NONINVASIVE IMAGING OF BREAST CANCER

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ABSTRACT

With the development of molecularly targeted cancer therapies, it is highly advantageous to be able to determine their efficacy, to improve overall patient survival. Non-invasive imaging techniques are currently available for visualizing different pathological conditions of the human body, but their use for cancer monitoring is limited due to the lack of tumor-specific imaging probes. This review will attempt to summarize the current clinical diagnostic approaches for breast cancer detection, staging, and therapy assessment. In addition, I will present some novel concepts from the field of molecular imaging that form the basis of some of our research. We believe that this general imaging strategy has the potential of significantly advancing our ability to diagnose breast cancer at the earliest stages of the pathology, before any overt clinical symptoms have developed, as well as to better direct the development of molecularly-targeted individualized therapy protocols.

Keywords: breast cancer, imaging, magnetic resonance imaging, cancer staging, muc-1


Natural history of breast cancer

Breast cancer is the second leading cause of cancer-related deaths in women of the western world. In the USA alone over 180 000 new cases are diagnosed each year. Of these patients, approximately 25% will succumb to their disease despite aggressive diagnostic and therapeutic intervention. Ten per cent of breast cancers are attributed to inherited mutations in highly penetrant breast cancer susceptibility genes, two of which (BRCA1 and BRCA2) have been identified based on genetic linkage studies of affected families (43, 49). Certain evidence suggests that there may be additional, less prevalent high penetrance breast cancer susceptibility genes, but these remain to be identified. The vast majority, 90%, of breast cancers are sporadic and associated with a considerable degree of molecular and clinical heterogeneity. Despite this heterogeneity, the natural history of breast cancer involves a well-defined progression through molecular and pathological stages starting with hyperplasia/atypical epithelial hyperplasia, progressing to in situ carcinomas, invasive carcinomas, and finally metastatic disease. Despite the fact that each step in the progression of breast cancer has been linked to a multitude of molecular genetic changes, the precise pathogenetic significance of these changes is poorly understood (65).

Atypical Ductal Hyperplasia (ADH)

Atypical ductal hyperplasia (ADH) is a lesion characterized by abnormal cell layers within the duct or lobule (26). Atypical ductal hyperplasia is typically viewed as a risk factor for and not as a direct precursor of invasive breast cancer (90). The first studies, at the beginning of the 20th century, leading to the concept of ADH revealed the presence of various types of intraductal proliferations and attempts were made to categorize these; the term 'atypical hyperplasia' or 'hyperplasia with atypia' was introduced and was used mainly to indicate the presence of proliferations of epithelial cells with cytonuclear atypia (90). Originally, ADH was described as usually unifocal and small, often less than 1 mm (or two spaces) in size (61). Subsequently, attempts were made to refine this definition and minimize inter-observer variability. These include morphological characterizations of the boundaries of the lesion, the degree of ductal involvement, and the extent of the lesion (90). Still, it remains questionable whether these refinements lead to improved inter-observer variability in the diagnosis of the lesion, even in the presence of consensus diagnostic criteria (85).

The most frequent molecular changes observed in ADH are losses of 16q and 17p (26). However, Lakhani and colleagues (39) demonstrated that LOH identified at loci on 16q and 17p in ADH is also present in invasive carcinoma and DCIS with a similar frequency. These studies raised questions about the validity of separating ADH from DCIS (39).

In situ carcinomas (DCIS)

Early stage cancer (ductal carcinoma in situ, DCIS) is associated with genetic instability, as its most informative biomarker (65). Tumor progression from DCIS to invasiveness is driven by the sequential acquisition of various genetic changes in a single cell followed by clonal selection and expansion. DCIS is believed to be the true precursor of invasive ductal carcinoma (IDC) based on its frequent coexistence with invasive lesions and on its high rate of recurrence as an invasive tumor at its original site (80, 81). Also, the histology of microinvasive carcinomas, invasive cancer cells measuring less than 1 mm protruding from ducts of DCIS, is also consistent with DCIS being a precursor of invasive cancer (73). Recent comparative molecular analysis of DCIS and invasive tumors further
supports this hypothesis and confirms that most invasive
tumors arise through clonal evolution from pre-existing in situ
disease (33).

