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Titel

From "omics" to molecular imaging

Kurztitel

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Abstract:

There is an increasing 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 what can be achieved to date based on “-omics”, in connection to new technologies, to bring forward novel strategies for the detection and analysis of clinically relevant biomarkers for diagnosis and therapy by using in vitro assays and molecular imaging.

Contents

From “omics” to molecular imaging

1 Introduction

Detecting 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 assays and technologies emerging within genomics, proteomics and metabolomics, which can be also summarized as “-omics”.

The purpose of this article is to introduce what can be achieved to date based on “-omics”, in connection to new technologies, to bring forward novel strategies for the detection and analysis of clinically relevant biomarkers for diagnosis and therapy by using in vitro assays and molecular imaging.

2 Genomics

2.1 Genetic profiling of biomarkers

At the time of the publication of the human genome, the final number of sequenced genes was determined to be approximately 32,000. All humans share a similar genome, with at least 99.9% of the nucleotide coding being identical. The total number of base pairs in the human genome is slightly under 3 billion. Although there is remarkable similarity in the genome makeup among all humans, a 0.1% difference in genomes between individuals represents 3 million base pairs that are of importance in different phenotypes and in the development of disease. 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 total of 32,000 genes, about 1,200 genes are responsible for 1,600 disorders. The challenge now is to connect the proteins encoded by these genes and the final metabolites to describe how physiological processes are mediated and regulated to find biomarkers for diagnosing in vitro or in vivo and treating disease in a new era of personalized medicine (1, 2).

Genetic profiling allows 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 at a time(3, 4). 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 (5, 6).

A DNA microarray is an orderly arrangement of thousands of defined DNA probes immobilized on a glass surface. Labeled transcripts isolated from biological samples are

hybridized to the DNA microarray probes for determination of the transcript abundance or relative expressions. In conventionally used protocols, gene expression profiling is based on extracting total RNA from fresh, frozen or fixed biological samples. The extracted RNA is then reverse transcribed into cDNA via RT-PCR. Next, cRNA is generated from the cDNA using RNA polymerase II. This in vitro transcription of the cDNA into cRNA is carried out with modified fluorescence-labeled nucleotides which are incorporated into the cRNA. The cRNA is fragmented and hybridized for many hours to a DNA microarray residing on a nylon membrane, glass slide, or synthetic chip to allow hybridization of the sample target to the microarray probes. An expressed sequence tag allows for detection of the hybridized DNA of interest using a confocal laser scanner. 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.

2.2 Molecular imaging of gene expression

2.2.1 General aspects

Radiolabeled oligonucleotides have been proposed for diagnostic imaging and the therapy of tumors. Assuming a total human gene number between 30,000 and 35,000, calculations that take into account alternative splicing result in a mRNA number between 46,000 and 85,000. It is expected that an oligonucleotide with more than 12 (12-mer) nucleobases represents a unique sequence in the whole genome. Since these short oligonucleotides can easily be 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, stability in vivo, 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. (7, 8).

Positron-emission tomography (PET) and PET-CT have been used extensively for cancer diagnosis, for staging and for monitoring of therapeutic efficacy. PET has also been used in contemporary animal cancer models. The most frequently “biomarker” applied for PET imaging in oncology is F-18-fluorodeoxyglucose (F-18 FDG). However, other tracers and many other pharmaceutical drugs are also used in oncological imaging. 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 about the reported methods, research, and clinical applications have been reviewed by Shah et al.(7-9).

As an example for new developments in this field, selected applications of molecular imaging based on genomics are reported in the next sections.

