

**Viral transduction of the HER2-extracellular domain expands trastuzumab-based
photoimmunotherapy for HER2 negative breast cancer cells**

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Abstract

Purpose: The prognosis of HER2 positive breast cancer has been improved by trastuzumab therapy, which features high specificity and limited side effects. However, trastuzumab-based therapy has shortcomings. Firstly, HER2-targeted therapy is only applicable to HER2-expressing tumors, which comprise only 20-25% of primary breast cancers. Secondly, many patients who initially respond to trastuzumab ultimately develop disease progression. To overcome these problems, we employed virus-mediated HER2 transduction and photoimmunotherapy (PIT) which involves trastuzumab conjugated with a photosensitizer; trastuzumab-IR700, and irradiation of near-infrared light. We hypothesized that if the gene transduction technique together with PIT would expand the range of tumor entities suitable for trastuzumab-based therapy and improve its antitumor activity.

Methods: The HER2-extracellular domain (ECD) was transduced by the adenoviral vector, Ad-HER2-ECD and PIT with trastuzumab-IR700 was applied in the HER2-negative cancer cells.

Results: Ad-HER2-ECD can efficiently transduce HER2-ECD into HER2-negative human cancer cells. PIT with trastuzumab-IR700 induced direct cell membrane destruction of Ad-HER2-ECD transduced HER2-negative cancer cells.

Conclusion: Novel combination of viral-transduction of a target antigen and an antibody-based

PIT would expand and potentiate molecular-targeted therapy even for target-negative or -attenuated cancer cells.

Keywords: Photoimmunotherapy, HER2, breast cancer, adenovirus

Introduction

HER2 is a well-known oncogene, the overexpression of which is strongly associated with more aggressive tumors and poorer overall survival[26]. Strategies such as trastuzumab that target HER2 have greatly improved the prognosis of HER2-positive breast cancer through their high specific targeting ability [5]. However, the clinical efficacy of HER2-targeted therapy is limited to breast cancers that overexpresses HER2, which only account for 20 to 30% of all breast cancer[4, 25] and which are also inclined to develop resistance[16]. In addition, although trastuzumab is known to have few side effects apart from cardiac toxicity, trastuzumab is usually used together with an anti-tumor drug [24, 27], because its effect is not strong enough when used as a sole agent.

Photoimmunotherapy (PIT) was developed as a new type of molecular-targeted cancer therapy. PIT uses specific monoclonal antibodies that are targeted towards cell surface receptors and that are conjugated to the photosensitizer phthalocyanine dye, IR700, which is cytotoxic upon irradiation with near-infrared (NIR) light [15]. Trastuzumab-IR700 (Tra-IR700) was designed to specifically target HER2-expressing cells. Cell death is only induced when the cells are bound by Tra-IR700 and irradiated with NIR light. Death is presumably induced by cell membrane damage due to local expansion of heated water [15].

Modified adenovirus type 5 vectors have been widely used as a platform for the delivery of genes of interest into various types of human cells. In a previous study, we constructed a replication-deficient adenoviral vector containing a gene that encodes the HER2 extracellular domain (Ad-HER2-ECD) [35]. We demonstrated that infection with Ad-HER2-ECD resulted in expression of the HER2-ECD on the surface of HER2-negative or trastuzumab-resistant HER2-attenuated cancer cells. The expressed HER2-ECD did not trigger HER2 signaling pathways because of the lack of the HER2 intracellular kinase domain [35].

We hypothesized that if the Ad-HER2-ECD mediated gene transduction technique was used together with molecular-targeted cancer therapy using PIT, the range of tumor entities suitable for trastuzumab-based therapy would be expanded and the problem of resistance and narrow indication could be overcome.

Materials and methods :

Cell lines and cell cultures

Three human mammary gland adenocarcinoma cell lines, SKBR3, MDA-MB-231 and MCF7, were obtained from the American Type Culture Collection. SKBR3, MDA-MB-231 and MCF7 were cultured in McCoy's 5A medium, Leibovitz's L-15 Medium and Dulbecco's modified

Eagle's medium (DMEM) supplemented with 2 mmol/ml L-glutamine, respectively. The human osteosarcoma cell line Saos-2 was kindly provided by Dr. Kyo (Kanazawa University, Ishikawa, Japan), and the cells were propagated as monolayer cultures in DMEM. Penicillin (100 units/ml), streptomycin (100 µg/ml), and 10% fetal bovine serum were added to the medium. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂.