Over the past 25 years, there has been a dramatic
improvement in our ability to detect DCIS. Still, our
understanding of the pathophysiology of this disease is still
poorly defined. This is complicated by the fact that DCIS is not
a single disease. Rather, it is a heterogeneous group of lesions.
Some DCIS, if untreated, will rapidly progress to invasive
cancer, while others will change very little in 5-20 years.
The challenge is to be able to predict the risk of recurrence
(particularly of the invasive lesions) and manage the patients
accordingly, sparing some unnecessary aggressive treatment.
Currently, there are several DCIS classification schemes
aiming to predict clinical outcome. A classification scheme
used until about a decade ago categorizes DCIS lesions based
on their growth pattern and architectural features. The major
problem with classification based on architecture is the lack
of reproducibility (intra- and inter-observer) and intra-tumoral
heterogeneity (65).

A sub-category of the architectural classification scheme is
based on the discovery that cytological features, particularly
nuclear grade, correlates better with outcome (5). High-grade
DCIS is associated with the highest rate of local recurrence
(25-30%), low-grade tumors have very low recurrence (0-
5%), while intermediate-grade tumors have a recurrence rate
somewhere between (10-15%) in 12 years of median follow-up
(65). However, determining the nuclear grade is still subjective
and, although nuclear grade seems to be a predictor of local
recurrence, it is unable to predict whether the recurrence will
be in situ or invasive carcinoma (65).

In addition to histologic features, surgical margins are
important predictors of local recurrence as well. However,
determination of the margins can be difficult in extensive
lesions and requires the careful examination of several blocks
and many slides from every tumor. Many times the patient
undergoes several excisions in order to achieve clear margins
(65).

A newer classification scheme is based on genetic and
epigenetic alterations in the lesions. Alterations of several
known tumor suppressor genes and oncogenes have been
studied in breast carcinomas including DCIS lesions. These
studies have confirmed that low-grade and high-grade tumors
are biologically different starting from the earliest identifiable
stages and have suggested that the progression from atypical
cystic lobules to low grade DCIS and low-grade IDC on the
one hand, and atypical ductal hyperplasia, high-grade DCIS,
and high grade IDC, on the other follow distinct molecular
pathways (48, 60). For example, high-grade DCIS lesions
and high-grade invasive carcinomas are more frequently
estrogen and progesterone receptor negative, have erbB2
amplification or overexpression, p53 mutation, decreased bel-
2 levels, hypermethylation of a distinct set of genes, and higher
proliferation/apoptosis rates than low-grade ones (65).

Invasive carcinomas
Distinguishing features of full-blown breast cancer include
sustained cell proliferation, disregard of growth and
differentiation controlling signals, resistance to apoptosis,
ability to invade surrounding tissue and induction of
angiogenesis (65). Pre-invasive cells express almost all of
the features associated with a full-blown cancer phenotype.
However, they lack the ability to invade surrounding tissue, a
property which has been related to tumor size. When growing,
a tumor accumulates genetic alterations, which may allow the
emergence of different cell sub-populations sharing essentially
the same ‘portrait’, but exhibiting minor phenotype differences.
One may speculate that a local complex cooperation between
these sub-populations might favor invasion. A growing
in situ tumor is also believed to exert a mechanical stress on its
neighboring basement membrane. Moreover, breast cancer
cell accumulation in a confined space might lead to local
concentrations of various secreted molecules (for instance
metalloproteinases (MMPs)) high enough to overcome the
mechanical and molecular resistance expressed (for instance
through secretion of MMP inhibitors) by the surrounding
normal cells (38).

