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RESEARCH ARTICLE

Dynamics of IGF-1R Expression During Endocrine Breast Cancer Treatment

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Abstract

Purpose: The aim was to assess changes in insulin-like growth factor 1 receptor (IGF-1R) expression with immunoSPECT/CT and to study the dynamics of IGF-1R expression of human breast tumors during endocrine treatment.

Procedures: Mice with MCF-7 xenografts were treated with estradiol or tamoxifen, and IGF-1R expression was measured by immunohistochemistry and immunoSPECT/CT using ^{111}In-R1507 (anti-IGF-1R antibody). Moreover, IGF-1R expression was analyzed immunohistochemically on 22 human breast tumors, treated preoperatively with endocrine therapy.

Results: Estradiol resulted in an increased expression of IGF-1R, as measured by immunohistochemistry and immunoSPECT/CT. In contrast, tamoxifen resulted in a downregulation of IGF-1R, whereas this could not be measured with immunoSPECT/CT. A downregulation was also detectable in 9 out of 22 (41 %) human breast tumors after endocrine therapy.

Conclusions: Anti-estrogen treatment can cause a reduction in membranous IGF-1R expression. Based on these results, a combination of anti-IGF-1R antibodies with anti-estrogen therapy might not be a rational treatment strategy.

Key Words: IGF-1R, ER, Estradiol, Tamoxifen, SPECT, Breast cancer

Introduction

The insulin-like growth factor 1 receptor (IGF-1R) is a target for the treatment of breast cancer. Upon binding of insulin-like growth factors (IGFs) to IGF-1R, the phosphatidylinositol-3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways can be activated, resulting in proliferation and inhibition of apoptosis [1, 2]. In breast cancer, IGF-1R expression is positively correlated with the presence of the estrogen receptor (ER). Approximately 40 to 60 % of ER-positive tumors express IGF-1R, while expression in ER-negative tumors is only 10 to 20 % [3]. In general, IGF-1R correlates with good prognostic markers; however, in ER-negative tumors, its expression is associated with a worse prognosis [4, 5].

Cross talk between IGF-1R and ER has been described in multiple studies. For example, estradiol can increase the expression of IGF-1R [6]. IGF-1R is one of the target genes

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of ER, and upon binding of estradiol to ER, transcription and translation of IGF-1R can be induced [7]. Also, estradiol can stimulate cytosolic ER, which can directly cause phosphorylation of IGF-1R, which results in activation of downstream pathways [8]. On the other hand, anti-estrogens such as tamoxifen can downregulate the expression of IGF-1R [6], and anti-estrogens can alter the expression of other molecules in the IGF pathway such as IGF-2, insulin receptor substrate 1 (IRS-1), IGF binding protein 3 (IGFBP3), or IGF-2R [9].

The effects of estrogens and anti-estrogens on IGF-1R expression can be of clinical importance, as *in vitro* studies have shown that IGF-1R expression is necessary for antitumor activity of anti-IGF-1R antibodies [10, 11]. Therefore, patient selection for IGF-1R-targeted therapy may be based on receptor expression. Thus far, evaluation of IGF-1R expression is usually performed by immunohistochemistry on archival tumor material. However, immunohistochemistry is not a very suitable technique to measure receptor expression at several time points, or in several tumor lesions, as this would require multiple invasive procedures. In addition, target accessibility of the receptor for its therapeutic agent is not taken into account. Several factors may influence whether therapeutic agents will reach the tumor cells, such as blood vessel density, vascular permeability, and intratumoral interstitial fluid pressure [12, 13]. If target accessibility is low, therapeutic agents cannot reach the tumor and may be ineffective despite adequate expression of receptors on the tumor cells as determined by immunohistochemistry. Therefore, *in vivo* imaging of IGF-1R expression using radiolabeled anti-IGF-1R antibodies may be useful to select patients who are most likely to benefit from IGF-1R-targeted therapy.

Previously, we have developed a noninvasive imaging technique using R1507, a fully human monoclonal antibody directed against human IGF-1R. After radiolabeling R1507 with ^{111}Indium (^{111}In) or with ^{89}Zirconium (^{89}Zr), we were able to visualize IGF-1R-expressing breast cancer xenografts with immunoSPECT and immunoPET, respectively [14]. This study was performed in a triple-negative breast cancer model with constant IGF-1R expression. The aim of the current study is to study the dynamics of IGF-1R expression in estrogen receptor (ER)- positive breast cancer xenografts and human tumors upon endocrine treatment, and to assess the dynamics of IGF-1R expression *in vivo* using ^{111}In-R1507 immunoSPECT/CT.

Material and Methods

Cell Culture

MCF-7 cells were cultured and maintained as a monolayer in culture flasks in RPMI 1640 (GIBCO, BRL Life Sciences Technologies, The Netherlands) supplemented with 10 % fetal calf serum (FCS), 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37 °C in a humidified atmosphere with 5 % CO_2.