2.2.2 PET imaging agents for HSV1-tk expression in a human breast cancer model.

Testing potential radiotracers for PET imaging of HSV1-tk gene expression, Alauddin et al. [52] evaluated the radiolabeled probes FMAU (2'-deoxy-2'-fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHBG (9-(4-fluoro-3-hydroxy-methyl-butyl)guanine), and FHPG (9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]-guanine) as PET imaging agents for HSV1-tk expression in a human breast cancer model (10). In vitro accumulation of [14C]FMAU, [18F]FHBG, and [18F]FHPG in HSV1-tk-expressing cells was 14-16 times ($P<0.001$), 9-13 times ($P<0.001$), and 2-3 times ($P<0.05$) higher than in tk-negative control cells, respectively, between 30 min and 240 min. Accumulation of FMAU and FHBG in vector-transduced cells was 10-14 times and 6-10 times higher than in wild-type cells, respectively. At 2 hr, uptake of [14C]FMAU in tk-positive cells was 6.3 times and 60 times higher than that of [18F]FHBG and [18F]FHPG, respectively. In vivo, tumor uptake of [14C]FMAU in HSV1-tk-expressing cells was 3.7times and 5.5times ($P<0.001$) higher than that of tk-negative control cells at 1 hr and 2 hr, respectively. Tumor uptake of [18F]FHBG was 4.2 times and 12.6 times higher ($P<0.001$) than in tk-negative cells at the same time points. Incorporation of [14C]FMAU in tk-positive tumor was 18 times and 24times higher ($P<0.001$) than that of [18F]FHBG at 1 hr and 2 hr, respectively. According to the authors the micro-PET images supported the biodistribution results and indicated that both [18F]FMAU and [18F]FHBG are useful for imaging HSV1-tk expression in breast cancer.

2.2.3 Optical imaging of in vivo transgene expression

Iyer et al. (11) applied noninvasive imaging of enhanced prostate-specific gene expression using a two-step transcriptional amplification-based lentivirus vector. 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 (fl) reporter gene in living mice. The fl 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 were 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 high levels of fl expression in prostate tumors. Injection of LV-TSTA directly into the prostate of male nude mice revealed efficient and long-term fl 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.

2.2.4 In vitro and in vivo molecular imaging of the human epidermal growth factor receptor 2 (HER2)

In solid tumors the predominant genetic mechanism for oncogene activation is through amplification of genes. The human epidermal growth factor receptor 2 (synonyms: ErbB2, c-erbB2, HER-2/neu, HER-2, HER2, 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 and is also commonly amplified in other forms of cancer (12). 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

In addition to Herceptin, 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 pre-clinical potential for HER2 amplified carcinomas.

Concerning assays for HER2 amplification, IHC and FISH have been approved for trastuzumab selection by the FDA. Because CISH features the advantages of both IHC (routine microscope, lower cost, familiarity) and FISH (built-in internal control, objective scoring, more robust DNA target), CISH is an important candidate for FDA approval.
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 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 Herceptin 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 be determined by immunoassay (HER-2/neu Microtiter ELISA assay and HER-2/neu ADVIA Centaur® (Bayer HealthCare, MA, USA). The literature concerning HER2 has been reviewed by Carney et al. and Ross et al. (12, 13) .
Within the frame of these developments, molecular imaging for detection of HER2 in vivo is on its way. There are different vitro and in vivo experimental approaches, including near-infrared optical imaging, magnetic resonance (MR): and positron emission tomography (PET).

2.2.4.1 Near-infrared optical imaging of HER2-over-expressing tumours

Hilger et al. (14) evaluated in vitro and in vivo imaging of HER2-over-expressing tumours using near-infrared optical imaging. A fluorochrome probe was designed by coupling Cy5.5 to anti-HER2 antibodies. Cells over-expressing (SK-BR-3 cells) or normally expressing (PE/CA-PJ34 cells) the HER2 protein were incubated with the probe. After removing unbound probe molecules, fluorescence intensities were determined (a.u.:

arbitrary units). Cells were additionally investigated using FACS and laser scanning microscopy. The probe was also injected intravenously into tumours bearing SK-BR-3 (n=3) or PE/CA-PJ34 (n=3). Whole-body fluorescence images were generated and analysed. The incubation of SK-BR-3 cells with the probe led to higher fluorescence intensities [2,133 (+/-143) a.u.] compared to controls [975 (+/-95) a.u.]. The results from FACS and immunocytochemical analysis were in agreement with these findings. A distinct dependency between the fluorescence intensity and the cell number used in the incubations was detected. In vivo, the relative fluorescence intensities in SK-BR-3 tumours were higher than in PE/CA-PJ34 tumours at 16-24 h after probe application. HER2-over-expressing tumours were depictable in their original size. Labelling of HER2 with Cy5.5 is suitable for in vitro and in vivo detection of HER2-over-expressing tumour cells

