'Omics'-based imaging in cancer detection and therapy

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Genomics, proteomics and metabolomics, which can be also summarized as 'omics', have become increasingly interrelated with imaging. Gene expression profiling may be assessed using high-density microarrays for the detection of overexpression patterns, followed by the development of histochemical assays. Next, antibodies to the gene-corresponding proteins (for example, receptors) can be produced, leading to serum immunoassays for follow-up as well as to antibody-guided *in vivo* imaging or therapy. *In vivo* imaging for cancer detection and/or therapy can be performed by applying nonlabeled antibodies, by using radiolabeled antibodies for detection using single photon tomography or positron emission tomography (PET), or by other tracers, for example, for magnetic resonance imaging tomography (MRI, MRT). Protein profiles from protein chips can be derived from mass maps obtained through mass spectrometry (MS). Electrophoretic separation of proteins has also been combined with MS to produce a two-dimensional assignment of proteins within a complex mixture. Overexpression of tumor-related proteins can be used for the development of antibodies to develop noninvasive assays which can be used in the screening of risk groups as a basis for further investigation by invasive imaging methods. Metabolomic profiling by nuclear magnetic resonance spectroscopy can be applied for the detection of biomarkers of the metabolome. Metabolite profiles in cells, tissues, and organisms can be generated with nuclear magnetic resonance spectroscopy and MS. Metabolic information provided by magnetic resonance spectroscopic imaging (MRSI) combined with the anatomical information provided by MRI can significantly improve the assessment of cancer location and extent, and cancer aggressiveness. Biomarkers found by MRSI can lead to new PET tracers. This article provides examples and discusses some of the recent achievements to bring forward novel strategies for the diagnosis and therapy of cancer.

Keywords: genomics, HER2, magnetic resonance imaging, magnetic resonance spectroscopic imaging, metabolomics, microarray, NMP22, nuclear magnetic resonance, optical imaging, positron emission tomography, proteomics, reporter gene, reporter probe, risk group screening, single photon tomography

future medicine

The genes contained within the chromosomes of cells serve as the hereditary blueprints for the construction of proteins. Each gene specifies a distinct protein with a unique sequence of amino acids. This amino acid sequence ultimately dictates the three-dimensional structure of a protein, which determines its biochemical interactions and specialized function. It is estimated that of the 32,000 human genes, approximately 1200 are responsible for 1600 disorders. The challenge now is to connect the proteins encoded by these genes and the final metabolites to establish how physiological processes are mediated and regulated, in order to identify biomarkers for the *in vitro* or *in vivo* diagnosis and treatment of disease in a new era of personalized medicine [1,2]. Detecting cancer diseases at an early stage, predicting how they will behave and act in response to therapy, as well as the *in vitro* and *in vivo* identification of new targets for therapeutic intervention, are among the main areas of research that will benefit from the current rapid increase in the number of assays and technologies emerging within genomics, proteomics and metabolomics, which can be also summarized as 'omics'.

The purpose of this article is to introduce some of the recent biomarker achievements and their applications in imaging, which bring forward new strategies for the diagnosis and therapy of cancer.

Profiling of biomarkers by genomics, proteomics & metabolomics

Genomics

Genetic profiling enables the establishment of expression patterns for thousands of genes simultaneously from a single specimen. The initial gene microarray chips contained cDNA for a few thousand genes, but it is now possible to assess well over 20,000 genes per array. Gene expression profiling may be assessed by using high-density microarrays composed of cDNA spotted arrays on glass slides, spotted oligomeric DNA arrays, or commercially available oligomeric DNA chip arrays. Scanning of the DNA

microarray identifies the DNA probe hybridization levels, which reflect the gene expression levels in the samples investigated [3–5]. It is possible to determine whether certain genes are overexpressed or underexpressed in a specific tissue. Clinical information regarding the patient can then be correlated with gene expression.

The amplification of the human epidermal growth factor receptor (*HER2*) gene and overexpression of the protein is a good example for the interrelationship of gene expression and imaging. In solid tumors the predominant genetic mechanism for oncogene activation is through the amplification of genes. The *HER2* (synonyms: *ErbB2, c-erbB2, HER-2/neu, HER-2, her2*) oncogene is a proto-oncogene that encodes a transmembrane protein, which is considered to function as a growth factor receptor. Amplification of the *HER2* gene and overexpression of the protein have been identified in 10–34% of invasive breast cancers [6,7], and has also been found to be amplified in other forms of cancer [7,8]. Alongside its important role in tumor induction, growth, and progression, the HER2 molecule is also a target for a new form of antibody therapy, often combined with chemotherapy. Since 1998, breast cancer patients have been treated with considerable success with Herceptin® (trastuzumab, an anti-HER2 humanized monoclonal antibody), a recombinant antibody designed to block signaling through the HER2 receptor [9–11]. The most important prerequisite for the optimal efficacy of trastuzumab-based therapy remains a very strict selection of those patients with tumors that have HER-2/neu overexpression [12].

In addition to trastuzumab, a large number of various HER2-directed immunological and genetic approaches, targeting either the HER2 receptor, its signaling pathways, or both the HER2 receptor and the epidermal growth factor receptor (EGFR) together, have demonstrated promising preclinical potential for HER2 amplified carcinomas [13,14].

With respect to assays for *HER2* amplification, immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) have been approved for trastuzumab selection by the US Food & Drug Administration (FDA). Chromogenic *in situ* hybridization (CISH) features the advantages of both IHC (routine microscope, lower cost, familiarity) and FISH (inbuilt internal control, objective scoring and more robust DNA target).

It is important to distinguish between assays that depend on tissue obtained via invasive diagnostic procedures on a fixed date, such as IHC or FISH, and noninvasive serum assays that can be carried out at any time for assessment of the current state of the disease or its response to therapy. The noninvasive approach has the advantage of reflecting the current state of disease, can be evaluated quantitatively for the interpretation of therapy effects during follow-up, and is independent of surgery. The *HER2* oncogene and its p185 receptor protein are indicators of a more aggressive form of breast cancer. *HER2* status guides trastuzumab therapy, specifically directed to the extracellular domain (ECD) of the HER2 oncoprotein. The HER2 ECD is shed from cancer cells into the circulation, and its concentration can be quantitatively determined by immunoassay (HER-2/neu Microtiter enzyme-linked immunosorbent assay (ELISA) and HER-2/neu ADVIA Centaur® (Bayer HealthCare, MA, USA). The literature concerning HER2 has been extensively reviewed by Ross and colleagues and Carney and colleagues [6,14,15].