Radiolabeling

The human monoclonal antibody R1507 was obtained from Roche Diagnostics (Penzberg, Germany) and is directed against an epitope on the extracellular domain of the human IGF-1R. It does not cross react with the human insulin receptor (IR) and murine IGF-1R and IR. R1507 was conjugated with isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (ITC-DTPA, Macrocyclis, Dallas, TX) and labeled with ^{111}In as described previously [14, 15]. Radiochemical yield of ^{111}In-DTPA-R1507 (^{111}In-R1507) exceeded 95 % in all experiments. The immunoreactive of ^{111}In-R1507 exceeded 90 %, as described previously [14].

In Vitro Studies

*Scatchard Analysis*MCF-7 cells were cultured to confluency in six-well plates. To determine the dissociation constant (K_d) and receptor density of IGF-1R, cells were incubated in triplicate for 4 h at 4 °C in 1.5-ml binding buffer (RPMI 1640, 0.5 % BSA) with increasing concentrations ^{111}In-R1507 (0.3–3,000 pM, specific activity 0.4 MBq/μg). Nonspecific binding was determined by incubation in the presence of 300 nM of unlabeled R1507. After incubation, cells were washed twice with PBS and were lysed in 0.1 M of NaOH, and the cell-associated activity was measured in a gamma counter (Perkin-Elmer, Boston, MA, USA).

*Regulation of IGF-1R Expression by Estradiol and Tamoxifen*MCF-7 cells were cultured for 72 h in six-well plates with increasing concentrations of 17β-estradiol (0–10 nM) or tamoxifen (0–10 μM). Subsequently, cells were washed once with PBS and incubated for 4 h at 4 °C in a 1.5-ml binding buffer with 0.3 nM of ^{111}In-R1507 (0.4 MBq/μg). Nonspecific binding was determined by co-incubation with an excess of 300 nM of unlabeled R1507. After incubation and washing, cell-associated activity was measured in a gamma counter (Perkin-Elmer). To study the effect of ERs signaling on IGF-1R expression in time, MCF-7 cells were cultured in the presence of 17β-estradiol (10 nM) or tamoxifen (1 μM) for 4 to 96 h. Cell-associated activity was measured as described above.

Animal Studies

Animal experiments were performed in female BALB/c nude mice (non-ovariectomized) and were conducted in accordance with the principles laid out by the revised Dutch Act on Animal Experimentation (1997) and approved by the institutional Animal Welfare Committee of the Radboud University Nijmegen. At 6–8 weeks of age, experiments were started. Mice were inoculated subcutaneously with 5×10^6 MCF-7 cells (200 μl, mixed 1:1 with Matrigel, BD Biosciences, Pharmingen). Experiments were started when tumors reach a size of approximately 0.1 cm^3 (without estradiol, approximately 21 days after inoculation; with estradiol, approximately 14 days after inoculation).

*Modulation of IGF-1R Expression of MCF-7 Xenografts by Estradiol*Ten out of 20 BALB/c nude mice with MCF-7 tumors were implanted subcutaneously with a slow-release estradiol pellet (0.18 mg, 60 days, Innovative Research of America, Sarasota, FL) under general anesthesia (isoflurane/O_2). The other ten mice (control group) underwent a sham operation. At the same day of pellet implantation, five control mice and five mice with estradiol pellets received an intravenous injection in the tail vein of 30 MBq of ^{111}In-R1507 (specific activity 10 MBq/μg). Three days after implantation of the estradiol pellets, the remaining ten mice received 30 MBq of ^{111}In-R1507 intravenously. Animals were euthanized and SPECT/CT images were acquired with the U-SPECT-II (MILabs, Utrecht, The Netherlands), 3 days after injection of ^{111}In-R1507. [16]. Mice were scanned for 40 min using the 1.0-mm diameter pinhole rat collimator tube, followed by a CT scan (spatial resolution 160 μm, 65 kV, 612 μA) for anatomical reference. Scans were reconstructed with the MILabs reconstruction software, using an ordered-subset expectation maximization algorithm, with a voxel size of 0.375 mm. Representative cross sections located approximately in the center of the tumor were displayed. After scanning, the biodistribution of the radiolabel was determined *ex vivo*. Tumor, blood, muscle, lung, heart, spleen, pancreas, intestine, kidney, and liver were dissected and weighed. Activity was measured in a gamma counter. To calculate the uptake of radiolabeled antibodies in each sample as a fraction of the injected dose, aliquots of the injected dose were counted simultaneously. The results were expressed as percentage injected dose per gram (%ID/g).

*Modulation of IGF-1R Expression by Tamoxifen*Four groups of five mice bearing MCF-7 xenografts (with a subcutaneous estradiol pellet from the day of tumor cell inoculation) were treated for 5 days intraperitoneally with tamoxifen (6, 19, and 50 mg/kg body weight in corn oil) or vehicle only. After 2 days of tamoxifen treatment, mice were injected intravenously with 0.2 MBq of ^{111}In-R1507 (specific activity 0.4 MBq/μg). Three days later, mice were euthanized, and uptake of the radiolabel in necropsied tissues was determined. In a separate study, four groups of five mice were treated for 10 days with 19 mg/kg of tamoxifen or vehicle only. At day 7 of treatment, mice were injected with 0.2 MBq of ^{111}In-R1507 (specific activity 0.4 MBq/μg). Two groups were co-injected with an excess of unlabeled R1507 (300 μg) to study the nonspecific tumor uptake.