From a molecular standpoint, according to the multi-step
view, progression from pre-invasive tumor should be
accompanied by the sequential acquisition of phenotype
changes, allowing breast cancer cells to invade, disseminate
and colonize distant sites. Surprisingly, most investigations
have revealed that progression is not accompanied by major
changes in marker expression or grade. For instance, grade,
tumor marker (P53, ERBB2, KI-67, ER, PR, BCL2 and
angiogenesis) were compared in 194 pure DCIS, 127 small
invasive lesions, and 305 lesions with both an invasive and in
situ component. Grade concordance was high between in situ
and invasive components of the same tumor. All markers were
found to correlate with grade rather than with invasiveness.
None of these markers were clearly associated with the
progression from in situ to invasiveness (38). Based on these
observations, it has been suggested that there are two distinct
pathways in the multi-step model of breast cancer progression
to invasiveness: one comprising well-differentiated DCIS that
progress to grade I IDC, and the other encompassing poorly
differentiated DCIS that progress to grade III IDC (83). In the
low-grade arm, tumors are of low nuclear grade, usually ER
and PgR-positive, negative for Her-2 and basal markers, and
harbor low genetic instability and recurrent 16q loss. Whereas,
in the high-grade arm, the lesions show a higher degree of
nuclear atypia, are more frequently hormone receptor-
negative, frequently positive for either Her-2 or basal markers,
and are genetically advanced lesions, showing a combination
of recurrent genomic changes. These include loss of 8p, 11q,
13q, 14q; gain of 1q, 5p, 8q, 17q; and amplifications on 6q22,
8q22, 11q13, 17q12, 17q22-24, and 20q13 (15, 16, 68, 70, 77).

These findings indicate that breast tumor phenotype does
not change extensively during tumor progression from in
situ carcinoma to invasive carcinoma and that tumor grade
to a greater extent than histological subtype can be used as a predictive marker of tumor progression. However, nearly all of the studies described to date show percentages of concordance in the 65–95% range (38). This clearly points to the existence of a substantial number of cases in which progression (to invasiveness, to metastasis or recurrence) is in fact accompanied by qualitative changes in marker expression. One of the few markers whose abundance has been correlated with tumorigenesis and tumor stage and which is the subject of the current investigation is MUC-1. Its relevance to the transition from in situ to invasive disease has been addressed in detail below.

Metastatic Disease
Metastatic breast cancer cells are often believed to have accumulated phenotypic alterations, as they are associated with late stages in tumor progression. In addition, metastatic cells may colonize various tissues that are highly different from the breast (bone, lung, brain, etc.) after having completed all steps of a complex process including local invasion, intravasation, resistance to blood pressure, adhesion to blood vessels, and extravasation. This suggests that they have sequentially acquired specific adaptive properties and it has thus been hypothesized that metastatic and recurrent cells could express a phenotype significantly different from that observed in the primary tumor. Surprisingly, studies comparing the expression of various markers and the histological grade in primary tumors and their metastases have shown a high level of similarity between the two, with concordance values ranging from 75 to 95% (7, 13, 24, 36, 50, 56, 79, 82, 87).

In summary, many attempts have been made to identify molecular events accompanying the development and progression of breast cancer. In spite of this, the mechanisms underlying tumor invasion and dissemination remain largely unclear. One of the current progression models for ductal tumors proposes that carcinoma in situ may evolve into invasive ductal carcinoma and subsequently produce metastases through an accumulation of molecular abnormalities possibly allowing carcinoma and subsequently produce metastases through an accumulation of molecular abnormalities possibly allowing extensive phenotype changes and gain of aggressiveness. To describe this progression, the ‘clonal hypothesis’ has generally been well received in the breast cancer community.

However, the data presented here indicate that most breast carcinomas cannot be viewed as a collection of a few successive clonal populations being associated with the major stages of progression. Rather unexpectedly, in situ and invasive components of carcinomas appear very similar, and this similarity has also been repeatedly observed in metastases, regardless of their localization, and in recurrences. In fact, at any step of their progression, breast tumors may be rather considered as collections of cell sub-populations exhibiting the same general pattern of gross recurrent genetic alterations and sharing the same major phenotypic features. Still, although the tumor phenotype remains essentially stable, genetic alterations do accumulate during progression. Micro-heterogeneity exists, due to minor (low-frequency) DNA changes, generally restricted to small sub-populations of breast cancer cells. This could result in minor phenotype differences that can be explored as biomarkers of breast cancer progression (38).