Recombinant adenovirus

A replication-deficient adenoviral vector expressing the extracellular and transmembrane domains of HER2 (Ad-HER2-ECD) was constructed [35]. Briefly, the HER2-ECD expression cassette, which contains the human cytomegalovirus promoter, HER2-ECD cDNA and the SV40 early polyadenylation signal, was inserted between the XbaI and ClaI sites of pXCJL.1. The HER2-ECD shuttle vector and the recombinant plasmid pJM17 were cotransfected into 293 cells. The culture supernatant of 293 cells that showed the complete cytopathic effect, which contained virus progenies, was collected and used for subsequent production. The resultant virus was purified by ultracentrifugation in cesium chloride step gradients, and its titer was determined by a plaque-forming assay using 293 cells. The virus was stored at -80 °C until use. Ad-GFP was used as control adenovirus [32].

Western blotting analysis

Primary antibodies against HER2-ECD (Ab-20, Thermo Scientific) and β -actin (AC-15, Sigma Chemical, Co.) were used. Proteins were electrophoretically transferred to Hybond-polyvinylidene difluoride transfer membranes (GE Healthcare Life Science), incubated with primary antibody and then with peroxidase-linked secondary antibody according to the manufacturer's protocol. The Amersham ECL chemiluminescence system (GE Healthcare Life Science) was used to detect the peroxidase activity of the bound antibody. In experiments with replication-deficient adenoviral vector, cells were infected with Ad-HER2-ECD or Ad-GFP at a multiplicity of infection (MOI) of 50 for 48 h.

Flowcytometric analysis

To measure the expression of HER2-ECD in cells infected with Ad-HER2-ECD, cells were labeled with APC-conjugated mouse monoclonal anti-HER2-ECD antibody (R&D Systems Inc.) or APC-conjugated IgG2b as control (Miltenyi Biotec, Inc.) on ice for 45 min and were then analyzed using a FACS instrument (BD Biosciences). The intensity of staining was determined using the BD-FACS Software (Flow Jo 7.6.1).

Immunocytochemistry

Cells cultured on 4 chamber glass slides were fixed with 4% paraformaldehyde in PBS for 15 min and blocked with 3% bovine serum albumin for 30 min. Slides were then labeled with APC-conjugated mouse monoclonal anti-HER2-ECD antibody (R&D Systems Inc.) on ice for 45 min and were subsequently photographed using a Confocal Laser Scanning Biological Microscope with a 647 nm excitation filter (FV10i, Olympus, Tokyo, Japan).

Synthesis of IR700-conjugated Trastuzumab

Trastuzumab was obtained from Chugai Pharmaceutical Co. The IR700 dye (LI-COR Biosciences) was conjugated to trastuzumab following the manufacturer's instructions. Briefly, trastuzumab (1 mg) was incubated with IR700 (66.8 mg, 34.2 nmol, 5 mmol/L in DMSO) in 0.1 mol/L Na₂HPO₄ (pH 8.6) at room temperature for 1 hour. Conjugated Tra-IR700 was purified using a Sephadex G50 column (PD-10; GE Healthcare). Protein concentrations were determined using a Coomassie Plus Protein Assay Kit (Pierce Biotechnology) by measurement of light absorption at 620 nm (8453 Value System; Agilent Technologies).

Fluorescence microscopy

To detect specific antigen-mediated localization of IR700 on the surface of MCF7 and MDA-MB-231 cells, the cells were seeded at 2×10^4 /well on cover glass-bottomed dishes,

incubated for 24 h and infected with Ad-HER2-ECD at an MOI of 50 for 48 h. Tra-IR700 (10 $\mu\text{g}/\text{ml}$) was then added to the culture medium and incubated for 6 h at 37 °C. The cells were washed with PBS and fluorescence was observed under a Confocal Laser Scanning Biological Microscope (FV10i, Olympus, Japan) with a 647 nm excitation filter.

In vitro Photoimmunotherapy

Cells were seeded on 35 mm cell-culture dishes at 2×10^4 / well or 96 well black plates at 5×10^3 / well, and were infected with Ad-HER2-ECD or Ad-GFP at an MOI of 50 for 48 h. The medium was then replaced with fresh culture medium containing 10 $\mu\text{g}/\text{ml}$ of Tra-IR700 and was further incubated for 6 h at 37 °C. After washing with PBS, the cells were then irradiated with light from a red-light-emitting diode at wavelengths of 670–690 nm for 35 mm cell-culture dishes and at a wavelength of 625 nm for 96-well black plates. The filter set to detect IR700 when observed under a fluorescence microscope (IX71; Olympus, Tokyo, Japan) consisted of a 590–650 nm excitation filter and a 665–740 nm band pass emission filter. Power density ranged from 40 to 100 mW/cm^2 , as measured using an optical power meter (PM 100, Thorlabs, Inc.)