For immunoSPECT/CT studies, two groups of five mice with MCF-7 xenografts were treated with tamoxifen (19 mg/kg body weight or vehicle only). At day 7 of treatment, mice were injected intravenously with 22 MBq of ^{111}In-R1507 (specific activity 9.6 MBq/μg), and 3 days later, SPECT/CT images were acquired as described previously. In addition, the biodistribution of the radiolabeled antibody was determined *ex vivo*.

Immunohistochemistry of MCF-7 Tumors

IGF-1R and CD34 expression in MCF-7 xenografts was analyzed by immunohistochemistry. Tumors were fixed in 4 % formalin and embedded in paraffin. Antigen retrieval was performed in 10 mM of sodium citrate, pH 6.0 for 10 min at 99 °C. Endogenous peroxidase activity was blocked with 3 % of H_2O_2 in PBS (10 min at RT), and nonspecific binding was blocked by incubation with 20 % of normal goat serum (30 min at RT). Subsequently, tumor sections were incubated with rabbit-anti-IGF-1R (3027, Cell Signaling, Danvers, MA, overnight at 4 °C) or rabbit-anti-CD34 primary antibody (ab8458, Abcam, Cambridge, UK, 1 h at RT), followed by incubation with a goat-anti-rabbit-biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) for 30 min at RT. Finally, avidin-biotin enzyme complex (Vector Laboratories, Burlingame, CA) was applied for 30 min at RT, and 3,3′-diaminobenzidine (DAB) was used to develop the tumor sections.

IGF-1R expression was scored as negative (0), incomplete weak (1+), complete weak to moderate (2+), and strong (3+) membrane staining (Table 1). The mean vascular density (MVD) was scored as the number of vessels counted in three hot spot areas that contained the maximum number of vessels.

Analysis of IGF-1R Expression of Human Breast Tumors Treated with Endocrine Therapy

Biopsies and surgical material were obtained from patients that were included in the AFTER study (ClinicalTrials.gov Identifier NCT00738777). In short, the AFTER study is a preoperative window study to determine the molecular and biological changes in breast cancer after short-term endocrine therapy exposure. Patients with proven estrogen receptor-positive invasive adenocarcinoma (size ≥1 cm) and a WHO performance score of 0 or 1 were eligible to enter the study. After informed consent, patients were randomized to receive preoperative treatment for 2–6 weeks with tamoxifen (loading dose of 40 mg, two times a day, orally during 7 days, followed by 20 mg, daily, orally), anastrozole (1 mg, daily, orally), or fulvestrant (500 mg intramuscularly at day 1, 15, and 29). The clinical study was approved by the regional ethical review board (CMO).

IGF-1R membrane expression before and after endocrine therapy was analyzed by immunohistochemical analysis of tumor sections obtained from the biopsy at diagnosis and surgical resection material, respectively. First, endogenous peroxidase activity was blocked, and antigen retrieval was performed as described above. Subsequently, tumor sections were incubated with anti-IGF-1R (3027, Cell Signaling, overnight at 4 °C) followed by

Table 1. Scoring of membranous IGF-1R expression

Score	Overexpression assessment	Staining pattern
0	Negative	No staining is observed or membrane staining is observed in <10 % of the tumor cells
1+	Negative	A faint/barely perceptible membrane staining is detected in >10 % of tumor cells. The cells exhibit incomplete membrane staining
2+	Weakly positive	A weak to moderate complete membrane staining is observed in >10 % of tumor cells
3+	Strongly positive	A strong complete membrane staining is observed in >10 % of tumor cells

incubation with Poly-HRP-Anti-mouse/rabbit/rat IgG (DPVO110HRP, Immunologic, Duiven, The Netherlands) for 30 min at RT. DAB was used to develop the tumor sections. IGF-1R expression was scored as described previously (Table 1). Tumors with a membrane expression of 0 or 1+ were scored as IGF-1R negative, while 2+ or 3+ tumors were IGF-1R positive. Downregulation of IGF-1R expression was defined as a change from positive to negative.

Statistical Analysis

Statistical analyses were performed using SPSS software version 16.0 (Chicago, IL) and GraphPad Prism version 4.00 (San Diego, CA) for Windows. Differences in uptake of radiolabeled antibodies were tested for significance using the nonparametric Kruskal-Wallis and Mann–Whitney *U* test. Differences in IGF-1R expression before and after endocrine therapy of patients included in the AFTER study were tested using the related-samples Wilcoxon-signed rank test. All tests were two sided, and a *p* value below 0.05 was considered significant.

Results

In Vitro

Scatchard Analysis ^{111}In-R1507 exhibited a K_d value of 0.69 nM (95 % CI 0.54–0.96 nM), and the IGF-1 receptor density of MCF-7 cells was 34,000 receptor/cell (95 % CI 29,000–46,000 receptor/cell, Supplemental Fig. 1).

