

Breast Cancer Treatment in the Era of Molecular Imaging

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Key Words

Breast cancer · Molecular imaging · PET · MRI ·
Optical imaging · Receptor

Summary

Molecular imaging employs molecularly targeted probes to visualize and often quantify distinct disease-specific markers and pathways. Modalities like intravital confocal or multiphoton microscopy, near-infrared fluorescence combined with endoscopy, surface reflectance imaging, or fluorescence-mediated tomography, and radionuclide imaging with positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are increasingly used for small animal high-throughput screening, drug development and testing, and monitoring gene therapy experiments. In the clinical treatment of breast cancer, PET and SPECT as well as magnetic resonance-based molecular imaging are already established for the staging of distant disease and intrathoracic nodal status, for patient selection regarding receptor-directed treatments, and to gain early information about treatment efficacy. In the near future, reporter gene imaging during gene therapy and further spatial and qualitative characterization of the disease can become clinically possible with radionuclide and optical methods. Ultimately, it may be expected that every level of breast cancer treatment will be affected by molecular imaging, including screening.

Schlüsselwörter

Brustkrebs · Molekulare Bildgebung · PET · MRT ·
Optische Bildgebung · Rezeptor

Zusammenfassung

Molekulare Bildgebung verwendet auf bestimmte Moleküle gerichtete Sonden, um bestimmte krankheitsspezifische Marker und Stoffwechselwege zu visualisieren und oft auch zu quantifizieren. Modalitäten wie die intravitale konfokale oder Multiphotonenmikroskopie, die Fluoreszenzbildgebung im nahen Infrarot, entweder als oberflächengewichtete Reflexionsbildgebung, als Endoskopie, oder als Fluoreszenz-medierte Tomographie, und Isotopenbildgebung mit Positronenemissionstomographie (PET) und Single-Photon-Emissionstomographie (SPECT) werden in immer größerem Ausmaß für Kleintiere genutzt. Die Anwendungen umfassen Selektionierungen mit hohem Durchsatz, Medikamentenentwicklung und die Beobachtung von Gentherapieexperimenten. In der klinischen Behandlung von Brustkrebs sind PET und SPECT sowie Magnetresonanz-basierende molekulare Bildgebung bereits für das Staging von Fernmetastasen und für den intrathorakalen Lymphknotentatus etabliert, sowie für die Patientenselektion hinsichtlich Rezeptor-gerichteter Therapien und um frühe Informationen über die Effektivität einer Behandlung zu erlangen. In der nahen Zukunft können die Bildgebung von Reporter-Genen während der Gentherapie und weitere räumliche und qualitative Charakterisierung der Erkrankung mit isotonenbasierten und optischen Methoden klinisch möglich werden. Letztlich kann erwartet werden, dass jede Ebene der Brustkrebsbehandlung von molekularer Bildgebung beeinflusst wird, einschließlich des Screenings.

Introduction

Current concepts of clinical diagnosis and treatment are increasingly influenced by the continuous progress in cellular and molecular biology techniques. The understanding of the molecular mechanisms underlying many pathologic processes, the capability to decipher and translate whole genomes, the identification of a multitude of receptors, enzymes and other molecules as effectors for targeted diagnosis and therapy have altered our understanding and management of disease today. Over the past 2 decades, imaging has played an ever increasing role in these processes by bridging the gap between new methods discovered at the bench on a molecular or cellular level and their direct, non-destructive visualization in a living macroscopic organism. Consequently, molecular imaging has become a rapidly emerging and heavily funded research discipline. While there is (and will be) no entirely satisfying definition discriminating it from 'morphologic' or 'physiologic' imaging, most authors agree that molecular imaging comprises the visualization (and often quantification) of biologic processes at the cellular or subcellular level by defined molecular interactions of an imaging probe with a target [1, 2].