Within the frame of these developments, molecular imaging for detection of HER2 *in vivo* is on its way. There are different *in vitro* and *in vivo* experimental approaches, including and positron emission tomography (PET), magnetic resonance (MR) and bioluminescence.

Proteomics

In order to compare protein profiles from tumor samples and tumor-free samples, tumor-specific proteins can be selected, sequenced and identified in databases. The field of proteomics has expanded rapidly since the completion of the human genome, and the realization that genomic information alone is insufficient to comprehend cellular mechanisms. In recent years, new proteomic microfluidic and array technologies have been developed. These novel approaches may help to solve the technical challenges faced in proteomics. The microarray format is frequently used to carry out high-throughput experiments to discover proteins in a proteome. Microarrays facilitate large-scale studies, due to the miniaturization, automation, and parallelism that the microarray format provides, making it the universal biochemistry platform of choice. The applications for protein microarrays in cancer include protein expression profiling, serum-based diagnostics, biomarker discovery, protein–protein binding assays, drug target binding, receptor epitope binding, and epitope mapping.

For the detection of proteins, samples are generally pretreated to remove lipids, immunoglobulin and human serum albumin. Following this,

fractionations are made by sodium chloride (NaCl) gradients. The fractions can be subsequently analyzed by electrophoresis and/or applied to protein chips. Protein profiles from protein chips can be derived from mass maps obtained through mass spectroscopy.

Electrophoretic separation of proteins has also been combined with MS to produce a two-dimensional assignment of proteins within a complex mixture. In this procedure protein mixtures are initially resolved first by charge, or isoelectric point, and then by relative molecular mass to reduce the complexity of each protein into resolved spots. Individual spots are then sequentially analyzed by MS, either matrix-assisted laser desorption/ionization time of flight MS (MALDI-TOF-MS), surface-enhanced laser desorption/ionization time-of-flight MS (SELDI-TOF-MS), or electrospray ionization tandem MS (ESI-MS/MS).

Proteomics provides a good foundation for the identification of novel cancer biomarkers and the early detection of cancer [16]. An example is the application of the nuclear matrix protein 22 (NMP22) in urology. This marker indicates early stage urinary bladder cancer and has become an important aid as an indication for imaging bladder cancer by cystoscopy [17–21].

Metabolomics

Nuclear magnetic resonance (NMR) spectroscopy has made important contributions to the metabolic profiling of biomarkers of the metabolome. This technique is based on the fact that certain isotopes posses the property of magnetic spin, causing their nuclei to behave in a similar manner to a tiny bar magnet. When they are placed in a magnetic field, the magnets either align with or oppose the external magnetic field. By applying a radio frequency to the nuclei, one can cause the nuclei to flip into the other magnetic state and the differences in the populations between these two magnetic energy states can be detected as a radio wave as the system returns to equilibrium. Molecular imaging in whole-body MR tomographs has employed so far the spin-tracer nuclei ^{1}H, ^{13}C, ^{19}F and ^{31}P. The combination of high-resolution magnetic resonance spectroscopy (MRS) with MR imaging methods for the purpose of chemical shift imaging or spectroscopic imaging (SI) permits the acquisition of molecular images of the human body noninvasively and *in vivo*. These data show the distribution of specific intracellular metabolites of low molecular mass and high mobility, for example, cholines or high-energy phosphates, within a tissue region or a whole organ. Phosphor NMR provides information on the energy status of the tissue (adenosine triphosphate) and its pH-value [22,23].

Solid-state 'magic angle spinning' NMR techniques have existed for decades. However, they have only recently been applied to intact tissues. By spinning the sample at a very fast rate (≥2 kHz) and a specific angle ($\theta = 54.7°$), magic angle spinning dramatically reduces chemical shift anisotropy and dipole–dipole interactions such that solution-like spectra with narrow line widths can be obtained. Since high-resolution magic angle spinning spectroscopy (HR-MAS) is nondestructive, samples can subsequently undergo histopathological, and genetic analyses, thereby providing the previously missing link between the pathological, molecular, and metabolic assessment of the malignant tissues. HR-MAS has already been utilized successfully to study several types of cancer (cervical, prostate, glioma, melanoma, and breast), yielding promising new biomarkers of cancer and its response to therapy [24–28].

Metabolomic profiling refers to the study of global metabolite profiles of small molecules generated by the process of metabolism in cells, tissues and organisms. In contrast to former studies of single or a few metabolites and their associated pathways over the past two decades, modern metabolomics focuses on complete metabolite profiles in a sample isolated from biofluids or from tissue biopsies. These are represented by analytical spectra, which are compared using statistical techniques such as pattern recognition. Metabolite profiles in cells, tissues and organisms can be generated with NMR spectroscopy and MS. Magnetic resonance spectroscopic imaging (MRSI) provides a noninvasive method of detecting small molecular markers within the cytosolic and extracellular spaces of the prostate and is performed in conjunction with high-resolution anatomic imaging. Biomarkers obtained from metabolomics profiling can also be useful for PET-imaging.

Gene-expression imaging

Imaging using the reporter gene expression

Radiolabeled oligonucleotides have been proposed for diagnostic imaging and the therapy of tumors. Assuming a total human gene number of between 30,000–35,000, calculations that take into account alternative splicing result in a mRNA number between 46,000–85,000. It is

expected that an oligonucleotide with more than 12 (12-mer) nucleotides represents a unique sequence in the whole genome. Since these short oligonucleotides can be easily produced, antisense imaging using radiolabeled oligonucleotides can, in principle, offer a huge amount of new specific tracers. Prerequisites for the use of radiolabeled antisense oligonucleotides are ease of synthesis, *in vivo* stability, uptake into the cell, interaction with the target structure, and minimal nonspecific interaction with other macromolecules. For the stability of radiolabeled antisense molecules, nuclease resistance of the oligonucleotide, stability of the oligo-linker complex, and a stable binding of the radionuclide to the complex are required [29,30].