Imaging of breast cancer
Considering the life-saving potential of surgical and chemo-/radiotherapeutic intervention, the most reliable predictor of overall cancer survival and therapeutic outcome in breast cancer patients is the stage at which the tumor is diagnosed. There is a wealth of imaging modalities capable of evaluating the tumor and the tumoral response to therapy. Mammography and sonography are the methods most widely utilized in routine clinical practice. These modalities primarily evaluate tumor volume and number of lesions. Other modalities, not only based on measurements of the size and the number of lesions, but on a functional analysis, such as uptake of contrast media and richness of neo-vascularization (MRI), or on the detection of a physiopathological tumoral activity (scintigraphy, PET) are emerging as valuable tools for breast cancer diagnosis and staging (59).

Mammography and Ultrasound
Conventional mammography and ultrasound are the methods most used for the initial staging and the assessment of tumor response to chemotherapy. The main parameter assessed by these modalities is relative tumor volume. The accuracy of this measurement depends on the contrast between the tumor and the surrounding normal tissue and increases with the difference in densities or echogenicity between tumor and surrounding tissue, especially when the limits of the tumor are sharp. A major shortcoming in the application of mammography for tumor staging stems from the fact that mammography does not allow assessment of the histological grade or prediction of the histopathological response but rather relies on the measurement of tumor size, which is a relatively late marker of disease progression or therapeutic response (59).

Reports on the reproducibility and reliability of ultrasound for breast tumor detection and monitoring are controversial. During treatment, the tumoral density decreases on successive mammograms. This modification of tumoral echogenicity induced by chemotherapy limits the reproducibility of the measurements after treatment (6). There seems to be consensus that ultrasound is a good modality for measuring edema and for examining lymph nodes (sensitivity 72%–84%, specificity up to 97% with high frequencies probes) (14, 96).

Magnetic Resonance Imaging
MRI allows morphological analysis of tumors and kinetic study of the contrast enhancement reflecting the richness of the vascularization. Most authors find an excellent correlation between the macroscopic tumor size and the tumor size established by MRI and think this method is better than mammography and mammography combined with ultrasound. Furthermore, MRI is useful in distinguishing benign from malignant lesions based on the architecture of the enhancement (72). For vascular assessment of the tumor, the diagnostic
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Mammography
Mammography uses technetium-99m methoxyisobutyl-isonitrile (MIBI). The uptake of this tracer by the tumor after intravenous injection is superior to that of the normal breast tissue. This uptake would be due to the hypervascularization and alteration of the cellular membrane and metabolism. This marker has a relationship with tumor physiopathology, thus it is useful to evaluate the response to chemotherapy. Images are analyzed both qualitatively and quantitatively with determination of the uptake ratio between the lesion and the normal tissue (L/N ratio) (59). A good correlation has been established between L/N ratio and histological response (42).

Positron Emission Tomography
In the most common clinical scenario, PET utilizes $[^{18}F]$ Fluorodeoxyglucose (FDG) as a tracer of glucose metabolism of the cancer cell, in order to detect and monitor breast tumors. PET has numerous advantages over several of the other modalities. It relies on an earlier predictive biomarker of therapeutic response (glucose metabolism) (71) and allows a pre-surgical staging by detecting eventual metastasis and adenopathies. Coupling PET with CT, increases spatial resolution and allows the collection of morphological as well as metabolic data (59). Besides $[^{18}F]$Fluorodeoxyglucose, several other ligands targeting proliferative activity ($[^{18}F]$-FLT) and protein synthesis L-$[^{18}F]$alpha-methyltyrosine, $[^{11}C]$methionine, $[^{18}F]$fluoroalanine, and $[^{11}C]$tyrosine may complement the value of PET by providing unique information about biological characteristics of breast cancer across primary and metastatic tumor sites (10). In addition, radioligands have been developed to assess hormone (estrogen receptor), and cell-membrane (Her2/neu) receptor status, using nuclear molecular imaging. However, due to the fact that the expression level or availability of these receptors does not correlate with tumor stage, and is almost always known from a histopathology specimen, they add little to the initial diagnosis of breast cancer. The key potential role of these ligands is to assess recurrent breast cancer in women with a history of an ER-positive primary tumor (10).