Modulation of IGF-1R expression by Estradiol and Tamoxifen In Vitro IGF-1R expression of MCF-7 cells increased in a dose-dependent manner after incubation with estradiol (Fig. 1a, b). Cells treated for 72 h with 1 nM of estradiol showed IGF-1R expression which was twice as high as untreated cells. IGF-1R expression started to increase between 24 and 48 h of incubation. In contrast, IGF-1R expression of tamoxifen-treated cells was twice as low as the expression of untreated cells. A decrease in expression could be measured as early as 16 h after start of treatment (Fig. 1c, d).

In Vivo

Modulation of IGF-1R Expression of MCF-7 Xenografts by Estradiol Treatment of mice with estradiol resulted in an increased expression of IGF-1R on the cell membrane of MCF-7 xenografts. In Fig. 2a, typical examples of IGF-1R expression of estradiol-treated and untreated tumors are presented. IGF-1R expression of untreated tumors was generally scored as 0 or 1+, while estradiol-treated tumors exhibited an IGF-1R expression of 2+.

Accumulation of ^{111}In-R1507 was significantly enhanced in estradiol-treated tumors. After 3 days of treatment, tumor uptake in untreated versus treated tumors was 10.9±1.5 and 14.2±1.8 %ID/g (p=0.01), respectively. Tumor to blood ratio of the tracer also increased significantly by 50 %, from 1.2±0.2 to 1.8±0.1 (p=0.01, Fig. 2). There was a trend toward faster clearance of ^{111}In-R1507 from the blood in estradiol-treated mice compared to untreated mice. The concentration of ^{111}In-R1507 in the blood of untreated mice compared to treated mice was 9.3±1.5 and 7.9±0.7 %ID/g (p=0.08) (see also Supplemental Table S1 for a complete overview of the biodistribution data). A similar effect was observed after 6 days of estradiol treatment. Tumor to blood ratio increased significantly by 83 % from 1.2±0.2 to 2.2±0.3 (p=0.01) for untreated versus estradiol-treated mice. Again, ^{111}In-R1507 cleared more rapidly from the blood in estradiol-treated mice compared to untreated mice (see Supplemental Table S1).

The results from the *ex vivo* biodistribution were confirmed by the imaging studies (Fig. 2). SPECT/CT images showed enhanced uptake of ^{111}In-R1507 in tumors of the estradiol-treated mice. Quantitative analysis of the images revealed that mean uptake of ^{111}In-R1507 in tumors treated for 3 days with estradiol was 11.0±1.9 %ID/g, compared to 9.2±1.7 %ID/g for untreated tumors (p=0.35). After 6 days of estradiol treatment, tumor uptake increased by 42 % from 6.8±1.3 %ID/g (untreated tumors) to 9.7±0.8 %ID/g (estradiol-treated tumors) (p=0.009).

To study whether targeting of ^{111}In-R1507 was affected by changes in tumor vasculature caused by estradiol treatment, immunohistochemical analysis of CD34 was performed. No differences in CD34 expression were observed. The mean vascular density was similar in estradiol-treated and untreated xenografts (Supplemental Fig. 2).

Modulation of IGF-1R Expression of MCF-7 Xenografts by Tamoxifen Tamoxifen treatment of mice with MCF-7 xenografts that were grown in the presence of an estradiol pellet resulted in a reduced membranous IGF-1R expression. Non-treated xenografts exhibited an average IGF-1R expression of 2+, while the expression in tamoxifen-treated xenografts was scored as 1+. Typical examples are presented in Fig. 3a.

While immunohistochemistry showed reduced membranous IGF-1R expression after tamoxifen treatment, the *ex vivo* biodistribution studies in mice did not show reduced targeting of ^{111}In-R1507 following tamoxifen treatment. Tumor uptake of ^{111}In-R1507 in mice treated for 5 days with vehicle only, 6, 19, and 50 mg/kg body weight of tamoxifen was 16.4±3.2, 16.1±4.1, 14.1±1.9, and 17.1±5.8 %ID/g, respectively. Also, 10 days after tamoxifen treatment, tumor uptake was similar in treated versus untreated animals. In addition, nonspecific tumor uptake was not affected by tamoxifen treatment. Tumor uptake in mice co-injected with an excess of unlabeled R1507 was 10.5±2.8 and 10.8±3.8 %ID/g, for treated and untreated mice, respectively. A complete overview of the biodistribution data can found in Supplemental Table 1.