As an example of new developments in this field, applications of molecular imaging based on reporter gene expression are discussed in the following two sections.

Positron emission imaging using HSV1-tk reporter gene expression

Positron-emission tomography (PET) and PET-CT have been used extensively in cancer diagnosis, for staging and for monitoring of therapeutic efficacy. PET has also been used in contemporary animal cancer models. The most frequently applied biomarker for PET imaging in oncology is ^{18}F-fluorodeoxyglucose (^{18}F FDG). However, other tracers and many other pharmaceutical drugs are also used in oncological PET-imaging [31,32]. The manufacture of small-animal PET instruments with appropriate resolution for murine experiments has led to the establishment of noninvasive techniques for functional imaging. The development of PET reporter genes whose activity can be monitored in living animals, based on the reporter gene-dependent sequestration of positron-emitting PET reporter probes, led to innovative analyses of gene expression in transgenic animals, to methods for monitoring the location, magnitude, and duration of expression for gene therapy vectors, and to the ability to noninvasively track the targeting, viability, and expansion of cellular therapeutics. Technical data regarding the reported methods, research, and clinical applications have been reviewed by Shah and colleagues [33].

Testing potential radiotracers for PET imaging of herpes simplex virus type 1 thymidine kinase (*HSV1-TK*) gene expression, Alauddin and colleagues evaluated the radiolabeled probes 2'-deoxy-2'-fluoro-5-methyl-1-β-D-arabino-furanosyluracil (FMAU), 9-(4-fluoro-3-hydroxy-methyl-butyl)guanine (FHBG), and 9-([3-fluoro-1-hydroxy-2-propoxy]methyl)-guanine (FHPG) as PET imaging agents for *HSV1-TK* expression in a human breast cancer model [34]. *In vitro* accumulation of ^{14}C-FMAU, ^{18}F-FHBG, and ^{18}F-FHPG in HSV1-tk-expressing cells was 14–16×, 9–13×, and 2–3× higher than in TK-negative control cells, respectively, between 30–240 minutes. Accumulation of FMAU and FHBG in vector-transduced cells was 10–14× and 6–10× higher than in wild-type cells, respectively. At 2 h, uptake of ^{14}C-FMAU in TK-positive cells was 6.3× and 60× higher than that of ^{18}F-FHBG and ^{18}F-FHPG, respectively. *In vivo*, tumor uptake of ^{14}C-FMAU in HSV1-TK-expressing cells was 3.7× and 5.5× higher than that of TK-negative control cells at 1 and 2 h, respectively. Tumor uptake of ^{18}F-FHBG was 4.2× and 12.6× higher than in TK-negative cells at the same time points. Incorporation of ^{14}C-FMAU in TK-positive tumor was 18 times and 24 x higher than that of ^{18}F-FHBG at 1 and 2 h, respectively.

The micro-PET images supported the biodistribution results and indicated that both ^{14}C-FMAU and ^{18}F-FHBG may be useful for the imaging of HSV1-TK expression in breast cancer. In conclusion, ^{14}C-FMAU, which is not a PET-tracer (^{14}C was detected by other means than PET), has been successfully evaluated as a potential PET radiotracer for imaging *HSV-TK* gene expression *in vivo* [34,35].

Optical imaging using luciferase (fl) reporter gene expression

Iyer and colleagues applied noninvasive imaging of enhanced prostate-specific gene expression using a two-step transcriptional amplification-based lentivirus vector [36]. Noninvasive evaluation of gene transfer to specific cells or tissues will allow for long-term, repetitive monitoring of transgene expression. Tissue-specific promoters that restrict the expression of a transgene to tumor cells play a vital role in cancer gene therapy imaging. In their study, the authors developed a third-generation HIV-1-based lentivirus vector carrying a prostate-specific promoter to monitor the long-term, sustained expression of the firefly luciferase (*FiLU*) reporter gene in living mice. Expression of the *FiLU* gene in the transcriptionally-targeted vector is driven by an enhanced prostate-specific antigen promoter in a two-step transcriptional amplification (TSTA) system. The efficiency of the lentivirus (LV-TSTA)-mediated gene delivery, cell type

specificity, and persistence of gene expression was evaluated in cell culture and in living mice carrying prostate tumor xenografts. *In vivo* bioluminescence imaging with a cooled charge-coupled device camera revealed significantly higher levels of *FiLU* expression in prostate tumors. Injection of LV-TSTA directly into the prostate of male nude mice revealed efficient and long-term *FiLU* gene expression in the prostate tissue for up to 3 months. These studies demonstrate the significant potential of TSTA-based lentivirus vectors to confer high levels of tissue-specific gene expression from a weak promoter, while preserving cell type specificity and the ability to image noninvasively the sustained, long-term expression of reporter genes in living animals.

Genomics-based protein imaging

Molecular imaging of proteomics expression

Radiolabeled pharmaceuticals for scintigraphic imaging by single photon emission computed tomography (SPECT) were initially applied for the imaging of receptors.

At present, some of these imaging radiopharmaceuticals have been differentially labeled for new imaging techniques, particularly PET or PET/CT. Compared with scintigraphic imaging and PET, PET/CT offers both functional analysis and structural localization of a bound radiopharmaceutical. PET provides a powerful means to noninvasively image and quantify protein expression and biochemical changes in living subjects at nano- and picomolar levels. Progress has been made within the area of HER2 receptor imaging for breast cancer. In the following sections, some of the recent results are described including PET, MR and bioluminescence.

Positron-emission-tomography HER2 receptor imaging

PET provides an effective means of both diagnosing/staging several types of cancer and evaluating efficacy of treatment. PET *in vivo* HER2 receptor imaging was reported using either small engineered antibody fragment specific for the HER2 receptor tyrosine kinase in mouse tumor xenografts [37–39], or a Fab2 fragment of the anti-HER2 antibody trastuzumab labeled with ^{68}Ga for *in vivo* imaging animal tumors [40].

