REVIEW ARTICLE

Towards detecting the HER-2 receptor and metabolic changes induced by HER-2-targeted therapies using medical imaging

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ABSTRACT. HER-2/neu (a receptor for human epidermal growth factor) is involved in cell survival, proliferation, angiogenesis and invasiveness. It is overexpressed in about 25% of breast cancers. Overexpression of HER-2 is associated with response to the anti-HER-2 antibody trastuzumab (herceptin). However, HER-2 expression can be heterogeneous within the primary tumour and can also exhibit discordant expression between a primary tumour and its metastases, bringing into question the practice of HER-2 screening to determine whether a patient is a candidate for trastuzumab using material obtained only from the primary tumour. Medical imaging modalities using HER-2-targeted tracers (or contrast agents) facilitate a global approach to the determination of HER-2 expression across all detectable tumour lesions, and could provide a more reliable indication of the patient's likely response to trastuzumab treatment. Here, I review the development and pre-clinical (and occasional clinical) assessment of HER-2-targeted tracers. I discuss studies in which established imaging tracers, such as ^{11}C -choline, have been used to determine response to trastuzumab in a range of medical imaging modalities, including positron emission tomography (PET), single photon emission tomography (SPECT), MRI and optical imaging.

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HER-2/neu (a receptor for human epidermal growth factor (EGF)) is a member of the HER family of class 1 growth factor receptor tyrosine kinases. It is involved in cell survival, proliferation, angiogenesis and invasiveness. HER-2 is overexpressed in a variety of tumour types, especially in breast cancer, where amplification of the gene encoding HER-2 is found in about 25% of cases [1]. Overexpression of HER-2 is associated with aggressiveness and poor prognosis [2].

The HER family has four members, HER-1–4. Structurally, these receptors consist of an extracellular region for ligand binding that is linked via a trans-membrane region to an intracellular tyrosine kinase. Ligand binding on the extracellular domain enables the pairing of receptors to form either homo- or heterodimers. This pairing brings together two intracellular domains that then undergo transphosphorylation. Epidermal growth factor is the endogenous ligand for HER-1. HER-2 has no natural ligand but participates in the formation of heterodimers with other members of the HER family. HER-2 enhances the binding affinity of HER-1 for EGF.

The variety of responses associated with HER-2 receptors is brought about by activation of several intracellular signalling pathways, including phosphoinositide 3 kinase (PI3K)/Akt, Raf/mitogen-activated

protein kinase kinase (MEK)/mitogen-activated protein kinase (MAPK), Janus kinase (JAK)/signal transducer and activator of transcription-3 (STAT3), and Src phospholipase C γ . Which pathway is activated is determined by the combination of HER molecules making up the activated dimer [3].

Therapeutic intervention to block HER-2 signalling

A number of mechanisms to inhibit HER-2 signalling have been developed. These include receptor degradation, inhibition of tyrosine kinase [4] activity and antibody-based receptor blockade [5]. Cellular proteins are tagged for degradation by conjugation with ubiquitin. This process is exploited by the drug geldanamycin, which induces the destruction of HER-2 by promoting ubiquitination. Targeting of the extracellular domain of HER-2 with the anti-HER-2 antibody trastuzumab (herceptin) is an example of antibody-based receptor blockade. In 1998, the Federal Drug Agency (FDA) approved trastuzumab for the treatment of women with metastatic breast cancer involving tumours that over-express the HER-2 receptor. In combination with chemotherapy, trastuzumab has been shown to prolong the life of women with HER-2-positive metastatic breast cancer [5]. Trastuzumab is an immunoglobulin type G (IgG), and one of its modes of action is to identify the cell for antibody-dependent immune destruction. Cleavage

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of the extracellular domain of HER-2 by metalloproteases can occur, leaving a truncated receptor that enhances tyrosine kinase activity [6]. This proteolytic cleavage is inhibited by trastuzumab [7]. Trastuzumab binding to HER-2 also impairs intracellular signalling downstream from the receptor [8]. Trastuzumab may also increase endocytosis of HER-2, thereby reducing the density of this receptor in the cell membrane [9]. A number of small membrane-penetrating molecules that compete with ATP for the ATP-binding site on the intracellular tyrosine kinase catalytic region of the receptor have been developed. These include lapatinib, which inhibits the tyrosine kinase activities of both HER-1 and HER-2 receptors [4].