Cell viability assay

Cell viability was determined 3 days after PIT by using the Cell Proliferation Kit II (Roche

Molecular Biochemicals) with the sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate (XTT) assay, according to the manufacturer's protocol. Propidium iodide (PI), which is plasma membrane impermeable, was used to stain membrane-disrupted dead cells [10]. PI was added to the medium at an approximate final concentration of 1 µg/ml and was incubated at 37 °C for 30 min.

Statistical analysis

A comparison of continuous variables between two groups for in vitro assays was performed using the two-sided Student's *t* test. Differences between groups were considered to be statistically significant when the *p* values were <0.05.

Results

HER2-ECD expression induced by Ad-HER2-ECD

MCF7 and MDA-MB-231 breast cancer cells were chosen as HER2 negative breast cancer lines since their expression of HER2 is known to be almost negative, or barely detectable[19].

Initially, Ad-GFP was used as a substitute for Ad-HER2-ECD in preliminary experiments aimed at finding the optimal dose of Ad-HER2-ECD to transduce HER2-ECD into these cells and at examining the transduction efficiency of the adenovirus. We found that an MOI of 50 was the

optimal MOI, at which almost all cells were transduced with GFP proteins without cellular toxicity (supplementary figure 1). Based on these data, HER2-negative breast cancer cells were infected with Ad-HER2-ECD at an MOI of 50 for 48 h, to transduce HER2-ECD. Western blot analysis showed that Ad-HER2-ECD infection resulted in a marked increase in the expression of the 100-kDa HER2-ECD protein in HER2 low-expressing MCF7 and MDA-MB-231 breast cancer cells as compared to parental cells and Ad-GFP infected cells (Figure 1a).

Flowcytometric analysis further demonstrated the expression of HER2-ECD on the cell surface (Figure. 1b). The cell-surface expression of HER2-ECD in Ad-HER2-ECD-infected cells was also confirmed using immunocytochemistry (Figure. 1c).

Trastuzumab-IR700 binds to the transduced HER2-ECD

We next examined whether Tra-IR700 bound to the HER2-ECD that was overexpressed on MCF7 and MDA-MB-231 cells by Ad-HER2-ECD transduction. Because IR700 is fluorescent it can be directly detected by fluorescence microscopy allowing visualization of the cellular location of bound Tra-IR700 conjugates. MCF7 and MDA-MB-231 cells were transduced with HER2-ECD using Ad-HER2-ECD and the cells were then incubated with Tra-IR700 for 6 h. Fluorescent imaging confirmed that Tra-IR700 primarily bound to the cell-surface of infected, HER2-ECD transduced cells (Figure. 2).

Morphological changes in Ad-HER2-ECD-infected HER2 negative cells after trastuzumab-IR700 with PIT

The effects of Tra-IR700 and PIT treatment on the cell morphology of Ad-HER2-ECD-infected breast cancer cells over time were microscopically examined. Two different types of morphological changes were observed in MCF7 and MDA-MB-231 cells after irradiation with NIR light. In MCF-7 cells, bubbling at the cell surface preceded total cell shrinkage. In MDA-MB-231 cells in contrast, instead of bubbling, many granules appeared in the cytoplasm, followed by shrinkage of the cells (Figure 3a). Of note, these changes were observed within one minute after irradiation with NIR light. A higher amount of light (18 J) resulted in more exaggerated changes in morphology than a lower amount of light (6 J) (Figure 3a). Ad-HER2-ECD infected cells died within 72 h after Tra-IR700 with PIT (6 J), while untreated cells and Ad-GFP-infected cells survived (Figure 3b). These phenomena were captured as time-lapse movies (Supplementary movies 1, 2). We further confirmed cell death of the MCF7 and MDA-MD-231 cells at 72 h after irradiation with NIR light (18 J) by staining of cells whose membranes had been destroyed using PI staining (Figure. 3c). Morphological change and PI-staining of cells immediately following PIT were also reproduced in Ad-HER2-ECD-infected and PIT-treated HER2 negative osteosarcoma Saos-2 cells

(Supplementary figure 2).