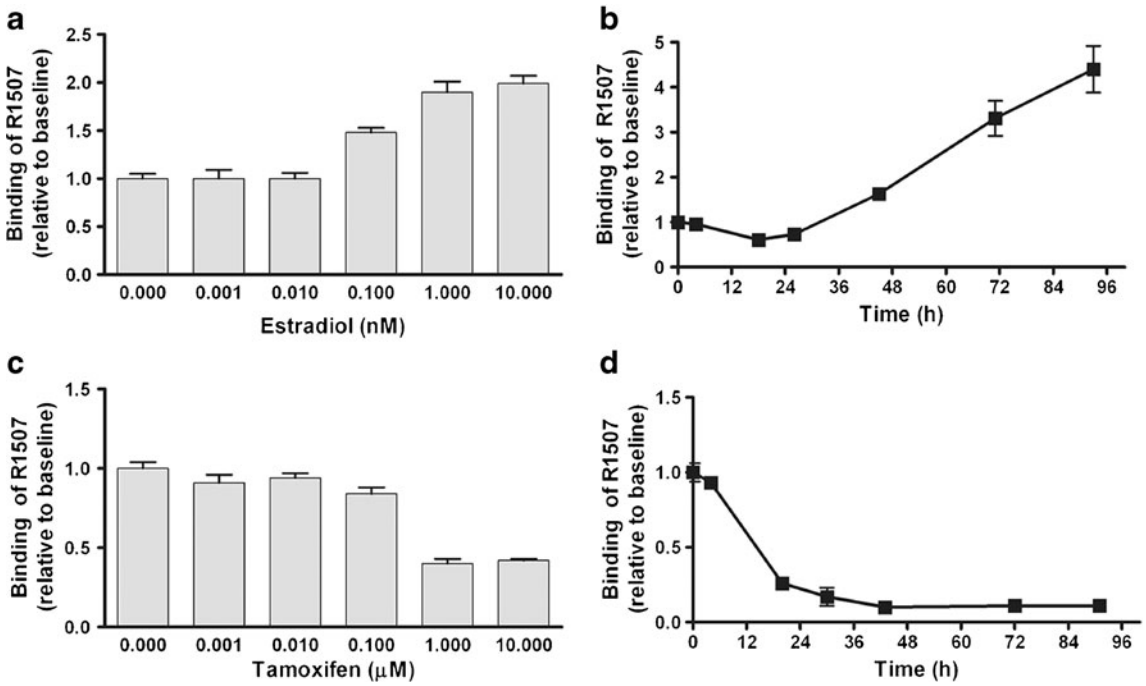

Fig. 1. The effect of estradiol and tamoxifen on IGF-1R expression of MCF-7 cells. MCF-7 cells cultured for 72 h **a** with increasing concentrations of estradiol or **b** with 10 nM of estradiol at various time points, showed enhanced binding of ^{111}In-R1507, compared with untreated cells. MCF-7 cells cultured for 72 h **c** with increasing concentrations of tamoxifen or **d** with 1 μM of tamoxifen at various time point, showed decreased binding of ^{111}In-R1507, compared with untreated cells. Binding is expressed relative to baseline, which is defined as binding of ^{111}In-R1507 to untreated cells.

ImmunoSPECT/CT in mice that were treated for 10 days with tamoxifen confirmed that treatment did not result in decreased tumor targeting of ^{111}In-R1507. SPECT/CT and biodistribution studies showed similar tumor uptake in

