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Radionuclide Imaging in the Post-Genomic Era

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Abstract The assessment of gene function, which follows the completion of human genome sequencing, may be performed using the tools of the genome program. These tools represent high-throughput methods evaluating changes in the expression of many or all genes of an organism at the same time in order to investigate genetic pathways for normal development and disease. They describe proteins on a proteome-wide scale, thereby, creating a new way of doing cell research which results in the determination of three dimensional protein structures and the description of protein networks. These descriptions may then be used for the design of new hypotheses and experiments in the traditional physiological, biochemical, and pharmacological sense. The evaluation of genetically manipulated animals or new designed biomolecules will require a thorough understanding of physiology, biochemistry, and pharmacology and the experimental approaches will involve many new technologies including in vivo imaging with SPECT and positron emission tomography (PET). Nuclear medicine procedures may be applied for the determination of gene function and regulation using established and new tracers or using in vivo reporter genes such as genes encoding enzymes, receptors, antigens, or transporters. Pharmacogenomics will identify new surrogate markers for therapy monitoring which may represent potential new tracers for imaging. Also drug distribution studies for new therapeutic biomolecules are needed at least during preclinical stages of drug development. Finally, new biomolecules will be developed by bioengineering methods, which may be used for isotope-based diagnosis and treatment of disease. J. Cell. Biochem. Suppl. 39: 1–10, 2002.

Key words: functional genomics; nuclear medicine; imaging; radionuclides

After the sequencing of the human genome has been completed, a tremendous amount of new research is needed to make sense of the sequence information which is now available. The basic challenges are: finding the genes, locating their coding regions, and predicting their functions. The next steps following gene identification are designed to figure out the properties that specific genes encode and what they do for a living organism. Gene mapping and sequencing delivers information about linkages, genome organization, protein complement, gene regulation, phylogeny, and evolution. One of the main problems is to package the huge amount of sequences and expression profiles generated by new array methods into useful biological knowledge [Eisenberg et al., 2000]. This may result in new diagnostic and therapeutic procedures, which include visualization of and interference with gene transcription and the development of new biomolecules useful for diagnosis and treatment.

ANALYSIS OF GENE EXPRESSION: FROM CHIPS TO IMAGES

The estimation of gene function using the tools of the genome program has been referred to as "functional genomics" which can be seen as describing the processes leading from a gene's physical structure and its regulation to the gene's role in the whole organism. Many studies in functional genomics are performed by analysis of differential gene expression using methods such as DNA chip technology. These methods are used to evaluate changes in the transcription of many or all genes of an organism at the same time in order to investigate genetic pathways for normal development and disease.

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A possible tool for the non-invasive detection of gene expression may be antisense imaging. Antisense RNA and DNA techniques have been originally developed to modulate the gene expression in a specific manner. These techniques originated from early studies in bacteria demonstrating that these organisms are able to regulate their gene replication and expression by the production of small complementary RNA molecules in an antisense (opposite) direction. Base pairing between the oligonucleotide and the corresponding target messenger RNA (mRNA) leads to highly specific binding and specific interaction with protein synthesis. Thereafter, several laboratories showed that synthetic oligonucleotides complementary to mRNA sequences could downregulate the translation of various oncogenes in cells [Zamecnik and Stephenson, 1978; Mukhopadhyay et al., 1991].

However, besides their use as potential therapeutics for specific interaction with RNA processing 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 which take into account alternative polyadenylation and alternative splicing result in a mRNA number between 46,000 and 85,000 [Claverie, 2001] which can theoretically be used for diagnostic or therapeutic purposes. It is expected that an oligonucleotide with more than 12 (12-mer) nucleobases represents a unique sequence in the whole genome [Woolf et al., 1992]. Since these short oligonucleotides can easily be produced antisense imaging using radiolabeled oligonucleotides may in principle offer a huge amount of new specific tracers. Prerequisites for the use of radiolalebeld antisense-oligonucleotides are ease of synthesis, stability in vivo, uptake into the cell, accumulation of the oligonucleotide inside the cell, interaction with the target structure, and minimal non-specific 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. In this respect, modification of the phosphodiester backbone such as phosphorothioates, methylphosphonates, peptide nucleic acids, or gapmers (mixed backbone oligonucleotides) result in at least a partial loss in cleavage by enzymes such as RNAse H.