Determining HER-2 status by immunochemistry and fluorescence *in situ* hybridisation

At present, HER-2 status is measured in tissue obtained from the primary tumour using either immunohistochemistry (IHC) to determine protein expression or fluorescence *in situ* hybridisation (FISH) to determine gene amplification. Protein expression levels are scored as 0 or 1+ (zero or low), 2+ (intermediate) or high 3+. It is common practice to send scores of 2+ on IHC for FISH analysis, and patients whose tissue is positive on FISH (*i.e.* amplification >2.0) are offered trastuzumab. There are, however, high levels of interobserver variation in the IHC scoring of 2+. Techniques such as automated image analysis methods for digital imaging are being used to reduce this source of variation [10].

Discordance between HER-2 expression in primary and metastatic lesions

In a recent study comparing HER-2 positivity (scoring 2+ or 3+ on IHC) in primary and metastatic lesions from 382 patients with breast cancer, 140 primary lesions were found to be HER-2 positive (HER-2 score of 2 or 3+) whereas metastasis from 90 of these patients were HER-2 negative (HER-2 score of 0 or 1+) [11]. Of the 242 patients with HER-2-negative primary tumours, 37 had a HER-2-positive metastasis. Lower et al [11] cited several other studies demonstrating discordance between the HER-2 expression of primary and metastatic lesions. These findings have obvious implications regarding the validity of making herceptin treatment decisions based on the HER-2 status of the primary tumour alone.

In view of the heterogeneity in HER-2 expression sometimes observed between samples taken from the same breast tumour [12], and the well-established finding that HER-2 expression in tumour cells in the primary tumour frequently differs from that associated with their metastasis, a technique that determines HER-2 expression in all lesions within the body is particularly attractive. Medical imaging techniques using tracers that specifically target the HER-2 receptor can achieve this goal. This paper is a review of the current developments in tracer design and the use of medical imaging modalities to detect HER-2 expression and response to trastuzumab treatment.

Targeted imaging

Targeted (molecular) imaging is the detection of markers such as cell-surface receptors or components of molecular pathways using tracers consisting of a targeting component and a label that facilitates detection of the tracer. The type of label used depends on the medical imaging technique employed: a radioisotope for detection with positron emission tomography (PET) or single photon emission tomography (SPECT) cameras; a paramagnetic contrast agent for detection with MRI; an echogenic material for detection with ultrasound; or a fluorescent probe for optical imaging.

Targeting moieties for the HER-2 receptor

To achieve good images with enhanced contrast using targeting tracers, the majority of the tracer that has not become associated with the target cell at the time of the scan should have cleared from the bloodstream. The problem with full-sized antibodies is that, because of their large size (>150 kDa), they are cleared only slowly from the bloodstream. The renal threshold for filtration is about 50 kDa, so molecules smaller than 50 kDa will be rapidly cleared from the bloodstream via renal excretion. This is particularly important for imaging with radioisotopes with short $t_{1/2}$ such as ^{18}F (110 min) and $^{99\text{m}}\text{Tc}$ (6 h), which are the most commonly used isotopes in PET and SPECT imaging, respectively.

Fragment antibodies can be produced by protease trimming of whole antibodies, leaving a single variable region of about 30 kDa that has an antigen affinity similar to that of the original whole antibody [13]. These smaller molecules also overcome the problem of poor tumour penetration exhibited by full-sized antibodies.

In the past 10 years, Affibody® molecules have been developed. These are scaffold-targeting proteins consisting of 58 amino acids with 13 surface residues that are randomised and then selected for target affinity [14]. These high-affinity molecules have a molecular weight of about 7 kDa and can be conjugated to radionuclides directly or via chelating groups [15]. Several HER-2 Affibodies have been developed with very high affinity (in the pmol/l range) for the HER-2 receptor. They include monomeric $Z_{\text{HER2:342}}$ and dimeric ($Z_{\text{HER2:4(2)}}$ and $Z_{\text{HER2:342(2)}}$) versions.

