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Prospects of Molecular Imaging in Neurology

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Abstract Molecular imaging aims towards the non-invasive kinetic and quantitative assessment and localization of biological processes of normal and diseased cells in vivo in animal models and humans. Due to technological advances during the past years, imaging of molecular processes is a rapidly growing field, which has the potential of broad applications in the study of cell biology, biochemistry, gene/protein function and regulation, signal transduction, characterization of transgenic animals, development of new treatment strategies (gene or cell-based) and their successful implementation into clinical application. Most importantly, the possibility to study these parameters in the same subject repeatedly over time makes molecular imaging an attractive technology to obtain reliable data and to safe recourse; for example, molecular imaging enables the assessment of an exogenously introduced therapeutic gene and the related alterations of endogenously regulated gene functions directly in the same subject. Therefore, molecular imaging will have great implications especially when molecular diagnostic and treatment modalities have to be translated from experimental into clinical application. Here, we review the three main imaging technologies, which have been developed for studying molecular processes in vivo, the disease models, which have been studied so far, and the potential future applications. J. Cell. Biochem. Suppl. 39: 98–109, 2002. © 2002 Wiley-Liss, Inc.

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Molecular imaging is an exciting and rapidly evolving field based on concerted research and technical developments spanning multiple disciplines. The ultimate goal of molecular imaging is the *non-invasive* localization and quantification of gene expression, protein function, and profiling of signal transduction pathways in vivo, (i) to get further insight into the molecular pathophysiology of animal models of human diseases; (ii) to speed up the development of new drugs; and (iii) to facilitate the design and implementation of improved patient-tailored therapies. Imaging molecular processes in vivo may allow a detailed kinetic analysis (over time) of both, endogenous and exogenous, gene expression in living animals, and in the clinical setting.

Non-invasive imaging of endogenous gene expression may reveal insights into the molecular basis of disease pathogenesis and into the extent of treatment response. On the other hand, when exogenous genes are introduced to ameliorate a genetic defect or to add an additional gene function to cells, imaging techniques may reveal the assessment of the location, magnitude and duration of therapeutic gene expression and, preferably, its correlation to the therapeutic response. Moreover, marker gene expression under control of a specific promoter may allow a direct visualization of promoter and related transcriptional activator/repressor functions. For this purpose, specific reporter genes are linked to genetic regulatory elements, and their expression can reveal spatial and temporal information about a variety of biological processes at the level of transcription.

Especially for the development and successful implementation of new gene- and cell-based therapies, imaging of endogenous effector gene expression and imaging of cells as well as

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vector-mediated gene expression in vivo becomes of critical importance. In a primate model of Parkinson's disease, for example, Kordower et al. [2000] non-invasively assessed the therapeutic effect of lentiviral vector-mediated transduction of glial cell line-derived neurotrophic factor (GDNF) by measuring the improvement of endogenous enzymatic activity of aromatic aminoacid decarboxylase (AADC) by positron emission tomography (PET) and [^{18}F]FDOPA as specific substrate for AADC. The improvement of lenti-GDNF-mediated nigrostriatal function as measured by [^{18}F]FDOPA–PET in vivo could be correlated to independent measures, such as functional improvements of motor tasks (as assessed in vivo) as well as to positive GDNF-expression and an increased number of tyrosine hydroxylase expressing nigrostriatal neurons (as assessed post mortem) indicating the relevance of molecular imaging of an endogenous effector gene (AADC) in vivo for successful implementation of this gene therapy paradigm. Moreover, in a phase-I/II clinical gene therapy trial of patients with recurrent glioblastoma, our group was able to non-invasively assess the transduced "tissue dose" of vector-mediated therapeutic gene expression by 2′-fluoro-2′-deoxy-1ß-D-arabinofuranosyl-5-[^{124}I]iodo-uracil ([^{124}I]FIAU) and PET [Jacobs et al., 2001c]. The imaged "tissue dose" of therapeutic gene expression could be correlated to the induced therapeutic effect by measuring the metabolic activity of the tumor using 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) and *methyl*-[^{11}C]-L-methionine ([^{11}C]MET) and PET. Both radiotracers served as direct measures of expression of cellular hexokinase and amino acid transporters and as indirect measures for the proliferative activity of the tumor, respectively. These two examples demonstrate, that molecular imaging of exogenous and endogenous gene expression is directly involved in the successful establishment of safe and efficient gene therapy protocols in the clinical application. This is in line with the Recombinant DNA Advisory Committee (RAC) of the NIH which as a reflection to the first gene therapy death called for better assays for measuring transgene expression in cells and tissues in vivo [Hollon, 2000].