Evidence has been presented of a receptor-coupled endocytosis of low capacity as the mechanism by which oligonucleotides enter cells [Loke et al., 1989; Iversen et al., 1992]. Subcellular fractionation experiments showed a sequestration of the oligonuleotides in the nuclei and the mitochondria of HeLa cells [Iversen et al., 1992]. This fractionation, the problems with the in vivo stability of the oligonucleotides as well as the stability of the hybrid oligo-RNA structures are severe obstacles to successful imaging of gene expression. Furthermore, binding to other polyanions such as heparin based on charge interaction may cause unspecific signals.

However, accumulation of ^{111}In-labeled c-myc antisense probes with a phosphorothioate-backbone was reported in mice bearing c-myc overexpressing mammary tumors [Dewanjee et al., 1994]. Tumor imaging was also possible with a transforming growth factor α antisense oligonucleotide or antisense phosphorothioate oligodeoxynucleotide for the mRNA of glial fibrillary acidic protein [Urbain et al., 1995; Cammilleri et al., 1996; Kobori et al., 1999]. Also in rat glioma cells permanently transfected with the luciferase gene autoradiography showed accumulation of a ^{125}I-labeled antisense peptide nucleic acid targeted to the initiation codon of the luciferase mRNA [Shi et al., 2000]. Furthermore, positron emission tomography (PET) was used for the assessment of the biodistribution and kinetics of ^{18}F-labeled oligonucleotides [Tavitian et al., 1998]. In addition, ^{90}Y labeled phosphorothioate antisense oligonucleotides may be applied as targeted radionuclide therapeutic agents for malignant tumors as was done for a phosphorothioate antisense oligonucleotide complementary to the translation start region of the N-myc oncogene mRNA [Watanabe et al., 1999]. The resulting ^{90}Y antisense oligonucleotide hybridized specifically to a complementary phosphorodiester sense oligonucleotide.

However, current data show that the transcriptome obtained with mRNA profiling for the characterization of cellular phenotypes does not faithfully represent the proteome because the mRNA content seems to be a poor indicator of the corresponding protein levels [Anderson et al., 1997; Futcher et al., 1999; Gygi et al., 1999]. Direct comparison of mRNA and protein levels in mammalian cells either for several genes in one tissue or for one gene product in

many cell types reveal only poor correlations (0.5 or lower) with up to 30-fold variations. This might lead to misinterpretation of mRNA profiling results.

Furthermore, a substantial fraction of interesting intracellular events is located at the protein level, for example, operating primarily through phosphorylation/dephosphorylation and the migration of proteins. Also proteolytic modifications of membrane-bound precursors appear to regulate the release of a large series of extracellular signals such as angiotensin, tumor necrosis factor, and others. mRNA is much more labile than DNA leading to spontaneous chemical degradation and to degradation by enzymes. Degradation may be dependent on the specific sequence, resulting in non-uniform degradation of RNA, which introduces quantitative biases that are dependent on the time after the onset of tissue stress or death. In contrast, proteins are generally more stable, and exhibit slower turnover rates in most tissues.

Expression profiling approaches data would be more useful, if mRNA samples could be enriched for transcripts that are being translated [Pradet-Balade et al., 2001]. This can be achieved by fractionation of cytoplasmic extracts in sucrose gradients which leads to the separation of free mRNPs (ribonucleoprotein particles) from mRNAs in ribosomal pre-initiation complexes and from mRNAs loaded with ribosomes (polysomes). Since only the polysomes represent actively translated transcripts, this fraction should be directly correlated with de novo synthesized proteins. This method assumes that translational control predominantly occurs at the initiation step. Thus polysome-bound mRNA profiling should provide a closer representation of the proteome than does profiling of total mRNA. Although this hypothesis is supported by measurements of total protein synthesis rates and overall polysome-bound mRNA levels [Smith et al., 1999] it remains to be proven on a proteome-wide scale. Furthermore, polysome-bound mRNA profiling cannot be used to study changes affecting protein levels by proteolysis, post-translational modifications, subcellular localization, or protein degradation. This type of analysis is restricted to proteomics.