Targeting strategies

The imaging of cell-surface molecules *in vivo* can be carried out using directly labelled tracers or by a two (or sometimes three)-step procedure in which the target molecule is pre-targeted with a conjugate consisting of the targeting moiety fused with a member of an affinity pair, such as biotin-avidin. Biotin and avidin have a very high mutual affinity, so administration of the complementary member conjugated to a radionuclide (after a period of time sufficient to allow blood clearance of the antibody-conjugate) will allow it to seek out and become associated with the pre-targeted antibody. By comparison with directly labelled tracers, pre-targeted molecules have been shown to produce cleaner images that have lower background activity [16].

Imaging modalities and tracers targeting HER-2

Single photon emission tomography (SPECT)

SPECT is a nuclear medicine imaging technique that can provide three-dimensional (3D) images by detecting gamma rays that are emitted from a targeting moiety labelled with a gamma-emitting nuclide, most commonly $^{99}\text{Tc}^{\text{m}}$ ($t_{1/2}=6\text{ h}$) but sometimes ^{111}In ($t_{1/2}=67\text{ h}$) (which requires a longer period between administration and imaging). The SPECT camera is rotated around the patient, acquiring information every 5° or so. A 3D image is then reconstructed from the complete 360° data set.

Several studies have described the labelling and characterisation of HER-2 targeting molecules with $^{99}\text{Tc}^{\text{m}}$ or ^{111}In [13, 17–20]. Tang et al [13] labelled a fragment antibody (Fab), formed by papain digestion of trastuzumab, with $^{99}\text{Tc}^{\text{m}}$ using the bifunctional agent hydrazinenicotinamide (HYNIC). One functionality of HYNIC conjugates to the N-terminal end of a protein or peptide, whereas the other functionality is a chelating group that enables HYNIC to chelate $^{99}\text{Tc}^{\text{m}}$. The Fab retained its affinity for the HER-2 receptor and was rapidly cleared from the bloodstream. Injection of this targeting molecule into mice bearing BT-474 xenografts, which overexpress HER-2, achieved a tumour uptake of 10.7% of the injected dose (ID) per gram and a tumour-to-blood ratio of 3:1 after 24 h.

In both a pre-clinical [17] and a clinical [20] study, trastuzumab was labelled with ^{111}In by conjugation with the chelating group diethylene triamine pentaacetic acid (DTPA). The pre-clinical study determined the uptake of ^{111}In -labelled trastuzumab (72 h after tracer administration) by a panel of breast tumour lines varying in HER-2 receptor density, which were grown as xenografts in mice [17]. When tracer incorporation was expressed as tumour uptake, the relationship between tumour-associated activity and HER-2 receptor density was poor. However, when uptake was corrected for blood pooling of tracer and for non-specific uptake determined using ^{111}In -DTPA-mIgG, HER-2 receptor density was shown to correlate strongly with tumour-associated activity. McLarty et al [17] also determined the sensitivity to trastuzumab of each of the breast tumour cell lines grown as xenografts in nude mice by determining a tumour growth inhibitory ratio, which was based on size after intraperitoneal (IP) trastuzumab treatment (4 mg kg^{-1} then weekly 2 mg kg^{-1} for 4 weeks) and the pre-treatment size. They found that the only significant growth inhibition was in cells expressing 2+ HER-2 levels. Cells expressing HER-2 at levels of 0, 1+ or 3+ did not exhibit growth inhibition with trastuzumab. The uncorrected uptake of ^{111}In -DTPA-trastuzumab was greatest for the 2+ HER-2 cell line (MDA-MB-361 tumours). In the only clinical study to date to have attempted imaging of the HER-2 receptor, Perik et al [20] reported that (after trastuzumab treatment) ^{111}In -DTPA-trastuzumab could detect new lesions in 13 of 15 patients with HER-2-positive metastatic breast cancer. Three of these patients exhibited cardiotoxicity, but this was not predicted with the ^{111}In -DTPA-trastuzumab scan.