2.2.4.2 MR 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 are reported about successful application of in vitro and in vivo MR HER2 imaging of the HER2 receptor in breast cancer cells using targeted iron oxide nanoparticles (15, 16), in vitro molecular imaging of tumor antigens using immunospecific MR in vitro imaging by oxide nanoparticles against the HER2 tyrosine kinase receptor (17), and by in vivo imaging of natural killer cells directed against HER2 receptors in mice with HER2-positive mammary tumors (18).

In the study of Artemov et al. (15, 16) 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 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 FACS analysis. In these experiments, iron oxide nanoparticles were attached to the cell surface and were

not internalized into the cells, which is a major advantage for in vivo applications of the method.

Funovics et al. (17) designed, synthetized, and tested in vitro two novel monocrystalline iron oxide nanoparticles (MION) conjugated to antibodies against the HER2 tyrosine kinase receptor and the 9.2.27 proteoglycane sulfate. MION was synthetized by coprecipitation of iron II and iron III salts in 12-kD dextran solution; antibody coupling was performed by reductive amination. The relaxivity of the conjugates was 24.1-29.1 mM(-1) s(-1), with 1.8 to 2.1 antibody molecules per nanoparticle. A panel of cultured melanoma and mammary cell lines was used for testing. The cells were incubated with the particles at 16-32 microg Fe/ml in culture medium for 3 h at 37 degrees C, and investigated with immune fluorescence, transmission electron microscopy (TEM), MRI of cell suspensions in gel atine, and spectrophotometric iron determination. All receptor-positive cell lines, but not the controls, showed receptor-specific immune fluorescence, and strong changes in T(2) signal intensity at 1.5 T. The changes in 1/T(2) were between 1.5 and 4.6 s(-1) and correlated with the amount of cell-bound iron (R = 0.92). The relaxivity of cell-bound MION increased to 55.9 +/- 10.4 mM(-1) s(-1). TEM showed anti-9.2.27 conjugates binding to the plasma membrane, while the anti- HER2-conjugates underwent receptor-mediated endocytosis. In conclusion, we obtained receptor-specific T(2) MR contrast with novel covalently bound, multivalent MION conjugates with anti-9.2.27 and anti- HER2to image tumor surface antigens. The authors claimed that this concept can potentially be expanded to a large number of targets and to in vivo applications.

Daldrup-Link et al. (18) 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 MR imaging. Parental NK-92 cells and genetically modified HER2 specific NK-92-scFv(FRP5)-zeta 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 MR imaging, Prussian blue stains and spectrometry. Subsequently, ferucarbotran-labeled NK-92-scFv(FRP5)-zeta (n=3) 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 MR imaging before and 12 and 24 h after cell injection (p.i.). MR data were correlated with histopathology. Both the parental NK-92 and the genetically modified NK-92-scFv(FRP5)-zeta 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 than ferumoxides (P<0.05). After intravenous injection of 5 x 10(6) NK-92-scFv(FRP5)-zeta 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 x 10(6) parental NK-92 control cells, not directed against HER2 receptors, did not cause significant signal intensity changes of the tumors. The authors reporte that the human natural killer 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 MR imaging. 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, to determine therapy response early and non-invasively.

2.2.4.3 PET 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 by using either small engineered antibody fragment specific for the HER2 receptor tyrosine kinase in mouse tumor xenografts (15, 16, 19), or an F(ab')2 fragment of the anti-HER2 antibody Herceptin labeled with 68Ga for in vivo imaging animal tumors (20).

