.5-fold enhancement was significant at 48 and 72 hours after irradiation with 10 Gy ($p < 0.05$). In RNA analysis no increased CEA expression was detectable.

Figure 2 depicts the course of ^{131}I -TF2 uptake in HT29 and T84 cells. After irradiation with 10 Gy, the ^{131}I -TF2 uptake increased during the first 24 hours up to $>200\%$ when compared to sham-irradiated cells. At all time points of assessment, antibody uptake was significantly increased in both cell lines after irradiation. The enhanced uptake

Figure 3. Mean apoptosis levels of irradiated cells standardized to those of sham-irradiated cells 24 hours and 48 hours after irradiation. Significantly increased apoptosis was detected in SW480 cells 48 hours after irradiation with 4 Gy ($n = 6$, $*p < 0.05$). Error bars = standard deviation (SD).

Abbildung 3. Mittlere Apoptoserate von bestrahlten Zellkulturen im Verhältnis zu der scheinbestrahlten Zellen 24 und 48 h nach Bestrahlung. Eine signifikante Zunahme der Apoptose wurde bei SW480-Zellen 48 h nach Bestrahlung mit 4 Gy nachgewiesen ($n = 6$, $*p < 0,05$). Fehlerbalken = Standardabweichung.

of ^{131}I -TF2 could be blocked by preincubation with 50-fold "cold" TF2. At least, a higher ^{131}I -TF2 uptake was significantly associated with the irradiation dosage in both cell lines.

The enhanced uptake of the pretargeting system was only partially associated with a higher percentage of CEA expression at the RNA level. In T84 cells, there were slightly elevated CEA expression levels detectable after irradiation with 10 Gy (Table 2).

Apoptosis

Only SW480 cells showed a significant increase in apoptosis ($p < 0.05$) occurring 48 hours after irradiation with 4 Gy (Figure 3). Compared to sham-irradiated cancer cells, there was no elevated apoptosis in HT29 and T84 cells after irradiation.

Discussion

In this study, we demonstrated a novel ^{131}I -TF2- ^{111}In -IMP-288 pretargeting system for semiquantitative evaluation of tumor cell viability after irradiation. In untreated cells, there was a linear correlation between individual tumor cell count and uptake detectable. In this simple and one-dimensional ^{131}I -TF2- ^{111}In -IMP-288 pretargeting system model, therapy-induced changes of CEA expression or binding affinity may not be considered.

Irradiation increased the specific uptake of the pretargeting system compared to sham-irradiated cells within the first few days in a dose dependent manner, up to 4-fold after a single dose of 10 Gy and 72 hours. Considering the mechanisms for this increase, we can exclude the contribution of CEA from nonviable cells by trypan blue exclusion. In addition, CEA from cell debris was removed by several washing steps in our protocol. An increased influx of the radiopharmaceutical into the cells after irradiation should be associated with an elevated rate of apoptotic cells. However, only in one cell line (Figure 3) was an increased rate of apoptosis detected after irradiation. A further factor might be an increased expression of CEA on the cell surface, based on an increased de novo synthesis or translocation of CEA. Our RNA analyses demonstrated a primarily posttranscriptional increase of CEA. The reasons for the increased expression of several targets for tumor imaging after irradiation are still unknown. However, it was demonstrated previously that the uptake of several pharmaceuticals was increased early after therapy [25, 30, 31]. Our findings are consistent with data describing an enhanced uptake of different radiopharmaceuticals induced by irradiation of different solid tumors [7, 12, 26, 44].

Patients in our planned clinical trial will be treated according to the CAO/AIO/ARO-04 protocol [21, 39]. Immuno-PET/CT will be performed pretherapeutically (PET/CT1) within the third week of RCT (PET/CT2) and immediately prior to resection of the rectal specimen (PET/CT3). In vitro experiments could not be projected directly to in vivo investigations. However, we suggest that CEA-mediated uptake on

PET/CT within the third week of therapy should not be dominated by an early increase of the uptake of the pretargeting system. CEA is a glycoprotein made by the cell and expressed on the cell surface. It will be released by damaged cells into the environment and will be absorbed quickly. Any decrease at this point in time should be a clear indication of tumor response. In addition metabolic downsizing of the primary tumor and metastatic spread should be quantitatively recorded within this approach.

Conclusions

Our in vitro testing showed that the novel ^{131}I -TF2- ^{111}In -IMP-288 pretargeting system specifically binds to CEA-expressing colorectal cancer cells without cytotoxic side effects. The uptake of the radioimmunoconjugate can be used for semiquantitative evaluation of tumor cell viability, especially after neoadjuvant radiochemotherapy.

Acknowledgments

This work was supported by the German Research Foundation (DFG) as a part of the clinical research group 179 (KFO179: Biological basis of individual tumor response in patients with rectal cancer). The authors thank Ms. Christa Angerstein for her technical support, and Drs. D.M. Goldenberg, Chien-Hsing Chang, William McBride, and Edmund A Rossi (Immunomedics, Inc., and IBC Pharmaceuticals, Inc., USA) for providing the antibody and peptide.

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