Quantitative evaluation of the cytotoxicity of Trastuzumab-IR700 with PIT for

Ad-HER2-ECD-infected HER2 negative cells

The specific cytotoxicity of Anti-HER2 PIT was quantitatively evaluated using the XTT assay of cell viability and HER2-transduced MCF7 and MDA-MB-231 cells (Figure 4a, 4b).

Treatment included seven control conditions in addition to Ad-HER2-ECD plus PIT. Only under the treatment condition that employed Ad-HER2-ECD together with Tra-IR700 mediated PIT (24 J for MDA-MB-231 and 36J for MCF-7), was cell proliferation significantly suppressed. In contrast, no significant cytotoxicity was observed under any other condition. Ad-HER2-ECD, Tra-IR700 or NIR irradiation did not harm the cells when each modality was given alone.

HER2-specific cytotoxicity of Tra-IR700 mediated PIT was also reproduced in the HER2 positive breast cancer cell, SK-BR-3 and in Ad-HER2-ECD-infected Saos-2 cells

(Supplementary figure 3a, b). These results indicated that Tra-IR700 mediated PIT selectively and specifically targets HER2-ECD transduced cancer cells.

Discussion

Over the past decades research has transformed the care of patients with breast cancer through

the application of basic science to the clinic[28]. Monoclonal antibodies currently occupy a key position among anti-cancer drugs due to their high specificity and efficacy, which can be achieved in spite of their low side effects. The typical clinical approach for HER2-positive breast cancer has been altered since the approval of trastuzumab[28]. However, for such molecular targeted therapy, the indication is limited to the populations which express the specific target antigen. In order to overcome this issue, we tried to artificially express the target antigen in target-negative cancer cells by viral transfection. We proved that viral transduction of HER2-ECD made even HER2-negative breast cancer cells sensitive to anti-HER2 photoimmunotherapy. Thus, this technique could expand the indication of antibody-directed therapy to not only target-positive but also to target-negative cancers.

In the present study, we have reproducibly expressed HER2-ECD on the cell membrane of HER2-negative MCF7 and MDA-MB-231 cells, as evidenced by immunocytochemistry as well as by flow cytometry. Although flow cytometric analysis suggested that both parental MCF7 and MDA-MB-231 cells express HER2, the 185 kDa HER2 protein could not be detected in these parental cells by Western blot analysis. These results are consistent with previous reports by other groups, which demonstrated that the levels of HER2 expression in these cell lines is low or negligible and that these cell lines are not sensitive to anti-HER2 therapy [8, 12, 31].

In addition to confirmation of HER2-ECD expression, we also confirmed that Tra-IR700

bound directly to transduced HER2-ECD by observation under a confocal fluorescence microscope. Since IR700 itself emits fluorescence [17] (Figure 2), Tra-IR700 proved to be a useful agent not only for treatment, but also for monitoring[14, 17, 20]. In our previous study, we demonstrated that antibody-dependent cellular cytotoxicity (ADCC) activity against HER2-negative cells or HER2-downregulated cells could be recovered by forced expression of HER2-ECD using Ad-HER2-ECD. At the same time, we confirmed that the expressed HER2-ECD did not activate the HER2 signal transduction pathway and not induce malignant transformation due to the lack of an intracellular kinase domain [35].

There are two main mechanisms by which Trastuzumab stops abnormal cell proliferation; one is through ADCC activity and the other is by blocking signal transduction pathways [7, 29].

However, trastuzumab resistance is known to occur. The cause of trastuzumab resistance appears to be multifactorial [16]. Thus, multimodal treatment strategies are theoretically needed.

Indeed, trastuzumab has been mainly used together with chemotherapy or with another monoclonal antibody [24, 27]. However, patients may ultimately suffer from the side effects of co-administered drugs [24, 27].

Cytotoxic agent-conjugated trastuzumab has been investigated to potentiate the antitumor activity of trastuzumab and to exploit the specific overexpression of HER2 on cancer cells[1, 2, 18]. One such agent is Tra-IR700, which was designed to induce cell death only when it is

bound to the target cells and when the cells are exposed to NIR [15]. Although the mechanism of cell death induced by PIT remains to be fully elucidated, it assumed to differ from that in conventional photodynamic therapy or in antibody-mediated cytotoxicity [15, 23]. The mode of cytotoxicity in HER2-ECD-targeted Tra-IR700-mediated PIT is also different from ADCC activity or from a block in signal transduction pathways. In the present study, the cytotoxic effect of Ad-HER2-ECD plus Tra-IR700 with PIT was clearly shown by cellular morphological changes and by quantitative analysis of cell viability (Figure 3, 4). Captured images of morphological change demonstrated that cell membrane injury occurred immediately after PIT, and time-lapse movies allowed continuous observation of morphological change (Supplemental movie 1, 2). Tra-IR700-mediated PIT directly affects the target cellular membrane and quickly damages it, suggesting that PIT for HER2-ECD can lead to target cell death regardless of intra-cellular conditions. This mechanism of cell death would suggest that this approach would overcome all modes of resistance known in HER2-targeted therapy including those mediated by altered signaling from receptor tyrosine kinases, constitutive phosphoinositide 3-kinase signaling, *PIC3CA* gene mutations and amplification of signaling by HER family receptors [6, 16, 36].