Robinson and colleagues [39] evaluated a small engineered antibody fragment specific to the HER2 receptor tyrosine kinase (C6.5 diabody) for its ability to function as a PET radiotracer when labeled with ^{124}I-iodine. Their studies revealed HER2-dependent imaging of mouse tumor xenografts with a time-dependent increase in tumor-to-background signal over the course of the experiments. Radioiodination via an indirect method attenuated uptake of radioiodine in tissues that express the sodium/iodide (Na/I) symporter without affecting the ability to image the tumor xenografts. In addition, they validated a method for using a clinical PET/computed tomography scanner to quantify tumor uptake in small-animal model systems; quantitation of the tumor targeting by PET correlated with traditional necropsy-based analysis at all time points analyzed. The authors claim that diabodies may represent an effective molecular structure for the development of novel PET radiotracers. Smith-Jones and colleagues [40] developed a method for imaging the inhibition of heat-shock protein 90 (Hsp90) by 17-allylaminogeldanamycin (17-AAG). It is known that 17-AAG causes the degradation of HER2 and other Hsp90 targets, and has antitumor activity in preclinical models. 17-AAG was the first Hsp90 inhibitor to be tested in a clinical trial. In order to assess the effect of this drug on its target in the patient, Smith-Jones and colleagues labeled a Fab2 fragment of the anti-HER2 antibody, trastuzumab, with ^{68}Ga, a positron emitter, which allows the sequential positron-emission tomographic imaging of HER2 expression. This method was used to quantify, as a function of time, the loss and recovery of HER2 induced by 17-AAG in animal tumors. This approach now enables noninvasive imaging of the pharmacodynamics of a targeted drug, and it is concluded that it will facilitate the rational design of combination therapy based on target inhibition.

Magnet-resonance HER2 receptor imaging

Molecular imaging of tumor antigens using immunospecific MR contrast agents is a rapidly evolving field, which can potentially aid in early disease detection, monitoring of treatment efficacy and drug development. Several different approaches have been reported on the successful application of *in vitro* and *in vivo* MR HER2 imaging of the HER2 receptor in breast cancer cells using targeted iron oxide nanoparticles [37,38]. In addition, the *in vitro* molecular imaging of tumor antigens using immunospecific MR imaging by oxide nanoparticles against the HER2 tyrosine kinase receptor [41], and *in vivo* imaging of natural killer cells directed against HER2 receptors in mice with HER2-positive mammary tumors [42] has also been reported.

In the study by Artemov and colleagues [37,38], HER2 receptors were imaged in a panel of breast cancer cells expressing different numbers of the receptors on the cell membrane. Commercially available streptavidin-conjugated superparamagnetic nanoparticles were used as a targeted MR contrast agent. The nanoparticles were directed to receptors prelabeled with a biotinylated monoclonal antibody and generated strong T(2) MR contrast in HER2-expressing cells. The contrast observed in MR images was proportional to the expression level of HER2 receptors determined independently with fluorescent-activated cell sorting (FACS) analysis.

Daldrup-Link and colleagues [42] labeled the human natural killer (NK) cell line NK-92 with iron-oxide-based contrast agents and monitored the *in vivo* distribution of genetically-engineered NK-92 cells, which are directed against HER2 receptors, to HER2-positive mammary tumors with MRI. Parental NK-92 cells and genetically modified HER2-specific NK-92-scFv(FRP5)-ζ cells, expressing a chimeric antigen receptor specific to the tumor-associated ErbB2 (HER2) antigen, were labeled with ferumoxides and ferucarbotran using simple incubation, lipofection and electroporation techniques. Labeling efficiency was evaluated by MRI imaging, Prussian blue stains and spectrometry. Subsequently, ferucarbotran-labeled NK-92-scFv(FRP5)-ζ or parental NK-92 cells were intravenously injected into the tail vein of six mice with HER2-positive NIH 3T3 mammary tumors, implanted in the mammary fat pad. The accumulation of the cells in the tumors was monitored by MRI before and 12 and 24 h post cell injection (p.i.). MR data were correlated with histopathology. Both the parental NK-92 and the genetically modified NK-92-scFv(FRP5)-ζ cells could be labeled with ferucarbotran and ferumoxides by lipofection and electroporation, but not by simple incubation. The intracellular cytoplasmatic iron-oxide uptake was significantly higher after labeling with ferucarbotran compared with ferumoxides ($p < 0.05$). Following intravenous injection of 5× 10(6) NK-92-scFv(FRP5)-ζ cells into tumor-bearing mice, MR showed a progressive signal decline in HER2-positive mammary tumors at 12 and 24 h (p.i.). Conversely, injection of 5× 10(6) parental NK-92 control cells, not directed against HER2 receptors, did not cause significant signal intensity changes of the tumors. The authors report that the human NK cell line NK-92 can be efficiently labeled with clinically applicable iron-oxide contrast agents, and the accumulation of these labeled cells in murine tumors can be monitored *in vivo* with MRI. This MR cell tracking technique may be applied to monitor NK-cell based immunotherapies in patients in order to assess the presence and extent of NK-cell tumor accumulations and, thus, determine therapy response early and noninvasively.

The success of cellular therapies will depend in part on the accurate delivery of cells to target organs. There is a recent paper that demonstrates the power of MRI in imaging of dendritic cell migration in human melanoma patients. In dendritic cell therapy in particular, delivery and subsequent migration of cells to regional lymph nodes is essential for effective stimulation of the immune system. De Vries and colleagues labeled autologous dendritic cells with a clinical superparamagnetic iron oxide formulation or ^{111}In-oxine which were co-injected intranodally in melanoma patients under ultrasound guidance. In contrast to scintigraphic imaging, magnetic resonance imaging (MRI) allowed assessment of the accuracy of dendritic cell delivery and of inter- and intra-nodal cell migration patterns. It could be shown that *in vivo* MR tracking of magnetically-labeled cells is feasible in humans for detecting very low numbers of dendritic cells in conjunction with detailed anatomical information. MRI cell tracking using iron oxides appears clinically safe and well suited to monitor cellular therapy in humans [43].