A number of groups have labelled HER-2 Affibodies with radionuclides including $^{99}\text{Tc}^{\text{m}}$ [19] and ^{111}In [15, 18]. Two of the studies [15, 19] illustrated the importance of the

chelating group on tracer performance. Tran et al [19] demonstrated that the conjugation of HER-2 Affibodies with different chelating groups produced tracers with different hydrophilicity, which affected their route of excretion. Minimising hepatobiliary excretion is important in tracer development, as it results in high accumulation of non-targeted radioactivity in the gut that obscures tumour detection in the abdominal region. Biodistribution studies in mice bearing SKOV3 xenografts demonstrated that hepatobiliary excretion of HER-2 Affibodies ($Z_{\text{HER2:342}}$) conjugated to mercaptoacetyl-glu-glu-glu- was lower than that of $Z_{\text{HER2:342}}$ conjugated to mercaptoacetyl-gly-gly-gly-. The *in vivo* targeting properties of mercaptoacetyl-glu-glu-glu- $^{99}\text{Tc}^{\text{m}}$ -maEEE- $Z_{\text{HER2:342}}$ showed a receptor-specific tumour uptake of $7.9\pm 1.0\text{ \%IA g}^{-1}$ and a tumour-to-blood ratio of 38 at 4 h post injection. Orlova et al [21] showed that the stability and labelling kinetics of $Z_{\text{HER2:342}}$ were better when the isothiocyanate derivative of CHX-A"-DTPA $Z_{\text{HER2:342}}$ was used rather than [^{111}In]benzyl-DTPA. At 4 h post injection, tumour-associated ^{111}In activity was $10.3\pm 3.6\text{ \%IA g}^{-1}$ and tumour-to-blood was ratio about 190.

In common with binding to many receptor types, ligand binding to HER-2 receptors leads to the internalisation of the ligand-receptor complex and its subsequent disassociation in the lysosomes. When radiolabelled tracers are present, this can lead to the release of non-specific radioactivity into the tissue, which could seriously impinge on image quality. Using both continuous and interrupted cell incubation procedures, Wallberg et al [18] showed that the internalisation of the anti-HER2 Affibody monomer ^{111}In -DOTA- $Z_{\text{HER2:342-pep2}}$ is slow. Of the ^{111}In activity that became associated with HER-2-expressing cells during incubation with this Affibody monomer, 60–80% is retained for 24 h during incubation in tracer-free medium.

Positron emission tomography

In PET, emitted positrons interact with an electron and annihilate within a couple of millimetres of the decaying nucleus, producing two almost antiparallel gamma rays. These are detected by the PET camera, which consists of a ring of detectors located around the patient. Coincident events, determined by sampling over about a 10 ns period, are then used to create lines of response that are used to determine the location of the emission.

PET is about an order of magnitude more sensitive than SPECT and can detect molecules in the pmol l^{-1} range. Positron-emitting nuclides include ^{11}C , which can be incorporated into compounds such as amino acids and choline to produce radiolabelled versions of these molecules. The most commonly utilised PET tracer is the ^{18}F -labelled glucose analogue fluorodeoxyglucose (FDG), in which the ^{18}F replaces an H atom of 2-deoxy-D-glucose. Other positron-emitting halides, such as ^{124}I and ^{76}Br , are also utilised. There are also many positron-emitting metals, including ^{68}Ga , which can be linked to larger molecules such as antibodies via chelating groups.

Choline metabolism is closely associated with membrane metabolism and intracellular signalling. The incorporation of radiolabelled choline by tumour cells

has been shown to be associated with proliferation [22], so it would be expected that therapy response might be accompanied by changes in the incorporation of [^{11}C]choline. Kenny et al [23] demonstrated that uptake of [^{11}C]choline by two patients responding to trastuzumab treatment was lower than pre-treatment uptake, suggesting that [^{11}C]choline-PET may be useful in detecting the response of breast cancer to trastuzumab treatment. However, the ^{11}C nuclide has a $t_{1/2}$ of only 20 min, so ^{11}C -choline needs to be produced on site, limiting the number of PET centres that could use this tracer. ^{18}F -FDG is a more practical tracer as ^{18}F has a $t_{1/2}$ of 110 min. However, a recent paper reported that ^{18}F -FDG-PET incorporation did not predict the response to treatment with trastuzumab of two HER-2 overexpressing xenografts [24]. The incorporation of ^{18}F -FLT was found to be decreased in one of the responding xenografts.