Cell death was induced only in breast cancer cells infected with Ad-HER2-ECD and treated by Tra-IR700 with PIT. In contrast, none of the other incomplete treatment conditions could induce

cell death. Thus target-specificity was proved to be very high. Furthermore, we also reproduced this effect in HER2 negative, SaOS2 osteosarcoma cells, highlighting the possibility that this approach could be applied to cells irrespective of the tissue of origin, as long as the adenovirus can infect the target cell.

Although targeting a forcibly-transduced target seems to be an attractive approach, the HER2-ECD as a defective antigen. Therefore, this approach might not be applicable to every anti-HER2 targeted therapy. Prior to employment of anti-HER2 PIT, we had exploited the trastuzumab-Saporin (Hum-ZAP: ADVANCED TARGETING SYSTEMS, San Diego, USA) conjugate, a so-called “immunotoxin”. However, that strategy was unsuccessful, probably because of a defect in trans-membrane internalization of HER2-ECD (data not shown). Thus, any toxins or cytotoxic drugs such as Trastuzumab-DM1 [3, 9, 12], which require intracellular uptake to exert their toxicity, may not be suitable for anti-HER2-ECD targeted therapy. In contrast, IR700 does not require intracellular trafficking because it can exhibit photo-toxicity at the surface of the membrane. This advantage of IR700 is therefore one of the reasons for the success of PIT in combination with anti-HER2-ECD targeted therapy.

Since PIT necessitates sufficient NIR irradiation to kill the cancer cells, the actual target lesion must be within the reach of NIR. NIR light has been shown to travel at least several centimeters through human tissues[33]. Clinical potential of this therapy is therefore assumed for superficial

cancer such as in a neoadjuvant setting for primary breast cancer, recurrences in the chest wall and metastatic lesion in the skin from breast cancer. As long as the device that emits NIR light can gain access to the cancer, even an intra-visceral approach through an endoscope might be possible [13]. Sano et al. recently reported that PIT enhanced local vascular permeability, which may enhance drug delivery through the so-called “enhanced permeability and retention” effect[21]. It is also expected that PIT might improve the efficacy of conventional chemotherapy. Furthermore, intrinsic ADCC activity can be expected simultaneously with PIT.

Next step will be proof of concept studies using HER2-negative breast tumors in mice. When this strategy is applied to a solid tumor model, success also depends on the penetration of adenoviral infection into tumor besides the reach of NIR light. Since Ad-HER2-ECD is a non-replicating adenoviral vector, intratumoral transduction might show at the less than ideal level[22]. Thus far, more appropriate viral and no-viral gene delivery systems have been extensively developed to be applied to preclinical and clinical studies[30, 34]. Therefore, in case of the application to in vivo study, the optimal vector such as a tumor-specific replicating adenovirus[11] might be preferred to deliver the gene of the target antigen.

In conclusion, we demonstrated that the combination therapy of Ad-HER2-ECD and Tra-IR700 with PIT effectively and selectively killed HER2-negative cells in vitro. This result suggests that viral transduction technology will enable expansion of the indication of

antibody-based therapy even to the target-negative group. Therefore, this strategy may also be applicable to other therapeutic antibodies that are currently being developed in rapid succession. The novel combination of gene transfer technology and antibody-based photoimmunotherapy is a promising approach for breaking the limitations of, and resistance to, cancer therapy

Abbreviations

DMEM: Dulbecco's modified Eagle's medium; ECD: extracellular domain; HER2: human epidermal growth factor receptor type 2; MOI: a multiplicity of infection; NIR: near infrared; PBS: phosphate-buffered saline; PI: Propidium iodide; PIT: photoimmunotherapy; SDS: sodium dodecyl sulfate; SQ: self-quenched; Tra-IR700: Trastuzumab-IR700; XTT: the sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1. HER2-extracellular domain (ECD) expression induced by Ad-HER2-ECD

(a) Western blot analysis showing expression of the 100-kDa HER2-ECD protein in

Ad-HER2-ECD infected cells but not in Ad-GFP infected or parental cells. Actin was used as a loading control.