Since protein levels often do not reflect mRNA levels, antisense imaging may not be a generally applicable approach. Polysome imaging with nuclear medicine procedures has not been tried to date or even may not be possible. Therefore, antisense imaging for the determination of transcription by hybridization of the labeled antisense probe to the target mRNA makes sense in cases where a clear correlation of mRNA and protein exists. Correspondingly successful imaging was possible in cases where the expression of the protein was proven [Dewanjee et al., 1994; Urbain et al., 1995; Cammilleri et al., 1996; Kobori et al., 1999; Shi et al., 2000]. If no correlation between mRNA and protein exists the diagnostic use of antisense imaging is questionable. It may be expected that rather therapeutic applications using triplex oligonucleotides with therapeutic isotopes would make sense. In that case, Auger electron emitters can be brought near to specific DNA sequences to induce DNA strand breaks at selected loci.

FUNCTIONAL STUDIES IN ANIMALS BEARING MUTATIONS

Usually, producing a knockout mutation or altering the expression of a gene gives rise of a phenotype that provides insights into the function of a gene. Besides these genotype-driven mutations there is increasing need for phenotype-driven mutations (e.g., using the alkylating agent *N*-ethyl-*N*-nitrosurea) to identify genes that are involved in specific kinds of disease. This approach needs no assumptions with respect to which genes and what kinds of mutations are involved in a particular phenotype or disease. In order to maximize the efficiency of the related experiments it will be necessary to develop multiple assays working at different levels of description (morphologic, physiologic, biochemical, or behavioral) to detect a large number of different phenotypes in a given set of mutagenized mice. The screening of a mutagenized population is usually done with phenotypically visible coat color markers in combination with selection genes as neomycin resistance gene and the Herpes Simplex Virus thymidine kinase (HSVtk) gene. However, using genes as HSVtk or others in combination with scintigraphic imaging as non-invasive in vivo reporters may be an attracting alternative.

USE OF IN VIVO REPORTER GENES

Commonly used reporter genes as β-galactosidase, chloramphenicol-acetyltransferase, green fluorescent protein (GFP), or luciferase play critical roles in investigating the mechanisms of

gene expression in transgenic animals and in developing gene delivery systems for gene therapy. However, measuring expression of these reporter genes often requires biopsy or death or cannot be used for the non-invasive imaging of deeper structures in the body. In vivo reporter genes can be visualized non-invasively using radiolabeled molecules. In this respect, genes encoding for enzymes, receptors, antigens, and transporters have been used. Enzyme activity can be assessed by the accumulation of the metabolites of radiolabeled specific substrates, receptors by the binding and/or internalization of ligands, antigens by binding of antibodies, and transporters by the accumulation of their substrates. Since expression of the HSVtk gene leads to phosphorylation of specific substrates and to the accumulation of the resulting negatively charged metabolite, this gene can be used as an in vivo reporter gene [Haberkorn et al., 1997; Tjuvajev et al., 1998; Alauddin et al., 1999; Gambhir et al., 1999; De Vries et al., 2000; Haberkorn and Altmann, 2001; Hustinx et al., 2001]. Using radiolabeled specific HSVtk substrates a significant higher uptake was found in HSVtk-expressing cells as compared to the wild type (wt) controls. There was a significant positive correlation between the percent injected dose of [^{131}I] 5-iodo-2′-fluoro-2′-deoxy-1-b-D-arabinofuranosyluracil (FIAU) and [^{18}F]fluoroganciclovir retained per gram of tissue and the levels of HSVtk expression. The amount of tracer uptake in the tumors was correlated to the in vitro ganciclovir (GCV) sensitivity of the cell lines, which were transplanted in these animals. A general problem is the fact that the affinity of these specific substrates for the nucleoside transport systems as well as for the enzyme is rather low which may be a limiting factor for cellular accumulation. Therefore, at present the ideal tracer for HSVtk imaging has not been identified and more efforts have to be done to synthesize radiolabeled compounds with improved biochemical properties.

In order to improve the detection of low levels of PET reporter gene expression a mutant Herpes Simplex Virus type 1 thymidine kinase (HSV1-sr39tk) has been used as an in vivo reporter gene for PET [Gambhir et al., 2000]. Successful transfer of this mutant gene resulted in enhanced uptake of the specific substrates [8-^{3}H]penciclovir, and 8-[^{18}F]fluoropenciclovir in C6 rat glioma cells with a twofold increase in accumulation compared with wt HSVtk-expressing tumor cells.