Molecular Imaging
The molecular imaging approach for detection, staging, and monitoring of breast cancer relies on the development of contrast agents specific to tumor antigens with relevance to tumor progression and therapeutic outcome. There are numerous examples of nuclear molecular imaging applications for the assessment of hormone and cell-surface receptor status (10, 22, 35, 58). In the field of MRI, there have been attempts to monitor the expression levels of the transferrin receptor (TfR), which is linked to tumor grade and metastatic potential (9, 32, 67). The transferrin receptor is a cell-surface internalizing receptor responsible for almost all iron sequestration in mammalian cells. Over-expression of the TfR on cancer cells is presumably secondary to increased cellular metabolism, for which iron is required. Conjugating ligands for the TfR to an MR contrast agent (superparamagnetic monocryalline iron oxide nanoparticles [MIONs]) selectively increased the uptake of MIONs into cells overexpressing the receptor, resulting in an altered MR signal. Several studies by us and others have shown that as little as a fivefold (37, 53) overexpression of the TfR on tumor cells can be detected using Tf-MION, which results in a 400-fold increase in MR imaging sensitivity in vivo. Molecular MRI has also been used for the detection of breast tumors based on their expression of the Her2 receptor (3, 4).

Another tumor antigen, which is the subject of our research is the underglycosylated MUC-1 antigen. We have extensively investigated the utility of a uMUC-1-targeting dual-modality (MRI/near infrared optical imaging) contrast agent (MN-EPPT) for specific tumor delineation in a variety of tumor models, as well as for monitoring tumor response to chemotherapy in pre-clinical pancreatic and breast carcinoma (46, 47, 54).

It is clear that the development of breast cancer has a well-defined molecular basis, which becomes apparent at the very onset of the pathology, before any anatomical or physiologic symptoms have developed. Due to its tumor-antigen targeted nature, molecular imaging has the potential to directly probe for these early events in vivo, in authentic physiologic environments, and over time. As a result, molecular imaging
has the potential to be the most accurate imaging modality for early breast cancer detection, staging, and evaluation of therapy.

**Imaging target- uMUC-1 tumor antigen**

In our search for a suitable imaging and therapeutic target, we focused our attention on epithelial cell mucin, the product of the MUC-1 gene. Mucin-1 is not a classic extracellular complex found on the mucous layers covering gastro-intestinal and respiratory tracts, but a transmembrane molecule, expressed by most glandular epithelial cells (2, 99). Several important features make mucin-1 an attractive molecule for targeted imaging of breast tumors.

First, MUC-1 is overexpressed on over 90% of human breast cancers (29, 55, 62, 95). Understandably, because of its abundant expression and remarkable relevance to the development of the pathology, MUC-1 is the most widely used serum marker for breast cancer (1).

Second, in adenocarcinomatous tissue the ordered architecture of the gland is lost, and basolateral and apical cell surfaces are not distinct. As a result, in cancer cells, which have lost polarity, MUC-1 is ubiquitously expressed all over the cell surface (8). Because of its rod-like structure, the molecule extends more than 100-200 nm above the surface, which is 5-10 fold the length of most membrane molecules (88). This feature makes this molecule an accessible target for imaging and, possibly, therapeutic probes.

Third, whereas in normal tissues mucin-1 is heavily glycosylated (50-90% of its molecular mass is due to carbohydrates), in neoplastic tissues, MUC-1 is underglycosylated. The biochemical basis of the altered glycosylation in tumor-associated MUC-1 has been well investigated. Normal mucin-1 consists of a tandemly repeated 20-amino acid sequence found in the extracellular portion of the molecule and present in 30-90 copies, comprising a variable number tandem repeat (VNTR) polymorphism. Serine and threonine residues on the protein backbone serve as attachment sites for oligosaccharide moieties bound to the amino acid residues by O-linkages through N-acetylglactosamine. Within each repeat sequence, there are five potential glycosylation sites. However, in the malignant state, the oligosaccharide chains are prematurely terminated by the addition of sialic acids limiting their branching potential (8). Reduced glycosylation permits the immune system to access the peptide core of the tumor-associated mucins (18). This modification of the antigen reveals epitopes associated with the core protein which in the normal cell are masked by glycosylation in tumor-associated MUC-1 has been well documented. For example, the peptide sequence N-acetylgalactosamine bound to the amino acid residues by β₁,3-sialyl linkage (80). This feature makes this molecule an accessible target for imaging and, possibly, therapeutic probes.