(b) Flowcytometric analysis showing HER2-ECD expression on the cell membrane. Cells were

labeled with APC-conjugated mouse monoclonal anti-HER2-ECD antibody (blue and red) or

APC-conjugated IgG2b as a negative control (green and yellow).

(c) Immunocytochemical analysis of the cell surface expression of HER2-ECD on

Ad-HER2-ECD-infected cells using an APC-conjugated anti-HER2-ECD antibody. Scale bars,

50 μm .

Figure 2. Trastuzumab-IR700 binds to the transduced cell surface HER2-ECD

Immunofluorescent analysis of Trastuzumab-IR700 (Tra-IR700) binding to HER2-ECD

transduced by Ad-HER2-ECD on the cell surface of breast cancer cells. Scale bars, 50 μm .

Figure 3. Microscopic analysis of the effect of Tra-IR700 mediated PIT on the cell morphology

and cell death of HER2-ECD-transduced HER2-negative cells

- (a) Phase contrast analysis of the morphology of the breast cancer cells MCF-7 and MDA-MB-231 immediately following PIT using 0, 6 or 18 J. Scale bars, 50 μm .
- (b) Phase contrast analysis of control, Ad-GFP-infected or Ad-HER2-ECD infected MCF-7 and MDA-MB-231 cells before and after 72 h treatment with Tra-IR700 mediated PIT using 6 J. Ad-HER2-ECD infected cells but not the other cells displayed cell shrinkage. Scale bars, 50 μm .
- (c) Analysis of cell death of HER2-ECD transduced cells following Tra-IR700 mediated PIT (12J) 72h after treatment. The PI staining indicates that plasma membrane destruction was induced by the treatment. Scale bars, 50 μm .

Figure 4. Effect of Tra-IR700 mediated PIT treatment of HER2-ECD transduced HER2-negative cells on cell viability.

Tra-IR700 mediated PIT was applied to the indicated cells along with seven control conditions and cell viability was quantified 72h after PIT (24 J for MDA-MB-231 and 36J for MCF-7) using the XTT assay. Only the group of Ad-HER2-ECD infected cells treated with Tra-IR700 mediated PIT showed target-specific cell death. (n =4, *p< 0.05 for Tra-IR700 mediated PIT treatment compared to control groups using Student's *t* test.)

Supplementary Figure 1.

Adenoviral transduction of GFP protein into breast cancer cells.

Fluorescence microscopic analysis of (a) MCF7 cells and (b) MDA-MB-231 cells that were transduced with GFP using Ad-GFP at the indicated MOIs. Most cells were transduced with GFP at an Ad-GFP MOI of 50.

Supplementary Figure 2.

Microscopic analysis of the effect of Tra-IR700 mediated PIT on Saos2 cells infected with Ad-HER2-ECD.

(a) Phase contrast analysis of the morphology of Ad-HER2-ECD-infected Saos2 cells immediately after PIT using 6 J or 24 J, and 24 h after PIT using 24 J.

(b) PI staining of Ad-HER2-ECD-infected Saos2 cells 5 days after Tra-IR700 mediated PIT using 15 J. PI staining indicates plasma membrane destruction of Saos2 cells following treatment.

Supplementary Figure 3.

Effect of Tra-IR700-mediated PIT on cell viability of SK-BR-3 and Saos2 cells.

(a) HER2-positive SK-BR-3 cells were treated with trastuzumab or with Tra-IR700 in

combination with NIR irradiation as indicated and cell viability was measured using the XTT assay after 72h. The suppression of cell viability by Tra-IR1700 plus NIR (24 J) was significantly higher than that induced by trastuzumab or by any of the control groups.

(b) Saos2 cells transduced with Ad-HER2-ECD were treated with Tra-IR700 mediated PIT () or with seven control conditions and cell viability was quantified after 72h using the XTT assay. Only the Ad-HER2-ECD transduced cells treated with Tra-IR700-mediated PIT (24 J) showed cell death that was significantly higher than that induced in any of the other groups.

Supplementary movies

Morphological change induced immediately after Tra-IR700 mediated PIT in HER2-ECD transduced breast cancer cells captured as a time-lapse movie.

The cellular membrane injury that was caused in 1. MDA-MD-231 cells and 2. MCF7 cells immediately after Tra-IR700 mediated PIT is shown.