Attachment of isotopes of iodine (e.g. ^{124}I , ^{123}I or ^{125}I) to peptides and proteins, including antibodies, can be achieved by conjugation of tyrosine residues with N-succinimidyl 4-iodobenzoate (PIB). Orlova et al [21] compared the uptake and retention of trastuzumab with that of an HER-2 Affibody labelled with radioiodide using PIB. They found that the tumour-to-organ ratio in nude mice bearing HER-2 overexpressing xenografts was higher for the Affibody than for trastuzumab at all time points measured (6, 24 and 72 h after administration) because of the more rapid blood clearance of the smaller molecule. Mume et al [25] also labelled trastuzumab with a positron-emitting nuclide. They used the prosthetic group N-succinimidyl 5-bromo-3-pyridinecarboxylate to attach the positron-emitting halide ^{76}Br . They were able to demonstrate good binding to HER-2 overexpressing SKOV3 cells *in vitro*, but the cell-associated activity was reduced to 33% after 24 h interrupted incubation.

A fragment of trastuzumab has also been labelled with ^{68}Ga using DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) as the chelating group [26]. This compound has been shown to image the HER-2 receptor successfully in HER-2 overexpressing xenografts, despite its short $t_{1/2}$ of ^{68}Ga (68 min).

The most ideal PET isotope is ^{18}F because it has low positron energy (0.64 MeV), so annihilation occurs within 1 mm of emission. ^{18}F is also a pure positron emitter and so has minimal scatter and gamma (non-true) coincidences. Kramer-Marek et al [27] labelled $\text{Z}_{\text{HER2:342}}\text{-Cys}$ Affibody with ^{18}F by conjugation to the prosthetic group N-2-(4- ^{18}F fluorobenzamido)ethylmaleimide ($^{18}\text{FFBEM}$). The tumour-to-blood ratio in xenograft-bearing nude mice was 7.5 at 1 h after injection, rising to 27 at 4 h after injection. Cheng et al [28] labelled anti-HER-2 monomeric and dimeric Affibodies with ^{18}F using site-specific oxime chemistry. When tested in mice bearing SKOV3 tumours, they found that the ^{18}F -labelled monomer was cleared more rapidly from the bloodstream, exhibited higher tumour uptake and achieved better tumour-to-normal tissue ratios than the ^{18}F -dimer. Similarly, using iodinated versions of monomer and dimers of $\text{Z}_{\text{HER2:342}}$, Tolmachev et al [29] recently demonstrated that the tumour uptake of the monomeric form was greater than that of the dimeric form at all time points studied post injection.

MRI

MRI is based on differences in the relaxation of protons in water molecules in different tissues. During an MRI scan, protons are lined up with a strong homogeneous magnetic field (B_0 field) within the scanner. Radiofrequency (RF) pulses are then briefly applied, causing the protons to adjust their alignment. When this RF is turned off, the protons realign with the B_0 field, producing a signal. The time taken for realignment is influenced by the environment of the proton and is the source of contrast within the image. The technique is intrinsically insensitive (detecting molecules at tens of $\mu\text{mol l}^{-1}$ to mmol l^{-1}) in comparison with radiotracer techniques) but can be improved by the use of contrast agents. These generally have paramagnetic qualities and reduce the relaxation of protons in their vicinity, so enhancing contrast.

The most common MRI contrast agent is gadolinium (Gd), and tissues that are enhanced with Gd appear very bright on T_1 weighted images. Receptor targets are generally expressed at relatively low concentrations in tissues, and their visualisation requires the presence many contrast agents attached to a single antibody. Zhu et al [30] attempted to use MRI to image HER-2 pre-targeted with biotin-trastuzumab using avidin conjugated with dendrimers, which are tree-like nanoparticles with multiple branches, each of which was functionalised with Gd-chelating groups. The avidin-dendrimer conjugate was found to accumulate in tumours but this was due to the enhanced permeability and retention (EPR) of the tumour vasculature and not due to specificity for the HER-2.

Another development in enhancing the signal in MRI is the SPIO (super paramagnetic iron oxide) nanoparticle, which contains several thousand iron atoms. Using SPIO-streptavidin conjugates, Artemov et al [31] detected HER-2 receptors that were pre-targeted with biotinylated trastuzumab. SPIO particles need to be surface coated to overcome problems of toxicity, phagocytosis by macrophages and biocompatibility. Using dextran-coated SPIOs conjugated to trastuzumab, Chen et al [32] imaged xenografts derived from HER-2-overexpressing cells in nude mice in a 3.0T magnetic resonance scanner.