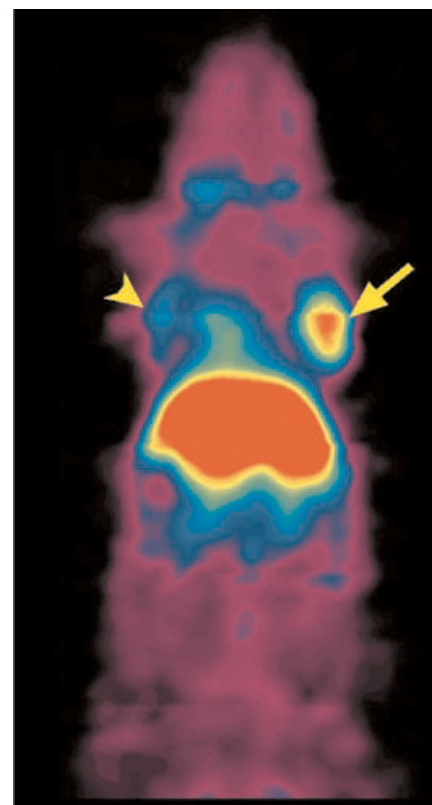
Traditionally, imaging was aimed at visualization of anatomic (e.g. tumor) or physiologic (e.g. hypervascularization) changes to identify pathologies or to assess reaction to treatment. Such changes, however, occur only as late manifestations of the cellular and molecular alterations causing the disease. The visualization of these early changes would allow for earlier detection and intervention at a stage where the result is more likely and easily influenced [3]. A number of different imaging modalities are currently used in the treatment of breast cancer in oncologic research or in the clinical setting.

Radionuclide Imaging Modalities

The two main modalities for molecular imaging using radioactive isotopes are single-photon emission computed tomography (SPECT), and positron emission tomography (PET). In SPECT, a rotating gamma camera visualizes a single high-energy photon from a gamma emitter. In PET, a coincidence camera visualizes only pairs of high-energy photons travelling in opposite directions which have been created by the annihilation of a positron from a beta⁺ emitter and an electron from the surrounding matter. PET can more easily be corrected for the attenuation effects of the surrounding tissue and is about an order of magnitude more sensitive than SPECT, mainly because of the lack of collimators which are inherently necessary for SPECT imaging.

The most common form of molecular PET imaging employs a labeled glucose analog, ¹⁸fluorodeoxyglucose (FDG). This molecule is actively transported into the cell by the Glut-1 glucose transporter where it is irreversibly phosphorylated by hexokinase, trapping it within the cell where it does not pro-

Fig. 1. Micro-PET image of a mouse injected with 26μCi of a ⁶⁴Cu-labelled anti-CEA minibody. Note the uptake in the CEA-positive tumor in the left shoulder (arrow), while no significant uptake occurs in the CEA-negative control tumor in the right shoulder (arrowhead). Unspecific uptake occurs in the liver. Adapted, with permission, from [53].



ceed further down the glycolytic pathway. The glucose transporter is overexpressed in many cancers including breast cancer, resulting in increased FDG uptake compared to normal tissue. [4].

In other approaches, several intra- and extracellular proteins (enzymes or receptors) [5] have been targeted, mostly with either radiolabeled ligands (fig. 1) or substrates that change their membrane permeability upon interaction with a target enzyme [6, 7]. While extracellular targets face less penetration barriers and therefore the probes have more favorable pharmacokinetics, the reaction product with intracellular targets is less likely to interact with the immune system and more likely to locally accumulate [1, 8]. In addition, the interaction with an enzyme can provide an important amplification step because enzymes can repeatedly react with multiple substrate molecules [5–7, 9].

Magnetic Resonance Imaging

Magnetic resonance (MR) imaging visualizes the relaxation time of hydrogen atoms, mainly in body water, in a static magnetic field. This time is altered by the chemical environment of the hydrogen atom (native imaging), and by local irregularities of the magnetic field, induced by the presence of paramagnetic or superparamagnetic substances (contrast-enhanced imaging). Compared to radionuclide imaging, MR imaging offers higher spatial resolution and limited sensitivity. Consequently, a high-