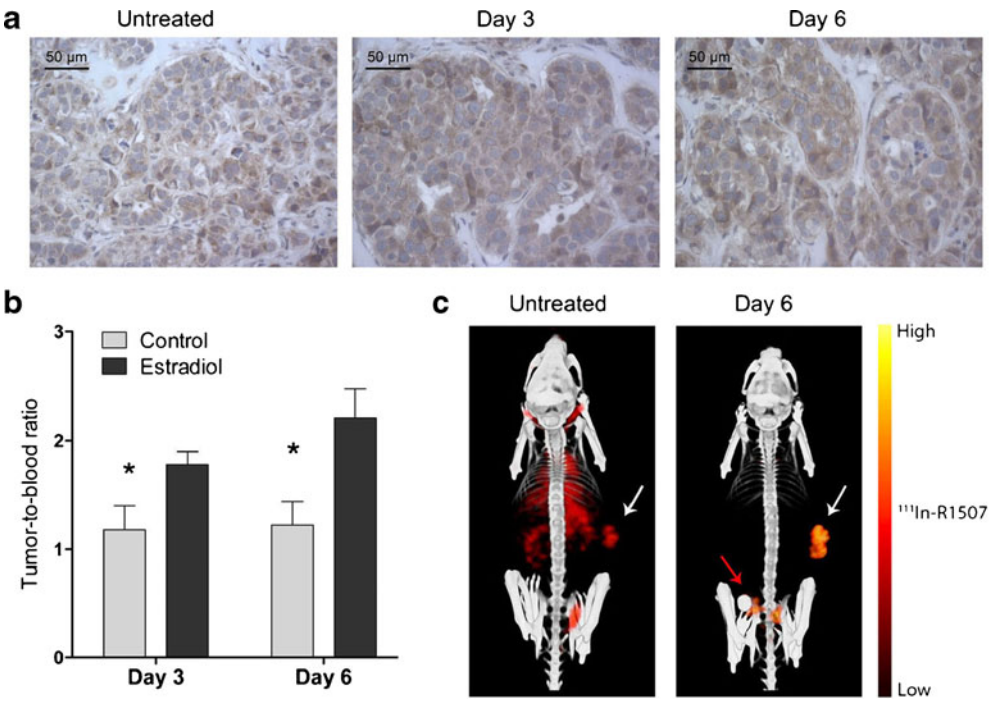

Fig. 2. The effect of estradiol on IGF-1R expression and targeting of ^{111}In-R1507 to MCF-7 xenografts. Immunohistochemical analysis of MCF-7 xenografts treated with or without an estradiol pellet showed increased membranous IGF-1R expression (magnification 40×, **a**). Targeting of ^{111}In-R1507 was significantly increased after estradiol treatment. Tumor to blood ratios (*$p <$ 0.05) are depicted in (**b**), and ImmunoSPECT/CT images of mice bearing MCF-7 xenografts (with or without estradiol pellet for 6 days) are depicted in (**c**). *White arrows* indicate the MCF-7 xenograft, *red arrows* indicate the estradiol pellet.

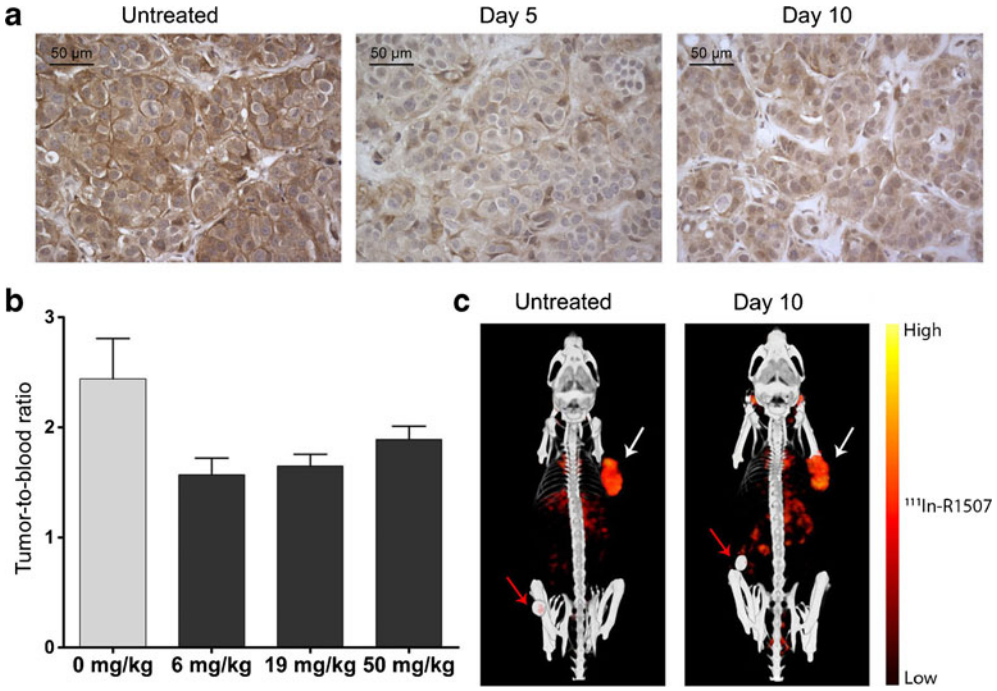

Fig. 3. The effect of tamoxifen on IGF-1R expression and targeting of ^{111}In-R1507 to MCF-7 xenografts. Immunohistochemical analysis of MCF-7 xenografts treated with or without tamoxifen showed reduced membranous IGF-1R expression (magnification 40×, **a**). Targeting of ^{111}In-R1507 was not significantly decreased after tamoxifen treatment. Tumor to blood ratios are depicted in (**b**), and ImmunoSPECT/CT images of mice bearing MCF-7 xenografts (with or without tamoxifen treatment for 10 days) are depicted in (**c**). *White arrows* indicate the MCF-7 xenograft, *red arrows* indicate the estradiol pellet.

tamoxifen-treated versus untreated animals (Fig. 3, Supplemental Table S1).

No differences were observed in the CD34 staining. The mean vascular density was similar in tamoxifen-treated and untreated xenografts (Supplemental Fig. 2).

Analysis of IGF-1R Expression of Human Breast Tumors Treated with Endocrine Therapy

Membranous IGF-1R expression was analyzed in samples from 22 patients treated preoperatively for 2 to 6 weeks with tamoxifen, fulvestrant, or anastrozole. Characteristics of the patient population are presented in Table 2. At diagnosis, 12 patients (55 %) presented with IGF-1R positive tumors. After anti-estrogen treatment, immunohistochemistry was performed on the surgical resection material. Only one patient (5 %) still had an IGF-1R positive tumor (Table 2). In total, IGF-1R expression was downregulated in nine patients (41 %) and did not change in another nine patients (41 %). No patient showed an upregulation of IGF-1R expression. For four patients (18 %), changes in expression could not be analyzed, because insufficient tumors cells were present in the tissue sections to reliably estimate IGF-1R expression. The decrease in IGF-1R expression was significant (p=0.003). In Fig. 4, typical examples of paired samples of IGF-1R expression before and after endocrine therapy are presented.