Robinson et al. (19) evaluated a small engineered antibody fragment specific for the HER2 receptor tyrosine kinase (C6.5 diabody) for its ability to function as a PET radiotracer when labeled with iodine-124. 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 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 athors claim that diabodies may represent an effective molecular structure for development of novel PET radiotracers.

Smith-Jones et al. (20) developed a method for imaging the inhibition of Hsp90 by 17-AAG. It is known that 17-allylaminogeldanamycin (17-AAG) causes the degradation of HER2 and other Hsp90 targets, and has antitumor activity in preclinical models. 17-AAG has been 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 et al. 2004 labeled an F(ab')2 fragment of the anti-HER2 antibody Herceptin with 68Ga, a positron emitter, which allows the sequential positron-emission tomographic imaging of HER2 expression and used this method to quantify as a function of time the loss and recovery of HER2 induced by 17-AAG in animal tumors. This approach now allows 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.

3. Proteomics

3.1 Proteomic profiling of biomarkers

By comparison of protein profiles from tumor samples and tumor-free samples, tumor-specific proteins can be selected, sequenced and identified in databases. For detection of proteins, samples are generally pretreated to remove lipids, immunoglobulin, and human serum albumin. Afterwards, fractionations can be carried out using NaCl gradients.

The microarray format is frequently used to carry out high-throughput experiments to discover proteins in a proteome. Microarrays allow large-scale studies, because the miniaturization, automation, and parallelism that the microarray format provides make it the universal biochemistry platform of choice. The application for protein microarrays in cancer includes protein expression profiling, serum based diagnostics, biomarker discovery, protein-protein binding assays, drug target binding, receptor epitope binding, and epitope mapping.

Biologists now need to evaluate complex protein samples on a more comprehensive scale. Shotgun proteomics refers to the direct analysis of complex protein mixtures to rapidly generate a global profile of the complement protein within the mixture. These techniques provide only an indirect estimate of dynamics in protein function. This proteomic strategy aims to catalogue the entire complement of protein products in a given sample.

Electrophoretic separation of proteins has 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-f light MS (SELDI-TOF-MS), or electrospray ionization tandem MS (ESI-MS/MS).

3.2. Molecular imaging of proteomics expression

3.2.1 General aspects

In the beginning, radiolabeled pharmaceuticals for scintigraphic imaging by single photon emission computed tomography (SPECT) were applied for imaging of receptors.

At present, some of these imaging radiopharmaceuticals have been differentially labeled for new imaging techniques, especially 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. As the field of molecular imaging develops, and as advances in the biochemistry, pharmacology, therapeutics, and molecular biology of disease are made, there is a corresponding increase in the number of clinically relevant, novel disease-associated biomarkers that are brought to the attention of those developing imaging probes for PET. New software and tracers have been developed: radioligands for steroid receptors, growth factor receptors, receptor tyrosine kinases, sigma receptors, tumor-associated enzymes, and gene reporter probes (21-27).

3.2.2 Assessment of receptor binding in vivo.

Radiopharmaceuticals of great potential or already in clinical use for imaging of cancer, inflammation, infection, and embolism are already available. First examples, based on genomic overexpression of HER2 in breast cancer have already been reported in this chapter within the genomics section.

In lung cancer, somatostatin receptor imaging by means of 99mTc-octreotide scintigraphy has proven useful for characterizing malignancy in solitary pulmonary nodules. Additionally, several radiopharmaceuticals targeting tyrosine kinase, e.g., 99mTc-labeled epidermal growth factor and 111In-diethylene triamine penta-acetic acid-trastuzumab, or