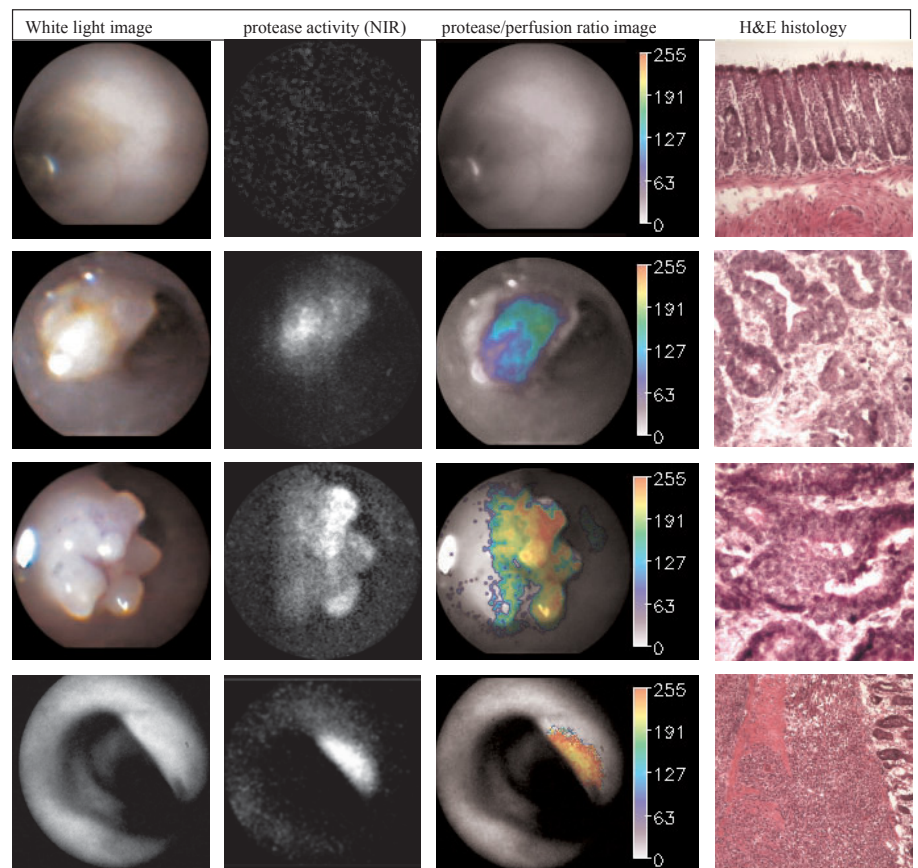


Fig. 2. In vivo mouse colonoscopy. In the first row, normal colon is shown. Neither in the NIR channel nor by ratio imaging a signal is detected. Second row, small APC^{Min/+} adenoma, 3rd row, large APC^{Min/+} adenoma, 4th row, invasive cancer. The NIR channel shows constantly areas of high signal intensity and high signal-to-background ratio corresponding to the areas of neoplasia. Ratio imaging shows moderately elevated values for small adenoma with moderate dysplasia, markedly elevated values for large adenoma with severe dysplasia, and high values for invasive cancer. The corresponding H and E sections demonstrate adenomatous formations with moderate or high dysplasia and tumoral invasion of the muscular layer. Adapted, with permission, from [21].

er molar concentration of the molecular imaging probe is necessary for efficient detection in the target tissue [10]. Usually, amplification strategies have to be incorporated into the design of a MR molecular imaging probe. This can be accomplished by targeting a receptor which undergoes endocytosis and receptor recycling upon interaction with the probe, thereby accumulating the probe in the cytoplasm [11]. A different approach is to employ a superparamagnetic nanoparticle, with between 20 and 50 binding sites per particle [12]. These sites can then be bound to antibodies, minibodies, or peptide fragments with a known high affinity to a cell membrane target [13]. By multivalent attachment, the probe reaches a disproportionately higher affinity to the target, thereby achieving a higher concentration [1].

Optical Imaging Modalities

Optical imaging visualizes bioluminescence or differences in absorption, reflection, or fluorescence of tissues. Newer techniques used in molecular optical imaging include diffuse optical tomography [14], molecular endoscopy, and intravital microscopy with confocal [15, 16] or multiphoton imaging [17–19].

Due to the absorption of light in living tissue, optical imaging methods generally suffer from limited penetration depths cur-

rently in the order of 1–10 cm. While a human whole-body optical imaging system will not be available in the foreseeable future, optical imaging is routinely practiced in small animals such as mice and rats [20]. In addition, proof-of-concept for minimally invasive approaches with fiberoptic systems or intraoperative applications exists. While some optical imaging methods such as fluorescence-mediated tomography are inherently quantitative, optical imaging offers the unique capability of a multichannel approach. By simultaneously imaging at 2 or more wavelengths, different probes can be visualized at the same time. This concept was used to determine the presence and at the same time to quantify the degree of dysplasia in precancerous and cancerous lesions in the murine colon in vivo (fig. 2) [21, 22].