Discussion

This study showed that estradiol induced an upregulation of membranous IGF-1R expression of MCF-7 cells and xenografts, while tamoxifen treatment resulted in a downregulation of IGF-1R expression. Furthermore, we showed that downregulation of IGF-1R expression also occurred in breast cancer patients after short-course preoperative anti-estrogen treatment.

Table 2. Patient characteristics

Number of patients	22
Age diagnosis (mean±SD)	61±9 years
Menopausal status	
Premenopausal	4 (18 %)
Postmenopausal	17 (77 %)
Male	1 (5 %)
Pathology (at diagnosis)	
ER positive	22 (100 %)
PR positive	18 (82 %)
HER2 positive	1 (5 %)
Treatment	
Tamoxifen	14 (64 %)
Fulvestrant or anastrozole	8 (36 %)
IGF-1R membrane expression pre treatment	
Negative (0, 1+)	8 (36 %)
Positive (2+, 3+)	12 (55 %)
Unknown*	2 (9 %)
IGF-1R membrane expression post treatment	
Negative (0, 1+)	19 (83 %)
Positive (2+, 3+)	1 (5 %)
Unknown*	2 (9 %)

*Insufficient tumor cells in biopsy or surgical material to accurately determine IGF-1R expression

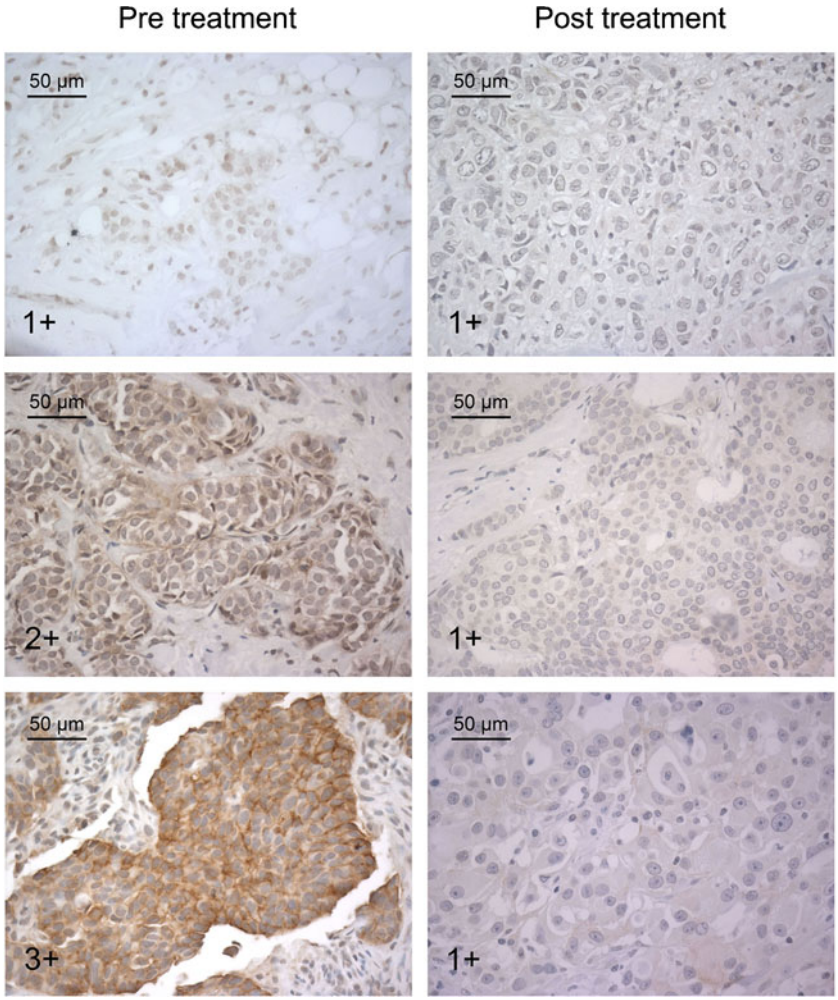

Fig. 4. IGF-1R expression of human breast tumors before and after endocrine therapy. Typical examples of downregulation of membranous IGF-1R expression of paired biopsy (pre treatment) and surgical resection material (post treatment) of patients who received anti-estrogen treatment. Scoring of IGF-1R membrane expression is depicted in the lower left corner.

Based on clear cross talk between IGF-1R and ER [6–9], it has been hypothesized that patients with ER-positive cancer may benefit from IGF-1R-targeted therapy and that the combination of IGF-1R and ER inhibitors may result in improved therapeutic efficacy. However, a clinical trial with the anti-IGF-1R antibody AMG479 failed to show clinical benefit in patients with ER-positive tumors who had progressed on previous endocrine therapy. The combination of AMG479 with exemestane or fulvestrant did not improve the progression-free survival compared to patients that received placebo and exemestane or fulvestrant [17]. Of note, preclinical studies have evaluated IGF-1R expression during tamoxifen resistance and reported reduced expression of IGF-1R mRNA [18]. Also, in a study in patients with recurrent breast tumors treated with tamoxifen for at least 6 months, IGF-1R expression was significantly lower in the recurrent lesions, compared to the primary tumors [19]. In fact, as shown in our study, already after a short course (2–6 weeks) of endocrine therapy, levels of IGF-1R expression may be reduced, and therefore, these patients may not benefit from IGF-1R-targeted therapy. Together, these results suggest that it might not be effective to combine endocrine therapy with IGF-1R inhibitors or to treat hormone-resistant breast tumors with anti-IGF-1R antibodies.