However, other genes may also be candidates for the in vivo detection of gene transfer. The dopamine D2 receptor gene represents an endogenous gene, which is not likely to invoke an immune response. Furthermore, the corresponding tracer 3-(2′-[^{18}F]-fluoroethyl)spiperone (FESP) rapidly crosses the blood–brain-barrier, can be produced at high specific activity and is currently used in patients. The tracer uptake in nude mice after transfection with an adenoviral-directed hepatic gene delivery system and also in transplanted stable tumor cells was proportional to in vitro data of hepatic FESP accumulation, dopamine receptor ligand binding, and the D2 receptor mRNA [MacLaren et al., 1999]. Also tumors modified to express the D2 receptor retained significantly more FESP than wt tumors. In modified non-small cell lung cell lines expressing the human type 2 somatostatin receptor and transplanted in nude mice images were obtained using an somatostatin-avid peptide (P829), that was radiolabeled to high specific activity with ^{99m}Tc or ^{188}Re [Zinn et al., 2000]. In the genetically modified tumors a fivefold to tenfold greater accumulation of both radiolabeled P829 peptides as compared to the control tumors was observed. The ^{188}Re-labeled peptide revealed similar results and has the additional advantage of energetic β decay with a potential use for therapy.

To overcome the limitation of low expression of tumor-associated antigens on target cells for radioimmunotherapy the gene for the human carcinoembryonic antigen (CEA) was transferred in a human glioma cell line resulting in high levels of CEA expression [Raben et al., 1996]. In these modified tumor cells, high binding of an ^{131}I-labeled CEA antibody was observed in vitro as well as by scintigraphic imaging.

Another approach is based on the in vivo transchelation of oxotechnetate to a polypeptide-motif from a biocompatible complex with a higher dissociation constant than that of a dicglycilcysteine complex. It has been shown that synthetic peptides and recombinant proteins like a modified GFP can bind oxotechnetate with high efficiency [Bogdanov et al., 1998]. In these experiments rats were injected i.m. with synthetic peptides bearing a diglycylcysteine (GGC) binding motif. One hour later ^{99m}Tc-glucoheptonate was injected i.v. and the

accumulation was measured by scintigraphy. The peptides with three metal-binding GGC motifs showed a threefold higher accumulation as compared to the controls. This principle can also be applied to recombinant proteins, which appear at the plasma membrane [Simonova et al., 1999].

Transfer of the human sodium iodide symporter (hNIS) gene into Moris hepatoma cells caused a significant increase in iodide uptake (by a factor of 84–235) with a peak after 1 h incubation. The radioactive iodide was concentrated in the cells to values up to 10^5-fold more than in the medium [Haberkorn et al., 2001a]. This is far more than in normal thyroid tissue where an I^- concentration gradient of 30-fold in FRTL5 cells and 20- to 40-fold in the thyroid gland in vivo has been described. However, a rapid efflux (80%) was observed in hNIS-expressing hepatoma cells during the first 10 min indicating that no organification of the radioactive iodide occurred. Animal studies with wt and hNIS-expressing tumors in rats showed similar results with a maximum uptake after 1 h (Fig. 1) and a continuous disappearance of the radioactivity out of the body as well as of the hNIS-expressing tumors [Haberkorn et al., 2001a]. Although the NIS activity is asymmetrical favoring iodide influx, there is obviously an efflux activity with the consequence that in cells that do not organify iodide the concentration of intracellular iodide will drop proportionally to the external iodide concentration. After administration of 0.4 mCi ^{131}I this resulted in an absorbed dose of 35 mGy (wt tumor) and 592 mGy (hNIS-expressing tumor). Therefore, the use of the hNIS gene alone for radioiodine therapy of non-thyroid tumors seems questionable, but the hNIS gene may be used together with ^{121}I, ^{124}I, or even with ^{99m}Tc-pertechnetate as a simple reporter system for the visualization of other genes in bicistronic vectors which allow co-expression of two different genes [Yu et al., 2000].