G-protein-coupled receptors, e.g., 99mTc-bombesin, 123I-vasoactive intestinal peptide, and 111In-tetraazacyclododecane tetra-acetic acid (DOTA)-cholecystokinin-B, are being explored for their diagnostic as well as treatment monitoring potential. With the aim of better evaluating the source of pulmonary embolism, as well as differentiating acute from chronic deep venous thrombosis, several radiolabeled peptides targeting the glycoprotein IIb/IIIa fibrinogen receptor found on activated platelets have been developed. Out of these, 99mTc-P280 is now approved by the FDA for scintigraphic imaging of suspected acute venous thrombosis in the lower extremities of patients. In the field of lung inflammation and infection, nonspecific 111In- and 99mTc-human polyclonal immunoglobulins have been successfully used to identify the presence and extent of Pneumocystis carinii, cytomegalovirus, Mycobacterium avium and fungal infections in patients with HIV infection. The clinical role of other radiopharmaceuticals, such as 99mTc-J001X, a nonpyrogenic acylated polygalactoside isolated from Klebsiella pneumoniae and binding with high affinity to CD11b and CD14 lipopolysaccharide receptors expressed on monocytes/macrophages, and 111In-octreotide, binding to upregulated somatostatin receptors on activated lymphocytes, needs to be further defined (28).

3.2.3 Profiling the activity of enzymes in vivo

The group around Spears and Cravatt (29) introduced the tag-free strategy for ABPP that utilizes the copper(I)-catalyzed azide-alkyne cycloaddition reaction ("click chemistry") to analyze the functional state of enzymes in living cells and organisms. Recently these investigators reported a detailed characterization of the reaction parameters that affect click chemistry-based ABPP and identify conditions that maximize the speed, sensitivity, and bioorthogonality of this approach. Using these optimized conditions, they compared the enzyme activity profiles of living and homogenized breast cancer cells, resulting in the identification of several enzymes that are labeled by activity-based probes in situ but not in vitro.

3.2.4 Molecular imaging by bioluminescence in cell cultures and in vivo imaging of small animals by PET

The study of small molecule-mediated interactions of proteins is important to understand abnormal signal transduction pathways in cancer and in drug development and validation.

Paulmurugan et al. (30)described a technique for molecular imaging of drug-modulated protein-protein interactions in living subjects. They used split synthetic renilla luciferase (hRLUC) protein fragment-assisted complementation to evaluate heterodimerization of the human proteins FRB and FKBP12 mediated by the small molecule rapamycin. The concentration of rapamycin required for efficient dimerization and that of its competitive binder ascomycin required for dimerization inhibition were studied in cell lines. The system was dually modulated in cell culture: at the transcription level, by controlling nuclear factor kappaB promoter/enhancer elements using tumor necrosis factor alpha, and at the interaction level, by controlling the concentration of the dimerizer rapamycin. The rapamycin-mediated dimerization of FRB and FKBP12 also was studied in living mice by locating, quantifying, and timing the hRLUC complementation-based bioluminescence imaging signal u sing a cooled charged coupled device camera. This split reporter system can be used to efficiently screen small-molecule drugs that modulate protein-protein interactions and also to assess drugs in living animals. Both are essential steps in the preclinical evaluation of candidate pharmaceutical agents targeting protein-protein interactions, including signaling pathways in cancer cells (30).

4. Metabolomics

4.1 Metabolomic profiling of biomarkers

Metabolomics refers to the study of global metabolite profiles of small molecules generated in the process of metabolism in cells, tissues, and organisms. In contrary to former studies of single or a few metabolites and associated pathways over the past two decades, modern metabolomics means focusing on complete metabolite profiles in a samples from biofluids or from tissue biopsies, 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 nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy. 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.

4.2 Molecular imaging of metabolomic expression

4.2.1 General aspects

Nuclear magnetic resonance spectroscopy 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 radiofrequency 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. NMR analysis for metabolomics has centered on [1]H and [13]C NMR spectroscopy. Solid-state "magic angle spinning" NMR techniques have existed for decades; however, they have only recently been applied to intact tissues [121,122]. 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 linewidths can be obtained. Since high-resolution magic angle spinning spectroscopy (HR-MAS) is nondestructive, samples can subsequently undergo histopathologic, and genetic analyses, thereby providing the previously missing link between the pathologic, molecular, and metabolic assessment of the malignant tissues. HR-MAS has already been utilized successfully to study several types of cancer (cervix, prostate, glioma, melanoma and breast), yielding promising new biomarkers of cancer and its response to therapy (31-35).