In addition to immunohistochemical studies, we used ^{111}In-R1507 immunoSPECT/CT to noninvasively study IGF-1R expression of hormone receptor-positive breast cancer xenografts in mice. After estradiol treatment, the uptake of ^{111}In-R1507 in MCF-7 tumors was significantly enhanced which correlated with increased levels of membranous IGF-1R as observed immunohistochemically. However, in this model, ^{111}In-R1507 immunoSPECT/CT could not detect the tamoxifen-mediated downregulation in membranous IGF-1R expression levels. A similar discrepancy between immunoSPECT/CT and immunohistochemical results has been obtained in a previous study in sarcoma xenografts [19]. This study showed that IGF-1R expression as measured by immunohistochemistry and Western blot did not always correlate with the targeting of ^{111}In-R1507 to sarcoma xenografts. However, accumulation of ^{111}In-R1507 in the tumor measured by ^{111}In-R1507 immunoSPECT/CT, rather than immunohistochemistry and Western blot, was predictive for response to anti-IGF-1R treatment [20]. These

results indicate that although receptor expression is an important factor for antibody tumor targeting and therapeutic efficacy, other factors should be taken into account as well. For example, previous studies have reported that IRS-1, IRS-2, IGF-2, or nuclear IGF-1R expression may be predictive for response to IGF-1R-targeted therapy [11, 21, 22]. In addition, antibody targeting can be affected by tumor vascular density, vascular permeability, interstitial fluid pressure, necrosis, and perfusion all contribute to the amount of antibody accumulating in a tumor [12, 13, 23]. Importantly, several types of therapy such as anti-angiogenic treatment directed at vascular endothelial growth factor (VEGF) can alter tumor vasculature in such a way that it could hamper effective drug delivery [24, 25]. Interestingly, several studies have also reported that hormones and endocrine therapy can have an effect on the VEGF pathway [26–28]. This suggests that tamoxifen treatment in our xenograft models may have altered tumor vascularization which might have affected targeting of ^{111}In-R1507 to MCF-7 xenografts.

Phase I/II studies with anti-IGF-1R antibodies have shown the safety and tolerability of targeting IGF-1R. Furthermore, these studies demonstrated a wide range of responses, from progressive disease to near complete response [29–31]. Although these results seemed promising, larger randomized phase III trials failed to show a clear benefit from targeting IGF-1R in combination with conventional treatment strategies [17, 32, 33]. However, these trials were conducted in unselected patient populations. Since early phase trials have shown meaningful single-agent responses, it is of importance to be able to identify those patients that potentially benefit from IGF-1R-targeted therapy. Therefore, *in vivo* imaging may be useful to select patients who are most likely to benefit from IGF-1R-targeted therapy.

The advantage of a therapeutic antibody such as R1507 as an imaging agent is that the targeting of the therapeutic drug can be imaged directly. However, when quick changes in receptor expression are being studied, intact antibodies such as R1507 are not the preferred tracer since they accumulate slowly in the tumor (3–7 days). Therefore, we have previously shown the feasibility of using R1507 F(ab')$_2$ fragments to image IGF-1R expression, which allows imaging as early as 6 h post injection [34]. Also, ^{111}In-IGF-1(E3R), an analog of IGF-1 which does not bind IGF-binding proteins, has been used to visualize IGF-1R expression of subcutaneous breast cancer xenografts with microSPECT. However, the tumor uptake and tumor-to-normal tissue contrast of ^{111}In-IGF-1(E3R) were lower than that of ^{111}In-R1507 IgG and ^{111}In-F(ab')$_2$ [35]. Another radiotracer that has been developed to target IGF-1R is the Affibody molecule $Z_{IGF\text{-}1R:4551}$. [36]. $Z_{IGF\text{-}1R:4551}$ has affinity for the murine and the human IGF-1R. Therefore, this tracer can be used to study targeting of human tumors and IGF-1R expressing normal tissue in mice bearing human tumor xenografts. SPECT imaging with $Z_{IGF\text{-}1R:4551}$ showed that IGF-1R expressing prostate cancer xenografts can be visualized as early as 1 h post injection, even while the targeting to normal IGF-1R expressing tissues was considerable.

Conclusion

Anti-estrogen therapy can downregulate membranous IGF-1R expression, both in the preclinical and clinical setting. In patients, this effect can occur within a few weeks after start of treatment. Targeting of ^{111}In-R1507 to IGF-1R expressing xenografts can be measured by immunoSPECT/CT. However, next to expression of IGF-1R, other factors may influence antibody accumulation in the tumor. Future research is warranted to determine which mechanisms are involved.

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Conflict of Interest. The authors declare that they have no conflict of interest.

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