The advent of probes that are optically silent and only become fluorescent upon interaction with one specific enzyme has considerably increased the signal-to-noise ratios by avoiding the enormous amount of unspecific signal during the application of the probe, which is common in most other imaging modalities [23].

Molecular Imaging in Breast Cancer Therapy

In the past years, new treatments have been developed that prolong survival, induce remission, and provide better quality

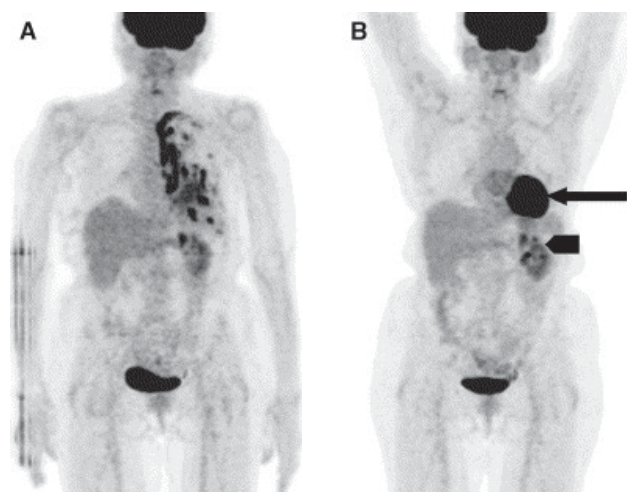


Fig. 3. Maximum-intensity projection of an FDG-PET scan with extensive left pleural involvement **A** before and **B** after 1 dose of fulvestrant, with resolved pleural uptake. Note unspecific cardiac uptake (arrow). Adapted, with permission, from [4].

of life for cancer patients [24]. The earliest studies on the use of FDG-PET in the diagnosis of breast cancer were in 1989 [25]. Since then, FDG-PET imaging has become the dominant molecular imaging modality for breast cancer, demonstrating its worth with advanced-stage disease, determining response to therapy, and in cases of suspected recurrent and metastatic involvement (fig. 3) [4, 26].

Axillary Node Staging

In the clinical setting, several studies have compared conventional MR imaging to FDG-PET in axillary lymph node staging. Generally, MR imaging showed higher sensitivity, and PET showed higher specificity in the detection of axillary nodal disease. This is due to the lower spatial resolution of PET, and the missed foci were correspondingly below 15 mm in size [27]. While there seems to be consensus that FDG-PET alone is not yet sensitive enough to allow the avoidance of axillary node dissection (AND) in negative studies [28], potentially the combination of sentinel node biopsy with FDG-PET may prove to have enough sensitivity for this purpose.

Optical imaging using near-infrared fluorescent (NIRF) macromolecular probes administered either intravenously or intracutaneously has given promising results in lymph node detection in the animal model [29]. However, due to its limited depth penetration, NIRF will likely have to be used in conjunction with endoscopic approaches or during partial dissection in human applications.

Staging Distant Disease

Contrary to its limited role in axillary staging, FDG-PET has been shown to be consistently superior to conventional imag-

ing modalities in staging of mediastinal, internal mammary, and distant disease [30–32] both in sensitivity and in specificity. In whole-body lymph node staging, the application of lymphotropic superparamagnetic nanoparticles with subsequent whole-body MR imaging has yielded excellent sensitivity and specificity in the detection of renal and prostate cancer metastases [33–35]. This concept can likely be transferred to breast cancer patients in the near future.

Molecular Imaging of the Estrogen Receptor

Hormonal therapy is one of the earliest and still most important targeted therapies of breast cancer. The estrogen receptor (ER) is up-regulated in most breast cancers, and halting the receptor-triggered cell proliferation is associated with tumor regression [36]. Current therapeutic concepts aim at either receptor blockade (tamoxifen), induction of ER down-regulation (fulvestrant), or by lowering ligand concentration (aromatase inhibitors) [37].

Since low or absent ER expression predicts a low likelihood of therapeutic response [38], molecular ER imaging could primarily function in directing hormonal therapy to receptor-positive tumors. A substantial percentage of patients with an ER-positive primary tumor can have one or more sites of ER-negative metastatic disease [39], thus, a complete ER status cannot be provided by biopsy in multicentric disease. A recent calculation assuming a hypothetical diagnostic algorithm including molecular ER imaging in the decision for hormonal therapy could double response rates without withholding appropriate therapy to potential responders [40].