Of all these reporter genes described, the sodium iodide symporter gene may present the advantage that it is not likely to interact with underlying cell biochemistry. Iodide is not metabolized in most tissues and, although sodium influx may be a concern, no effects have been observed to date [Haberkorn et al., 2002]. The HSVtk gene may alter the cellular behavior towards apoptosis by changes in the dNTP pool [Oliver et al., 1997], antigens may cause immunoreactivity, and receptors may result in second messenger activation such as triggering signal transduction pathways. However, these possible interactions have to be studied in detail in future experiments. For the dopamine 2 receptor system a mutant gene has been applied which shows uncoupling of signal transduction [Liang et al., 2001].

ANALYSIS OF GENE REGULATION BY REPORTER ASSAYS

Functional genomics partly relies on a comparison of sequences near coding regions in

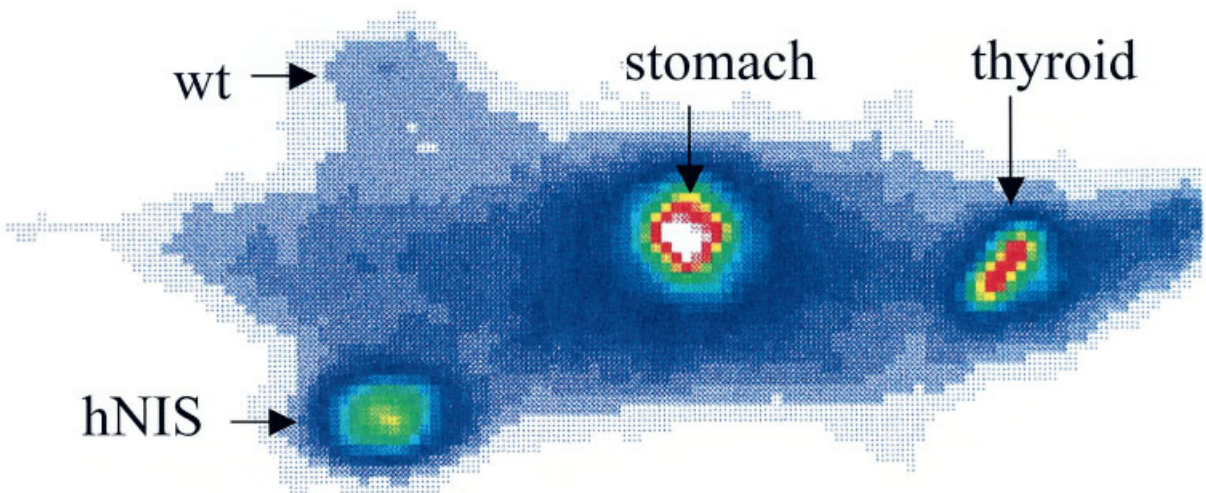

Fig. 1. Scintigraphic image of a tumor bearing male young adult Copenhagen rat subcutaneously transplanted with human sodium iodide symporter (hNIS)-expressing or wild type (wt) prostate adenocarcinoma cells at 1 h after injection of $^{131}I^-$.

diverged organisms assuming that nucleotides conserved in non-coding regions between these pairs of organisms identify functional sites which typically are response elements for regulatory proteins. Among genes, which exhibit correlated expression patterns across a large variety of biological conditions, a significant fraction is expected to be co-regulated, i.e., responsive to common expression factors. However, the prediction of promoter locations and properties as well as analyses for the precise identification of intron/exon architecture and boundaries of gene transcripts still face unacceptable uncertainties [Claverie, 1999; Werner, 1999].

With the increasing availability of intrinsically fluorescent proteins that can be genetically fused to virtually any protein of interest, their application as fluorescent biosensors has extended to dynamic imaging studies of cellular biochemistry even at the level of organelles or compartments participating in specific processes [Fred et al., 2001]. On the supracellular level, fluorescence imaging allows the determination of cell-to-cell variation, the extent of variation in cellular responses, and the mapping of processes in multicellular tissues. Furthermore, visualization of intracellular gradients in enzymatic activities, such as phosphorylation and GTPase activity, can now be related to morphogenetic processes, where the distribution of activity shapes the cellular response.