Proteomics-marker selected imaging of urinary bladder cancer

Cystoscopy (the 'gold standard'), and urine cytology are the standard tools for monitoring superficial bladder cancer. The sensitivity of cystoscopy as an imaging method is limited to the tumors that can be visualized, and the sensitivity of cytology is relatively low in low-stage/low-grade tumors. This approach is costly, time consuming and, in the case of cystocopy, invasive and uncomfortable. Additionally, other imaging methods such as transabdominal ultrasonography and intravenous urography might be made in the diagnosis of bladder carcinoma in those patients. This above mentioned procedure may be completely changed by a new, proteomics-derived nuclear matrix biomarker.

The nuclear matrix is involved in the control and coordination of gene expression during differentiation. Nuclear matrix proteins have

already been shown to be a tool to detect cancer in a variety of tumor entities, and the development of tumor-associated nuclear matrix proteins has facilitated the clinical management for early diagnosis of some types of primary cancer. Demonstration of tissue- or cell type-specific expression of tumor-specific nuclear matrix proteins has led to the development of nuclear matrix assays for diagnostics [16].

NMP22 is a nuclear protein that was analyzed and isolated in a proteomic isolation approach by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). It is responsible for chromatid and daughter cell separation in cellular replication. Significantly, the expression of NMP22 has been reported to demonstrate a 25-fold increase in bladder tumors compared with normal bladder epithelia [44]. NMP22 has now been developed as a marker for urinary bladder cancer, and it has already been approved by the US FDA as an adjunct to cystoscopy and to aid in the diagnosis, follow-up and screening of urinary bladder cancer and employs void urinary samples [20,21].

NMP22 is a noninvasive test for voided urinary samples. Two completely different test kit systems have been developed, a laboratory-based ELISA test, and an instrument-free point-of-care (POC) format test kit (BladderChek®, Matritech, Inc., MA, USA). The automated DPC laboratory version enables a much faster performance within 30–60 minutes. The Matritech-POC test (NMP22 BladderChek) is an instrument-free assay employing four drops of voided urinary samples without stabilization and is performed in approximately 30 minutes. The overall concordance between the two assay formats is 95%.

In a study by Ponsky and colleagues [17], 608 patients were investigated using the quantitative NMP22 assay. Of those 608 patients, 529 (87%) presented with *de novo* hematuria or chronic voiding symptoms without a diagnosis of bladder cancer, 79 (13.0%) patients were being monitored with a known history of bladder cancer. In 135 patients with increased NMP22 values, the 46 tumors identified were accompanied by 89 false-positive values yielding a specificity of 83.9% and a positive predictive value of 34.1%. These false-positive results were divided into six clinical categories. Exclusion of these categories improved the specificity and positive predictive value of NMP22 to 99.2 and 92.0%, respectively, yielding results similar to urinary cytology (99.8% and 94.1%). Awareness and exclusion of the categories of false-positive results can increase the specificity and positive predictive value of NMP22, enhancing the clinical use of this urinary tumor marker. The NMP22 assay was superior to voided urine cytology in the detection of transitional cell carcinoma (TCC) of the bladder.

Corresponding results were reported for the qualitative NMP22 BladderChek test [20,21]. In a study by Grossman and colleagues [20], participants included 1331 patients at elevated risk for bladder cancer. Inclusion criteria for the study were factors such as history of smoking or symptoms including hematuria and dysuria. Bladder cancer was diagnosed in 79 patients. The NMP22 BladderChek assay was positive in 44 of 79 patients with cancer, whereas cytology test results were positive in 12 of 76 patients. The specificity of the NMP22 BladderChek assay was 85.7% compared with 99.2% with cytology. The proteomic marker detected four cancers that were not visualized during initial endoscopy imaging, including three that were muscle invasive and one carcinoma *in situ*. This demonstrates that the noninvasive POC assay for elevated urinary NMP22 protein levels can increase the accuracy of imaging with cystoscopy, with test results available during the patient visit. In a different study, excluding patients with urocystitis, stones, urinary tract infections and incorporated catheters, and prior to endoscopy, 212 hematuria patients in 16 urologic practitioners sites were investigated [Peter Oehr. Unpublished study]. In a subgroup of 113 patients, NMP22 BladderChek and cytology diagnoses were determined and evaluated simultaneously, NMP22 BladderChek had 86% sensitivity and 98% specificity compared with cytology with 57 and 97%, respectively. The positive predictive values were 86 and 73%, the negative predictive values were 98 and 94% for Bladderchek and cytology, respectively. When both tests were positive, every patient turned out to have urinary bladder cancer (8 of 14 clinically positive cases). Under circumstances where both tests were negative in the tumor-negative group (99 cases), the result was a true negative in 94 cases, and false negatives in only two cases. According to this study, combined use with cytology can result in a highly reliable screening. Under circumstances where both tests were positive, no false positive results arose, making this assumption a 100% tumor inclusion criterion in 67% of the patients who had developed a yet undetected urinary bladder cancer. In addition, a negative predictive value of 98% for NMP22 BladderChek is an excellent exclusion criterion. As a conclusion, NMP22 BladderChek can change the routine

patient management of hematuria patients with suspected urinary bladder cancer with respect to the indication of cystoscopy.

Raitanen and colleagues found that, in routine follow-ups of 446 consecutive patients being monitored for bladder cancer in a prospective multicenter study, cystoscopy had an overall sensitivity of only 93.6% of the recurrent tumors [45]. This indicates that even negative cystoscopy patients have to be considered to be at risk. There is also an additional risk for false-negative results of cystoscopy in hematuria patients with bladder cancer of the upper urothelial tract. These combined risks provide realistic reasons and strongly favor a change in the official standard patient procedures, namely to first use the NMP22 BladderChek in combination with cytology as a fast, inexpensive and noninvasive prescreening for selecting (including or excluding) cystoscopy and/or other additional imaging procedures in urinary bladder cancer.