Currently, no approved agent for PET ER imaging exists, but several compounds have been investigated [37]. Quantitative results correlating with in-vitro assays have been achieved with 16-fluoroestradiol-17 β , an estrogen analog [41].

Molecular Imaging in Breast Cancer Research

Perhaps the most important potential contribution of molecular imaging in breast cancer treatment is to affect therapy by visualizing and quantifying fundamental tumor cell processes such as proliferation, apoptosis, angiogenesis, and hypoxia [24].

Angiogenesis Imaging

Angiogenesis, the tumor-driven formation of new vessels by the host, is a fundamental prerequisite for tumor growth and metastatic spread [42]. Several receptors are up-regulated by endothelial cells during angiogenesis, and have been visualized with a receptor-targeted approach. The $\alpha_v\beta_3$ integrin ($\alpha_v\beta_3$) has been targeted with nanoparticles linked to peptides with the R-G-D motif or conjugated to antibodies [43]. Such conjugates have also been effective in localizing melanoma micrometastases in mice [44]. Endothelial selectin, which is up-regulated in inflammation and tumor angiogene-

sis, has been successfully imaged in a similar approach using an optically active nanoparticle conjugated to a peptide sequence with high affinity to the receptor [45].

Apoptosis Imaging

Apoptosis or programmed cell death is as important as cell proliferation in the development, regeneration, and homeostasis of an organism. Carcinogenesis is as much a breakdown of apoptotic mechanisms as it is a result of growth stimulation [46]. In addition, the development of blocks in the apoptotic signal cascade is the main reason why tumors develop resistance to chemo- and radiotherapy [47, 48]. A marker for apoptosis is annexin V, a protein that binds to externalized membrane components characteristic for apoptotic cells. It has long been used in histopathology, and recently, the feasibility of radionuclide [49, 50] and optical in-vivo imaging of apoptosis has been demonstrated [51].

Gene Therapy Imaging

In cancer treatment, gene therapy is aimed at the introduction or alteration of the cellular genome in the target tissue in an effort to either correct specific genetic alterations, to induce tumor cell death, or to provide a point of attack for targeted therapies. Experiments have been conducted on suppression of oncogenic products, alterations of receptor expression [52, 53], activation of apoptosis or tumor suppressor genes [54–56]. In a phase I clinical trial, a viral transcriptional regulator was introduced into cancer cells by a liposomal delivery system and has been shown to effectively target and repress tumoral ErbB-2 overexpression and induce apoptosis [57]. Reporter gene imaging has formed a substantial part of gene therapy from its beginning. Briefly, the vector which introduces the therapeutic gene also includes a gene whose protein product is expressed under the same promoter and in the same location and concentration as the therapeutic gene. It can be visualized in a molecular imaging modality, thereby helping to determine the efficiency of transfection, and the

amount and location of the transfected tissue. Such reporter genes typically include luciferase for visualization by bioluminescence [58], or an intracellular enzyme which converts a prodrug which is able to cross the cell membrane into the active imaging probe which can not pass the membrane and accumulates in the cell. Prodrugs include radiolabeled uracil nucleoside derivatives or acylguanosine derivatives, which can be phosphorylated by the HSV-thymidine kinase [53].

A different approach of gene therapy termed molecular chemotherapy aims at introducing effective pathways for drug delivery into the tumor via receptor expression or the induction of intracellular enzymes [52]. These products can then be visualized by MR imaging, near-infrared fluorescence, or fluorescence-mediated tomography [9].

An inherent problem with gene therapy is that each cellular transfection in itself has no effect on the surrounding tissue, thus, the majority of the tumor cells must be transfected for a clinical effect. Molecular imaging can potentially aid in the refinement of such protocols.

Conclusion

In the near future, we anticipate a growing availability and prevalence of molecular imaging techniques in breast cancer therapy. FDG-PET is already established as an effective clinical tool for the staging of intrathoracic and distant disease, while it can not yet replace sentinel node biopsy or axillary dissection. Whole-body ER imaging may be superior to biopsy in selecting patients for hormonal treatment, and radionuclide and MR-based modalities will aid in the monitoring of clinical studies of gene therapy. In laboratory research, optical imaging provides a high-throughput modality for drug development and monitoring of therapeutic efficacy. It can be expected that methods of molecular imaging, probably with optical probes, will ultimately even provide reliable screening tools for breast cancer.

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