Magnetic resonance can also be used to determine, non-invasively, the concentration of metabolites containing nuclei with spin $1/2$, which include phosphorus. Using ^{31}P nuclear magnetic resonance (NMR) spectroscopy, a number of studies have shown that the tumour content of phosphomonoesters (PMEs), which appear as a PME peak in tumour ^{31}P NMR spectra, change in response to therapy [33]. Rodrigues et al [34] applied this technique to HER-2-overexpressing tumours treated for 21 d with trastuzumab and found that the PME peak was increased after therapy.

Optical probes

The identification of malignant tissue *in vivo* is enhanced by the use of fluorescence-labelled probes, which can guide the surgical removal of tissue. Coincident administration of different coloured probes that target several cell

surface determinants can further facilitate tissue discrimination. Pre-clinical studies [35–37] have shown that cocktails of antibodies labelled with differently coloured fluorescent tags could differentiate between different tumour types. Thus, Longmire et al [35] recently demonstrated that the use of conjugates consisting of trastuzumab–rhodamine green to target HER-2 could discriminate HER-2-overexpressing cells from SHIN3–RFP (red fluorescent protein) tumour cells, which are engineered to express red fluorescence. Barrett et al [36] were able to detect and differentiate tumours overexpressing HER-1 and HER-2 24 h after administration of a mixture of Cy5.5-labelled cetuximab and Cy7-labelled trastuzumab. Koyama [37] used multifilter spectrally resolved optical imaging to detect tumours overexpressing HER-1, HER-2 and interleukin-2 receptor alpha-subunit receptor (IL-2Ra) grown as xenografts using cetuximab–Cy5 (targeting HER-1), trastuzumab–Cy7 (HER-2), and daclizumab–Alexa-Fluor-700 (IL-2Ra) labelled antibodies, respectively, in mice. Koyama [38] has also shown that a trastuzumab–RhodG conjugate could detect HER-2-overexpressing pulmonary metastasis in mice.

Fluorescent detection techniques using fluorescence-labelled trastuzumab [39] and HER-2 Affibody [40] have also been applied to the detection of tumour response to trastuzumab therapy. Gee et al [39] administered trastuzumab conjugated to the NIR (near infrared) dye CY5.5 to nude mice bearing xenografts derived from breast tumour cell lines expressing high and normal levels of HER-2. They showed that fluorescence detected by a whole-body multichannel imaging system on the surface of mice above the tumours corresponded with HER-2 expression.

In a further study of HER-2-overexpressing cells from xenografts, Gee et al [39] also found that the fluorescence from a further administration of tracer 14 days after initiating trastuzumab treatment was significantly lower than pre-treatment fluorescence levels, so predicting treatment response. They did not comment, however, on the potential interference from the non-labelled therapeutic trastuzumab that may have been present in the bloodstream during the follow-up scan but not during the pre-treatment scan.

Lee et al [40] compared the performance of three probes labelled with Alexa-Fluor-750 conjugate, including trastuzumab and albumin-binding domain (ABD)-fused ($Z_{HER2:342}$)₂ conjugate for imaging the HER-2 receptor. They found the optimal tracer accumulation by HER-2 expressing cells to be exhibited by the ABD-fused ($Z_{HER2:342}$)₂ conjugate. Importantly, through competition studies, they also found that the epitope recognised by the HER2-specific Affibody is distinct from the epitope recognised by trastuzumab. Thus, trastuzumab would not interfere with the binding of ABD-fused ($Z_{HER2:342}$)₂ conjugate, allowing its use to determine HER-2 expression levels during trastuzumab treatment.

Problems associated with optical imaging include limited tissue penetration of fluorescence and high tissue autofluorescence producing high background noise. The use of NIR light reduces the fluorescence background and enhances tissue penetration. Target-to-background signal levels can also be improved by the use of quenchers [41]. Ogawa et al [41] conjugated

trastuzumab to a fluorescent–quencher pair that is activated by dissociation of the quencher component in the cellular lysosomes after internalisation of the probe by HER-2-expressing cells. The pair produced images with high intracellular signal and low background fluorescence.