Fourth, the extracellular domain of MUC-1 extends 300-500 nm above the cell surface, thus interfering with the interaction between adhesion molecules on the tumor cell surface and their ligands on lymphocytes, aiding in the inaccessibility of tumor epitopes to immune recognition (34). Therefore, there is a lack or no response to immunotherapy and, hence, no tumor antigen downregulation. The expression of MUC-1 on tumor cells of epithelial cell adenocarcinomas remains homogeneous upregulated during the life of the tumor (63). Furthermore, the MUC-1 antigen is found both on primary tumors and tumor metastases (55). These features are important in designing probes for different stages of tumor progression. A number of investigations have focused on the potential to use the tumor-associated MUC-1 antigen for immunotherapeutic purposes. Multiple monoclonal antibodies have been produced using the PDTRP sequence of the tandem repeat as an immunogen (30, 51, 63, 64, 93, 94). The benefit of specific targeting is obvious, in that high selectivity and affinity of receptor ligands enable the use of low (nanomolar) doses of the compounds, which results in a high signal-to-noise ratio. However, when antibodies were used as targeting molecules, the immunogenicity and long plasma half-life of these proteins were detrimental (25). Consequently, the use of small peptides instead of antibodies or antibody fragments may eliminate these shortcomings, because peptide ligands are nonimmunogenic and combine high affinity and selectivity for receptors with more desirable pharmacokinetic properties.

In our studies we have traditionally used a synthetic peptide, EPPT1 (Y Corey PPTRTFAYWG), derived from the CDR3 V_{H} region of a monoclonal antibody (ASM2) raised against human epithelial cancer cells (30, 84). Analysis of the peptide structure revealed a β-strand type portion of the sequence as the active binding site (31). The EPPT1 synthetic peptides bind the glycosylated mucin-derived peptide PDTRP (VTSPDTPPAAGST). Deglycosylated mucin was found to have an ordered, rod-shaped structure defined by the presence of the APDTRP sequence as a knob protruding away from the rod. That property of the APDTRP sequence is the basis for its exclusive immunogenicity in the underglycosylated mucin-1 protein (17). In a previous study, the EPPT1 peptide, labeled with $^{99}$Tc, was used to image breast carcinomas in vivo (84). The $^{99}$Tc-labeled EPPT1 peptide is the basis for a commercially developed breast cancer imaging agent.

All of the features of the MUC-1 protein listed above (overexpression throughout the cytoplasm and the cell surface in tumor cells, aberrant glycosylation exclusively on tumor cells, and stability of the protein core) make this molecule an ideal candidate for a potential imaging target.

The capacity to noninvasively detect tumors based on the uMUC-1 antigen and the ability to quantitatively assess relative uMUC-1 availability in these tumors holds great potential as a tumor staging tool because MUC-1 undergoes well-defined molecular changes with tumorigenesis, manifested as an increase in expression, more extensive deglycosylation, and alterations in its subcellular localization (19, 20, 44, 97). In a recent systematic study of females with either no disease, benign disease, stage I, stage II, stage III, or stage IV breast cancer, MUC-1 serum levels reflected a distinction between different groups. MUC1 levels were slightly elevated in women with benign disease compared to healthy controls,
and very significantly elevated with more advanced stages of the disease (11). The value of MUC-1 for tumor staging is especially high because MUC-1 is expressed very early in the transformation process. In fact, MUC-1 overexpression has been implicated as a causative factor for tumorigenesis in a breast cancer model (76). In humans, aberrant expression of MUC1, has been found in atypical ductal hyperplasia lesions, implying that aberrant expression of the antigen indicates a higher risk for developing subsequent invasive breast carcinoma (52). MUC-1 is also found in ductal carcinoma in situ (21, 52) and, remarkably, its pattern of expression in tissue has direct relevance to the capacity to distinguish clinically-relevant functional subtypes of DCIS, making uMUC-1 an excellent predictive marker of tumor progression and local recurrence (21). An example of the value of MUC-1 in defining the differential diagnosis of early breast lesions is presented by a recent study which found differences in MUC-1 tissue distribution between patients with invasive micropapillary carcinoma (IMPC) of the breast, which is a special subtype of invasive ductal carcinoma (IDC) with a high potential for metastasis and poor prognosis and conventional IDC, which is less invasive (41). Similar conclusions about the value of MUC-1 in predicting the invasive potential of early ductal carcinoma in situ were based on the observation that elevated cytoplasmic expression of MUC-1 in human specimens of pure carcinoma in situ was associated with higher-grade forms of the disease (23).