However, for the examination of whole organisms and especially of organ systems in deeper parts of the body in vivo reporter systems are promising. Biological systems are more complex than cell cultures because external stimuli may affect and trigger cells. Therefore, non-invasive dynamic in vivo measurements are needed to study gene regulation in the physiological context of complex organisms. These in vivo reporters may be used also for the characterization of promoter regulation involved in signal transduction, gene regulation during changes of the physiological environment, and gene regulation during pharmacological intervention. This may be done by combining specific promoter elements with an in vivo reporter gene. Furthermore, the functional characterization of new genes will result in new diagnostic targets and possibly also in new tracers for their visualization which may be substrates for enzymes or transporters, ligands for receptors or antibodies for antigens. However, there may be concerns about the image resolution even when animal scanners are used. Therefore, autoradiography or FRET represent alternatives in cases where resolution at the micrometer range is required.

DESIGN OF NEW BIOMOLECULES FOR DIAGNOSIS AND THERAPY

Through the accumulation of genomics and proteomics data novel biomolecules may be discovered or designed on a rational basis in diverse areas including pharmaceutical, agricultural, industrial, and environmental applications. This can be achieved through directed genome evolution, metabolic pathway engineering, protein engineering, analyses of functional genomics and proteomics, high-throughput screening techniques, and the development of bioprocess technology [Ryu and Nam, 2000]. The products of this process will be monoclonal antibodies, vaccines, enzymes, antibiotics, therapeutic peptides, and others. Besides bioinformatics, functional genomics, proteomics, protein chemistry, and engineering, the methods involved include also recombinant techniques as random mutation DNA shuffling and phage-displays, metabolic pathway engineering including metabolic flux analysis, and bioprocess engineering which develops technologies needed for the production of the desired high-value biomolecules.

Two techniques are important for the design of new biomolecules: DNA shuffling and phage display libraries. DNA shuffling mimicks natural recombination by allowing in vitro homologous recombination of DNA [Stemmer, 1994; Kolkman and Stemmer, 2001]. Therefore, a population of related genes is randomly fragmented and subjected to denaturation and hybridization, followed by the extension of 5′ overhang fragments by Taq DNA polymerase. A DNA recombination occurs when a fragment derived from one template primes a template with different sequences. The applications of this method include improvement of enzyme properties, development of altered metabolism pathways, antibiotics and pharmaceutical proteins, development of plasmids or viruses for novel vaccines, and gene therapy applications. Genes from multiple parents and even from different species can be shuffled in a single step in operations that do not occur in nature but

may be very useful for the development of diagnostic and therapeutic approaches.

The principle of phage-displayed peptide libraries is the display of the peptide libraries fused with the carboxy-terminal domain of the minor coat protein, gene III protein fragment, on the surface of a filamentous phage. The relevant molecule is then directly detected and screened using the target molecules and amplificated after infection of *Escherichia coli*. This allows a rapid selection (within weeks) of particular clones from large pools ($>10^{10}$ clones), and determination of the amino acid sequence of a peptide displayed on a phage by sequencing the relevant section of the phage genome. This technique has been employed for searching antibodies, receptors for new drug discovery, and cancer therapy, either as an antagonist or an agonist of a natural ligand–receptor interaction [Smithgall, 1995; Zwick et al., 1998] and custom-made enzymes for gene therapy.

One of the main goals of biocatalyst engineering is to endow them with new features that are not found in natural sequences because they confer no evolutionary advantage [Arnold, 2001]. The design of a biocatalyst involves two main steps, which can be iterative: making a set of mutant biocatalysts and searching that set of mutants with the desired properties. In this stage of development, isotope based methods are needed to assess binding characteristics of antibodies or ligands or measure new transport or enzyme functions in vivo. Furthermore, in later stages the coupling of antibodies or peptides with α or β emitters may be used for therapeutic purposes.

THERAPY MONITORING

For therapy monitoring proteomics may deliver new means for the evaluation of therapeutic effects on the target tissue as well as on other tissues. This is based on the assumption that many therapeutic drugs act through mechanisms involving perturbations of protein expression and, therefore, a successful drug could be defined as one that restores the expression levels of a cell or an organ to the normal state [Anderson and Anderson, 1998]. Using this approach drugs can be compared with respect to their effectiveness in restoring the normal protein expression. These novel "surrogate markers" for functional measurements will provide quantitative data of the drug's influence on the disease process, whereas standard clinical trials depend primarily on improvements in clinical signs. This relies on strong functional relationships between drug treatment, protein expression, and resulting physiological effects where the real therapeutic mechanism often consists of modulations in protein gene expression occurring as a secondary result. Therefore, it is expected that drugs acting through similar mechanisms ought to produce similar gene expression effects and, conversely, different mechanisms should produce distinct expression effects.