Dual-modality probes

A number of studies have reported the synthesis of dual-modality imaging probes that allow the combined detection of target cells using combinations of PET, SPECT, MRI and optical imaging [42–44]. These probes enable whole-body imaging procedures to be followed up by intra-operative fluorescent imaging or detailed laboratory analysis of resected tissue. Several groups have described the synthesis and testing of combined SPECT and optical imaging probes [42–44] by labelling trastuzumab with chelating groups and fluorescent tags. Sampath et al [43] synthesised a dual-labelled agent ($(^{111}\text{In-DTPA})(n)$ –trastuzumab–(IRDye800)(m)) for the detection of lymph node metastasis from primary breast tumours. They found that the time for clearance of trastuzumab from uninvolved axillary lymph nodes in mice was lengthy, potentially compromising the use of ^{111}In but not of the IR-detectable label in detecting involved tumour tissue. In an earlier study, Sampath et al [44] found that the binding of ($^{111}\text{In-DTPA})(n)$ –trastuzumab–(IRDye800)(m) by HER-2-expressing SKBr3 cells was greater than that of HER-2-negative MDA-MB-231 cells. Tumour-to-muscle signals from fluorescence and ^{111}In activity were only 2.25 and 2.66, respectively, which is low for a potential imaging agent. However, pre-treatment of mice for 24 h reduced both fluorescence and ^{111}In incorporation by SKBr3 xenografts, indicating that the uptake was specific for HER-2.

Huh et al [45] prepared a dual-modality tracer to detect HER-2 receptor targeting using MRI (iron oxide nanocrystal) and optical imaging (fluorescent dye). Upon incubating four cell lines exhibiting a range of HER-2 expression levels with this tracer, they demonstrated increased darkening on T_2 weighted images corresponding with increased fluorescence and HER-2 expression.

Ultrasound

Ultrasound imaging detects boundaries between tissues, for example, between fluid and soft tissue or between soft tissue and bone, by detecting the reflection of high-frequency sound pulses that are administered by a probe. Contrast enhancement can be achieved by the administration of echogenic particles, such as microbubbles, which do not leave the bloodstream and can be used to demonstrate blood flow. SKBr3 cells, but not HER-2-negative cells, incubated with polylactic acid nanoparticles attached to herceptin have been shown to exhibit enhanced echogenicity [46]. These nanoparticles are bioavailable and are stable for days or weeks, suggesting that translation to the clinic could be achievable.

Concluding discussion

Since the development and clinical employment of trastuzumab in treating HER-2-positive breast cancer, there has been a significant interest in the development of HER-2-targeting tracers for use with a number of imaging modalities. Lengthy blood residence times and the poor tumour penetration of full-sized antibody molecules have been surmounted by the use of Affibodies, small affinity molecules that can be labelled with short-lived isotopes such as ^{18}F and $^{99\text{m}}\text{Tc}$. Another advantage of HER-2 Affibodies is that they identify and target a different epitope to that of trastuzumab, so enabling HER-2 receptor imaging in the presence of trastuzumab. Therapeutic elimination of HER-2-expressing cells could be detected by serial measurements using HER-2 targeting tracers during the course of treatment.

Tracers that are based on labelled trastuzumab are more suited to imaging modalities that are not time limited from the point of tracer administration. Such modalities include MRI and those based on fluorescence. Fluorescence imaging, which is being developed partly to facilitate intra-operative detection of tumour tissue and detailed laboratory analysis, also has the advantage that the tissue is exposed, so assisting in the elimination of background.

Imaging measures signal from whole or large regions of lesions, which has the advantage of averaging out tumour heterogeneities. However, the amount of tracer binding per volume of tumour (and hence signal intensity) will be influenced by intertumour variation in stromal bulk, which in breast cancer can range from 5% to 95% [47], so diluting out signal from tracer that is bound to tumour cells. The tumour contrast therefore needs to be high to ensure the correct identification of HER-2-positive tumours. In some of the studies reviewed, tracer performance achieved very high tumour-to-normal tissue ratios, suggesting that medical imaging could be utilised to identify confidently patients with HER-2-positive cancer.

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