In addition, the expression of MUC-1 not only in the early stages of disease but also as the tumor progresses from local to invasive is an independent predictor of clinical outcome. Among a panel of breast-cancer markers, MUC-1 was found to be the most accurate predictor of both relapse-free survival and overall survival in patients with invasive ductal breast carcinoma (91). As a molecule directly involved in cell adhesion and proliferation, it is not surprising that MUC-1 expression is highly correlated with both tumor growth and metastatic potential, making it an important predictive marker not only for the transition from early to locally advanced disease but also for the progression to advanced malignancy (57, 66, 75, 89).

In addition to being an excellent marker for the prediction and characterization of tumor progression, MUC-1 availability is also directly related to treatment outcome, making this target antigen one of the most widely used markers of therapeutic response and disease recurrence, particularly in patients with locally advanced and invasive disease (1). In patients treated with neoadjuvant chemotherapy, MUC-1 levels were found to be consistently reduced, suggesting that MUC1 can be explored as an intermediate biomarker for assessment of treatment and prognosis (27). Having in mind that MUC-1 is a causative factor for proliferation and metastasis, it is not surprising that downregulation of MUC-1 as a form of therapeutic intervention decreases the invasive potential of cancer cells, reduces metastatic burden, and improves survival (74, 78, 98).

 Whereas most currently available tests for tumor detection and staging based on uMUC-1 depend on either biopsy or serum specimens, these approaches have certain disadvantages compared to our method. Biopsy is invasive, whereas imaging would probe for molecular changes in breast tissue completely noninvasively. Serum testing is minimally invasive but it is somewhat indirect. It does not detect local events at the level of the native breast epithelial cell but rather examines the availability of antigen shed from epithelial cells which may become available in the circulation at sufficient quantities for detection relatively late. By contrast, our approach relies on direct monitoring of local breast tissue with high spatial and temporal resolution and adequate sensitivity to changes in antigen availability, allowing for the detection of the earliest molecular changes preceding the appearance of serum markers or overt symptoms.

In summary, a major focus of the breast cancer research and clinical community is on defining the functional, cellular, and molecular characteristics of breast lesions to augment and refine the classification of these lesions for determinations of risk of tumor progression and therapeutic outcome. One of the key tools for the accomplishment of these goals are provided by the field of non-invasive imaging and particularly molecular imaging. The specific value of non-invasive imaging is underscored by the capacity of this methodology to deliver quantitative information in intact authentic physiologic environments. As such, non-invasive imaging provides both a spatial and a temporal dimension in which biological processes can be monitored simultaneously or near-simultaneously.

What’s even more important and unique about noninvasive imaging is its innate capability to address biological questions on a systems level, thus embodying a new perspective (integration instead of reduction) to study biological systems. This integrative approach has the potential to not only verify biological theory generated through traditional reductionist methods but also to provide a new unprecedented understanding of how system properties emerge and how the interplay between causes and effects define the biological networks that ultimately build a living organism.

From a more applied perspective, noninvasive imaging represents one of the key tools in the hands of clinicians for the early diagnosis of disease based on anatomic, physiologic, metabolic, or molecular biomarkers, often flagging disease before any overt clinical symptoms have developed and at a point when effective treatment is still possible. The same principles underlie the value of noninvasive imaging for disease prevention, staging and prognosis, as well as for the design, testing, and optimization of therapeutic strategies.
REFERENCES