It should be mentioned that further promising competing assays have been developed, such as telomerase, or the genomic biomarker FISH-Urovysion™ (used for microscopic imaging in urinary samples after fluorescent staining of chromosomes). These assays can certainly improve the diagnostic specificity. Hallig and colleagues determined the sensitivity and specificity of various assays for the detection of urothelial carcinoma. A total of 280 voided urine specimens from 265 patients were obtained immediately before cystoscopy. Of the 265 patients, 75 had biopsy-proven urothelial carcinoma, and the sensitivity of the assays was determined from these patients. The overall sensitivity of UroVysion and telomerase was 81 and 46%, respectively. The specificity of the tests was calculated for 80 of the 265 patients in this study who had no history of urothelial carcinoma and negative cystoscopy findings despite common urological complaints. Most specific was UroVysion (96%), followed by telomerase (91%) [46]. At present, these assays are still expensive, and are only used in research.

Metabolomics-based imaging of cancer

Combined magnetic resonance imaging & MRSI in prostate cancer

According to the current literature, MRI and MRSI are emerging as sensitive tools for the noninvasive, anatomic and metabolic evaluation of cancer [26,47–49]. Good results have been reported for the diagnosis of prostatic cancer. MRI demonstrates zonal anatomy with excellent contrast resolution and can reveal tumors in areas not routinely sampled on biopsy and not palpable on digital rectal examination. In addition, MR images allow assessment of local extent (including extracapsular extension and seminal vesicle invasion) and thus can assist in local staging while providing surgeons and radiation therapists with a visual road-map for treatment planning. The addition of ^{1}H MRSI to MRI can improve prostate cancer detection and assessment of tumor volume; it also contributes indirectly to improved local staging. In addition, ^{1}H MRSI metabolic and volumetric data correlate with pathological Gleason grade, and may thus offer a noninvasive means to better predict prostate cancer aggressiveness. Combined MRI/^{1}H MRSI is currently of greatest value for high-risk patients [48].

Studies in preprostatectomy patients have indicated that the metabolic information provided by MRSI combined with the anatomical information provided by MRI can significantly improve the assessment of cancer location and extent within the prostate, extracapsular spread, and cancer aggressiveness. Additionally, pre- and post-therapy studies have demonstrated the potential of MRI/MRSI to provide a direct measure of the presence and spatial extent of prostate cancer after therapy, a measure of the time course of response, and information concerning the mechanism of therapeutic response. In addition to detecting metabolic biomarkers of disease behavior and therapeutic response, MRI/MRSI guidance can improve tissue selection for *ex vivo* analysis. ^{1}H HR-MAS spectroscopy provides a full chemical analysis of MRI/MRSI-targeted tissues prior to pathologic and immunohistochemical analyses of the same tissue. Preliminary (^{1}H HR-MAS) spectroscopy studies have already identified unique spectral patterns for healthy glandular and stromal tissues and prostate cancer [26]. The strength of the combined MRI/MRSI exam is demonstrated when changes in three or more metabolic markers (choline, polyamines and citrate) and imaging findings are concordant for cancer.

In addition to identifying unique spectral patterns for healthy glandular and stromal tissues and prostate cancer, ^{1}H HR MAS has determined the composition of the composite *in vivo* choline peak, and identified the polyamine spermine as a new metabolic marker of prostate cancer. The focus of future studies will be to increase the number of metabolic markers and to better understand the cause of these changes

through correlation with earlier protein and genetic changes.

PET-imaging based on magnetic resonance imaging findings in prostate cancer

The promising spectroscopy results concerning choline in prostatic cancer were reasons to investigate its potential as a new tracer for tumor imaging with PET-diagnostics using ^{11}C-choline. Yamaguchi and colleagues made a comparative study of ^{11}C-choline PET and MRI combined with proton MR spectroscopy in order to clarify the indication of ^{11}C-choline PET for localizing and evaluating cancer lesions in patients with prostate cancer [50]. They conducted a prospective comparison with MRI combined with proton MR spectroscopy. PET and MRI combined with proton MR spectroscopy were performed in 20 patients with prostate cancer. Correlations among the metabolite ratio of choline plus creatine to citrate (Cho+Cr/Ci) on MR spectroscopy, serum prostate-specific antigen (PSA) and maximum standardized uptake value (SUV[max]) of ^{11}C-choline were assessed. The location of the primary lesion was assessed by the site of SUV(max) and the laterality of the highest Cho+Cr/Ci ratio, and confirmed by examination of surgical pathology specimens. PET exhibited a diagnostic sensitivity of 100% for primary lesions, while the sensitivities of MRI and MR spectroscopy were 60 and 65%, respectively. Weak linear correlations were observed between SUV(max) and serum PSA ($r = 0.52$, $p < 0.05$), and between SUV(max) and Cho+Cr/Ci ratio ($r = 0.49$, $p < 0.05$). Regarding the localization of the main primary lesions, PET results agreed with pathological findings in 81%, while MR spectroscopy results were in accordance with pathological findings in 50%. This preliminary study suggests that ^{11}C-choline PET may provide even more accurate information regarding the localization of main primary prostate cancer lesions than MRI/MR spectroscopy.

Tian and colleagues attempted to assess and compare the usefulness of ^{11}C-choline-PET with that of ^{18}F-FDG-PET for the differentiation between benign and malignant in various tumors [51]. They examined 38 consecutive patients with various tumors, including seven patients with brain tumors, two with oral cavity tumor, two with esophageal cancer, six with lung cancer, 11 with bone tumor, nine with soft tissue tumors, and one with myeloma. The study demonstrated that ^{11}C-choline-PET is similar to FDG-PET in differentiation between malignant and benign lesions in various tumors. These results are promising and show that the metabolic information provided by MRSI, combined with the anatomical information provided by MRI, can significantly improve the assessment of new markers for imaging with other methods such as PET.