4.2.2 Combined magnetic resonance imaging and MRSI in prostate cancer

According to a review published by Kurhanewicz et al. (31), studies in pre-prostatectomy 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 (31). 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.

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. This is demonstrated in Fig. 1, which is reproduced from the review by Kurhanewicz et al. with the kind permission of the author and the publisher.

Preliminary [1]H HR-MAS spectroscopy studies have already identified unique spectral patterns for healthy glandular and stromal tissues and prostate cancer, 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 (31).

5 Discussion

This article introduced to some examples what can be achieved to date based on "-omics", in connection to new technologies, to bring forward novel strategies for the detection and analysis of clinically relevant biomarkers for diagnosis and therapy by using in vitro assays and molecular imaging.

Molecular imaging based on "-omics" has become an important tool to evaluate gene therapy. Many advances have been made in high-resolution in vivo imaging methods, including radionuclide imaging, such as positron emission tomography (PET) and single photon emission tomography (SPECT), magnetic resonance (MR) imaging and spectroscopy, and bioluminescence imaging and various fluorescence imaging techniques, such as fluorescence-mediated tomography (FMT) and near-infrared fluorescence (NIRF) reflectance imaging. A variety of factors determine the choice of specific imaging system, including the imaging requirements (single or repeated), intended use (animal or human), and spatial requirements (organs versus cellular resolution and depth).

The fast increase of techniques and results concerning genomic biomarkers for in vitro and in vivo cancer detection and the recent application of new in vivo gene expression markers show that different genomic approaches can effectively be used to detect specific cancer biomarkers. The genomic approach can further be applied for estimation of prognosis in cancer disease, for therapy planning, and for therapy monitoring in vitro and in vivo. However, the advances are still only a small step in the development of clinically applicable biomarkers. This is also the case for molecular imaging of gene expression.

The studies reported in this review demonstrate that molecular diagnostics already has 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, perform diagnostics in vivo, monitor treatment and test and develop new drugs.

New classes of biomarkers derived from mass spectroscopy 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 development of a modular platform technology with sufficient flexibility and design abstractions allowing for concurrent experimentation, testing, and refinement will help speed the progress of mass spectroscopy-derived proteomic pattern-based diagnostics from the scientific laboratory to the medical clinic.

The approach of HR-MAS spectroscopy demonstrates that it is possible to separate out metabolites for tumors. Therefore, this non-invasive technique is useful for detecting and monitoring metabolic changes in vitro and in vivo, whereas tissue-extraction procedures often dilute these metabolites. It seems that HR-MAS [1]H NMR spectroscopy will become increasingly important as a metabolic tool. It is also confirming the metabolic events measured, in vivo, by means of MRS. Like gene-expression profiling, this approach allows the screening of large populations and can be used for high throughput analysis. Metabolomic approaches generate highly reproducible datasets. Unlike DNA microarrays, costs are low per sample after the initial equipment purchase (36). 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 (see section 2.1.3: Molecular imaging of gene expression). Therefore, metabolic approaches and the detection of specific metabolic profiles will become a new important approach in detection of biomarkers for (early) detection of markers in vivo and in vitro.

6. Conclusions

Molecular imaging based on “-omics” has become an important tool in biology and medicine for non-invasive detecting and monitoring metabolic changes in vitro and in vivo. Advances have been made including tissue recognition by microcopy, radionuclide imaging, such as positron emission tomography (PET) and single photon emission

tomography (SPECT), magnetic resonance (MR) imaging and spectroscopy, bioluminescence imaging and various fluorescence imaging techniques, such as fluorescence-mediated tomography (FMT) and near-infrared fluorescence (NIRF) reflectance imaging.

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