Also measurement of the pattern of protein changes can be used to describe the mechanism of action. Proteomics offers the opportunity to obtain complimentary informations to genomic-based technologies for the identification and validation of protein targets in following time-dependent changes in protein expression levels which result from selective interaction with specific biological pathways and identifying protein networks (functional proteomics). Changes in protein expression or function could also serve as targets for non-invasive imaging procedures. Although nuclear medicine procedures are not large scale measurements by defining leading changes in protein content or function it may be possible to use radiolabeled ligands or substrates to assess the drug's effects on specific parts of the proteome. This may be done using established tracers for new therapies or using new tracers which have been identified either by functional studies of new genes or by analysis of changes in expression or functional patterns by the high-throughput methods of functional genomics.

As an example measurements of tumor perfusion and tumor metabolism have been performed to assess the efficacy of suicide gene therapy. Tumor perfusion, as measured in GCV treated HSVtk-expressing KBALB tumors after intravenous administration of [^{99m}Tc]HMPAO, increased by 206% at day 2 after the onset of ganciclovir treatment [Morin et al., 2000]. In the same animals, the accumulation of the hypoxia tracer [^{3}H]misonidazole decreased to 34% at day 3, indicating that the tumor tissue had become less hypoxic during ganciclovir treatment.

The ^{18}F-fluorodeoxyglucose (FDG) uptake has demonstrated to be a useful and very sensitive parameter for the evaluation of glucose metabolism during or early after treatment of malignant tumors. Dynamic PET measure-

ments of ^{18}FDG uptake in rats bearing HSVtk-expressing hepatomas revealed an uncoupling of FDG transport and phosphorylation with enhanced transport values and a normal phorphorylation rate after 2 days of GCV treatment [Haberkorn et al., 1998]. These tumors showed a significant increase of the glucose transporter 1 (GLUT1) as demonstrated by immunohistochemistry [Haberkorn et al., 2001b]. The increase in FDG transport normalized after 4 days whereas the phosphorylation rate increased. As underlying mechanism a redistribution of the glucose transport protein from intracellular pools to the plasma membrane may be considered and is observed in cell culture studies as a general reaction to cellular stress. Consequently, inhibition of glucose transport by cytochalasin B or competition with deoxyglucose increased apoptosis [Haberkorn et al., 2001b].

Besides these established monitoring procedures we may expect new biochemical pathways emerging from proteomics research leading to the use of radiolabeled substrates for enzymes, transport systems or specific structures on cell membranes.

CONCLUSION

A huge amount of new molecular structures have been cloned and are now available as potential novel diagnostic or drug discovery targets. Therefore, the target selection and validation has become the most critical component in this process. Ultimately, the disciplines of functional genomics and proteomics have their foundation in the physiological, biochemical, and pharmacological sciences. The evaluation of genetically manipulated animals or new designed biomolecules will require a thorough understanding of physiology, biochemistry, and pharmacology and the experimental approaches will involve many new technologies including in vivo imaging with MRI and PET [Ohlstein et al., 2000]. Nuclear medicine procedures may be applied for the determination of gene function and regulation using established or new tracers, for example, in knockout mice or in transgenic animals. The measurement of gene regulation may also be done using in vivo reporter genes such as enzymes, receptors, antigens, or transporters. Intracellular signaling has been visualized in vitro with combinations of specific regulatory elements (promoters, enhancers) and reporter genes such as the secreted alkaline phosphatase (SEAP) downstream of several copies of specific transcription factor binding sequences [Ohkubo et al., 2001]. This approach may be extended for in vivo detection using in vivo reporters instead of SEAP. Pharmacogenomics will identify new surrogate markers for therapy monitoring which may represent potential new tracers for imaging. Also drug distribution studies for new biomolecules are needed to fasten drug approval at least in preclinical stages of drug development. Finally, bioengineering will lead to the design of new biomolecules by methods such as DNA shuffling or phage display procedures which may be used for new approaches in isotope-based diagnosis and treatment of disease.

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