Discussion & conclusions

This article introduces some recent examples on the successful application of omics-based technologies in the development of novel cancer diagnostic imaging tools and the evaluation of cancer treatment. Molecular imaging based on omics is becoming an increasingly important new tool in the evaluation of diagnosis and therapy. *In vivo* imaging can provide noninvasive real-time information on the stage of disease and be applied in the evaluation of treatment response. Many advances have been made in applications using high-resolution *in vivo* imaging methods, including radionuclide imaging, such as PET and SPECT, MRI, spectroscopy and bioluminescence imaging.

The rapid development of new techniques and the generation of results concerning the use of genomic biomarkers for *in vitro* and *in vivo* cancer detection, accompanied by the recent application of new *in vivo* gene expression markers, demonstrates that different genomic approaches can be effectively used to detect specific cancer biomarkers. The genomic approach can be further applied for the estimation of prognosis in cancer disease, for therapy planning, and for monitoring therapy *in vitro* and *in vivo*. However, these advances represent only a small step in the development of clinically applicable biomarkers. This is also the case for the molecular imaging of gene expression. The studies reported in this review demonstrate that molecular diagnostics have already expanded from *in vitro* histopathology and assays in clinical chemistry to *in vivo* genetic imaging in nuclear medicine and biology, with the goal of understanding cancer, performing diagnostics *in vivo*, monitoring treatment and testing and developing new drugs.

New classes of biomarkers derived from MS analysis of the low-molecular-weight proteome have shown improved abilities in the early detection of disease and hence in patient risk stratification and outcome.

The approach of HR-MAS spectroscopy demonstrates that it is possible to separate out metabolites for tumors. Therefore, this noninvasive technique is useful for detecting and monitoring metabolic changes *in vitro*, whereas tissue-extraction procedures often dilute these metabolites.

As for gene-expression profiling, this approach allows the screening of large populations and can be used for high-throughput analysis. Metabolomic approaches generate highly reproducible data sets. In contrast to DNA microarrays, costs are low per sample after the initial equipment purchase [52]. Most important seems to be the MRI/MRSI approach, which can provide both quantitative biomarker and morphological real-time information *in vivo* in a noninvasive way, similar to *in vivo* gene expression by biomarkers combined with PET-CT. Therefore, metabolic approaches and the detection of specific metabolic profiles will become a new important approach in the early detection of biomarkers *in vivo* and *in vitro*.

Choline PET is a promising tracer in the diagnosis of prostate cancer, but its validity in local tumor demarcation, lymph node diagnosis and detection of recurrence has to be defined in future clinical trials.

An interesting final point is the fact, that a proteomics derived marker may change the standard protocol of *in vivo* bladder cancer diagnosis, possibly allowing the development of less invasive, faster, safer and cheaper diagnostic prescreening of patients at risk.

Outlook

With respect to therapy and imaging, in particular in the field of angiogenesis, promising omic-based therapeutics are currently evaluated in clinical trials. Angiogenesis has become an attractive target for anticancer drug development, based on its important roles in tumor growth, invasio, and metastasis. A potent stimulus of angiogenesis is vascular endothelial growth factor (VEGF) [53]. There are new agents developed to inhibit VEGF activity, such as Avastin® (bevacizumab) and SU5416. Avastin may have the potential to provide significant efficacy benefits for patients with metastatic renal cell cancer, non-small cell lung cancer, pancreatic cancer, and other tumor types when used first-line in combination with standard therapy [54]. These angiogenesis agents tend to be cytostatic, and therefore few responses are observed with conventional imaging by computerized tomography. Furthermore, toxicity with these agents is seen when the maximum-tolerated dose is combined with chemotherapy. Hence, there is a need to develop imaging strategies that can determine the minimum and optimum biologically active doses (for example, microdosing). There is increasing awareness of the need to obtain evidence of drug activity through the use of surrogate markers of the biologic mechanism of action during early clinical trials, in addition to determining the pharmacokinetics, toxicity profile, and maximum-tolerated dose. One of the major impediments to the rapid development of antiangiogenic agents in the past has been the lack of validated assays capable of measuring an antiangiogenic effect directly in patients. Recently, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) has emerged as a useful technique for noninvasive imaging of tumor vasculature in preclinical and clinical models. The major challenge is the standardization of the technique worldwide for the purpose of early clinical studies that are likely to be multicenter. Convincing data on correlations between changes observed through molecular imaging and changes in tumor angiogenesis, and hence tumor biology, are still lacking [55].

Choline-PET is a promising tool in imaging various tumors. However, this method is still controversial. Choline accumulation is found in the membranes of proliferating tissue, and as such is based on a completely different mechanism compared with the FDG-uptake, which relates to the energy-need of the tumor cell. That is why choline-PET may become a promising alternative for detection of tumors with low sugar uptake. Both methods are not tumor-specific. In contrast, tumor detection by specifically imaging somatostatin receptors using PET (for example, DOTA-TOC-PET) and SPECT has proven valuable in several clinical trials and should also be addressed as valuable approaches for the future. In particular, in this context there is a direct link to therapeutic application using somatostatin-receptor-specific radiotherapy.

Comparison of different biological sources using omics expression pattern profiling has become the new tool for the detection of cancer specific biomarkers, opening the avenue for mainly noninvasive personalized diagnosis and therapy by imaging methods.

However, by primarily measuring changes in transcript and protein abundance, conventional genomic and proteomic methods may fail to detect significant post-translational events that regulate protein activity and, ultimately, cell behavior. To address these limitations, activity-based proteomic technologies that measure dynamics in protein function on a global scale will be of particular value. To assess analysis of protein function, several proteomic methods are currently introduced to characterize the activity

of proteins on a global scale. These include large-scale yeast two-hybrid screens and epitope tagging immunoprecipitation experiments, which aim to construct comprehensive maps of protein–protein interactions. These methods reveal interactions that place functionally unclassified proteins in a biological context, interactions between proteins involved in the same biological function, and interactions that link biological functions together into larger cellular processes.

Another current approach which will play a role in the future of omics is computational biomarker prediction. The purpose is to show that computational methods can be useful in narrowing the search for biomarker candidates. The overall goal is to implicate peaks that have a high likelihood of being biologically linked to a given disease state, and thus narrow the search for biomarker candidates. Given that sample sizes for these types of experiments are typically small, and given that validation of any results produced requires laborious protein identification, the aim is to find the smallest set of peaks that yields reasonable (i.e., statistically significant) classification results. Once a small set of peaks is found that can be used to computationally predict phenotypes with high accuracy, these peaks should be analyzed further and the underlying biomarkers identified. The hope is that the subsequent functional study of these biomarkers will eventually lead to new biological insights into disease pathways and, ultimately, to new diagnostic tests and potential therapeutic targets.

In the screening or prescreening of risk groups using proteomic assays, NMP22 is now thought to possibly replace routine follow-up microscopic imaging by cytology for asymptomatic patients with initial low grade Ta tumors, for which cytology has a low yield and is not recommended. In this case, NMP22 is regarded complementary to, but not as a replacement for, imaging by cystoscopy (AUA News, the official newsmagazine of the American Urological Association, September/October 2005, vol. 10, issue 6). Independent of these present developments, the next step might be the general combined use of NMP22 and cytology together, either as an indication for imaging by cystoscopy, or as an aid to omit this invasive procedure.

It might be recommendable to inform the general population about genetic screening, selection of risk groups, and the chances of better survival of these groups by surveillance.

The management of genetic screening to select groups with an increased lifetime risk will become more important in the future, as the number of such detected gene mutations is increasing. An example are women with a documented mutation in breast cancer susceptibility genes (for example, *BRCA*). For those patients, intensified surveillance is recommended at a younger age than in women at average risk [56]. Recommended imaging refers to mammographic techniques, concomitant ultrasound and clinical breast examination. However, this is probably not sufficient to ensure an early diagnosis of familial breast cancer. If MRI and/or PET is integrated in surveillance programs, early diagnosis seems to be improved. The introduction of genetic testing combined with subsequent imaging is increasingly attracting general interest for purposes of disease prediction and susceptibility (risk) analysis in asymptomatic individuals. As an example, the diagnosis in patients who present clinically with dementia or Alzheimer's disease has shown that apolipoprotein E (*APOE*) *ε4* genotype is a well-established risk factor for Alzheimer's disease (AD) [57].

Although there is general consensus on the involvement of *APOE* in the pathogenesis of AD, a possible association with the disease progression is still controversial. Several recent publications indicate an involvement of the *APOE* genotype on disease course measures such as neuropsychological performance, reaction to treatment, and survival [58–60]. Correspondingly, current neuroimaging studies suggest a possible influence of the *APOE* genotype on cerebral perfusion deficits, progression of atrophy, and decline of cerebral glucose metabolism in patients with AD [61,62]. In contrast, others dispute an influence of the *APOE* genotype on disease course measures and on the cerebral glucose metabolism in patients with AD [63,64].

Associations between the *APOE* genotype and various medical conditions have even been documented at a very young age [65]. *APOE* genotype testing can identify a patient at risk, but not be used as a diagnostic test for predicting progression from normal to cognitive impairment or AD. On the other hand, previous studies with PET and functional magnetic resonance imaging have indicated differences in neural metabolism and activity between carriers of the *APOE ε4* allele and those who are not at risk for AD [66]. Based on these findings, the screening of a population for the *APOE ε4* allele and subsequent imaging of the carriers might be a method for early detection of AD [67,68]. A combination of both functional imaging and genotyping may allow an early high-risk or low-risk stratification

Highlights

- The advances described in this article represent a view into new approaches to develop cancer-specific and clinically applicable biomarkers for personalized diagnosis and therapy, and their noninvasive visualization by imaging methods.
- In order to detect cancer-related new biomarkers, the approaches in genomics, proteomics and metabolomics compare expression patterns from different biological sources (e.g., cancer and noncancer).
- Approaches in genomics and proteomics by microarray evaluation, in proteomics often combined with 2-dimensional electrophoresis and/or laser enhanced mass-spectrometry, lead to cancer related genetic or proteomic profiles. Approaches in metabolomics, for example, applying nuclear magnetic resonance spectroscopy, lead to detection of cancer related metabolic end product profiles.
- Genes, proteins or metabolites can be separated from these profiles and used as biomarkers to estimate cancer risk (genomic assays), and to detect and describe the state of a cancer disease (proteomics and metabolomics). Antibodies to those biomarkers can also be used for development of *in vitro* tumor marker assays.
- Early detection of cancer in risk groups by tumor marker assays can be used as an indication, and true negative findings at high specificity can be used to avoid invasive imaging, for example, nuclear matrix protein 22 (NMP22) in urinary bladder cancer.
- Antibodies to the detected biomarkers can also be used for *in vivo* tumor imaging to localize cancer by single photon scintigraphy (SPECT) or positron emission tomography (PET) for staging and prognostic evaluation of the detected cancer lesions.
- In certain cases, labeled or nonlabeled antibodies to those biomarkers are currently applied for cancer therapy.
- Metabolic information provided by magnetic resonance spectroscopic imaging (MRSI) combined with the anatomical information provided by magnetic resonance imaging can also significantly improve the assessment of cancer location and extent, and/or cancer aggressiveness.
- Metabolites detected by MRSI can be labeled with positron emitters and used for cancer detection by PET.
- Noninvasive evaluation of gene transfer to specific cells or tissues will allow for long-term, repetitive monitoring of transgene expression. Transfer or cotransfer of reporter genes used as genomic markers *in vivo* can be imaged noninvasively by methods using bioluminicence, positron emission or magnetic resonance and give qualitative as well as quantitative information about the individual effectiveness of gene transfer in gene therapy.

of patients with either very high sensitivity or very high specificity. This may be valuable, for example, for patient selection in scientific studies [67]. It might also become a basis for testing the effectiveness of new drugs to prevent the progress of this disease in individual patients. On the other hand, the premature introduction of genetic testing may include possible unwanted consequences, such as adverse selection in insurance markets, which have to be avoided. That is why, before the onset of any omics-based screening and subsequent imaging, it will be necessary to achieve a balance in the areas of genetics, imaging, data protection, counseling, ethics, and public policy, and to include leadership from related consensus projects [69].

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