CONCEPTS AND EXPERIMENTAL APPROACHES

Different approaches and technologies for non-invasive imaging of mammalian gene expression have been developed using either optical, radionuclide, or magnetic resonance (MR) imaging techniques (Fig. 1) [Chalfie et al., 1994; Tjuvajev et al., 1995; Bogdanov and Weissleder, 1998; Contag et al., 1998; Gambhir et al., 1998; Tavitian et al., 1998; Lewin et al., 2000; Weissleder et al., 2000; Ray et al., 2002]. Although optical imaging techniques, such as intravital microscopy of green fluorescent protein (GFP) expression or bioluminescence or near-infrared fluorescence, have excellent temporal resolution, the common disadvantages are the limited spatial resolution and depth penetration [Contag et al., 1998; Honigman et al., 2001; Weissleder and Mahmood, 2001]. Nuclear imaging techniques have a high sensitivity, where very low levels of specific tracer accumulation can be detected but have an inherently limited spatial resolution [Phelps, 2000; Haberkorn and Altmann, 2001; Blasberg and Tjuvajev, 2002; de Vries and Vaalburg, 2002]. Typically, the expression of enzymes and receptors is being assessed by PET, however, the expression of peptides and cell surface epitopes or the presence of DNA and mRNA may also be imaged by using the respective radiolabeled binding partners [Tavitian et al., 1998]. MR imaging techniques have a spectacular image

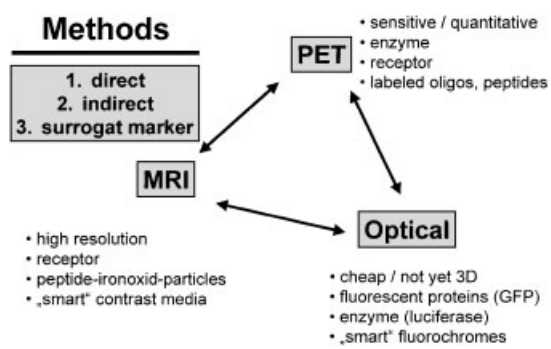

Fig. 1. Technologies in molecular imaging. PET, MRI, and optical imaging techniques have all been applied in the context of localizing and quantifying gene expression in vivo. Three paradigms have to be distinguished [Blasberg and Tjuvajev, 2002x]: (i) direct imaging of an exogenous or endogenous gene/protein by imaging the accumulation of a radiolabeled, paramagnetic or fluorescent binding partner or substrate; these genes can serve as marker genes for the (ii) indirect imaging approach, where a gene of interest can not be directly but indirectly visualized when it is proportionally co-expressed with an imaging marker gene (see also Figs. 2, 3); (iii) imaging of a surrogate marker, for example, accumulation of [^{18}F]FDG as surrogate marker for cell density of gliomas or loss of [^{18}F]FDG-activity as surrogate marker for the severity of degeneration in Alzheimer's disease.

resolution, however, temporal resolution is limited and molecular probe detection is several orders of magnitude less sensitive [Stegman et al., 1999; Bell and Taylor-Robinson, 2000; Weissleder et al., 2000]. MR imaging will be the method of choice for trafficking labeled cells at high-resolution, whereas quantification of gene expression will be difficult. Currently both, optical and MR imaging technologies, are further developed by designing "smart" contrast agents and fluorescent probes, respectively, which get only activated after a certain molecular step, for example, proteolytic cleavage by metalloproteinases, caspases, or other proteases [Weissleder, 2002].

PET Imaging of Gene Expression

PET allows the quantitative localization of expression of endogenous or exogenous genes coding for enzymes or receptors by measuring the accumulation or binding of the respective enzyme substrates or receptor binding compounds [Sokoloff et al., 1977; Phelps, 2000; Blasberg and Tjuvajev, 2002; Gambhir, 2002].

Neurologically important endogenous enzymes, receptors and membrane transporters, which can be non-invasively assessed on a routine basis in the clinical application by PET are: cellular hexokinase ([^{18}F]FDG); cellular thymidine kinase (3′-deoxy-3′-[^{18}F]fluoro-L-thymidine; [^{18}F]FLT); AADC ([^{18}F]FDOPA); acetylcholine esterase ([^{11}C]-*N-methyl*-4-piperidinylacetate; [^{11}C]MP4A); dopamine D2 ([^{11}C]raclopride); and benzodiazepine receptors ([^{11}C]flumazenil) as well as amino acid transporters ([^{11}C]methionine). These tracers are applied for early detection of Alzheimer's disease ([^{18}F]FDG, [^{11}C]MP4A), in the differentiation of Parkinson's disease from multiple systems atrophy ([^{18}F]FDOPA, [^{11}C]raclopride, [^{18}F]FDG), the grading of gliomas and differentiation of radionecrosis from recurrent tumor ([^{18}F]FDG, [^{18}F]FLT, [^{11}C]methionine), and in the assessment of neuronal integrity after stroke ([^{11}C]flumazenil, [^{18}F]FDG) [Herholz et al., 2000; Heiss et al., 2001; Hilker et al., 2001; Jacobs et al., 2002].

The unique ability of the exogenous herpes simplex virus type 1 thymidine kinase (HSV-1-TK) to selectively incorporate radiolabeled nucleoside analogues into DNA [Saito et al., 1982; Tjuvajev et al., 1995; Wiebe et al., 1997; Gambhir et al., 1998] allows the non-invasive imaging of *tk*-gene expression in distinct regions within a transduced tissue [Tjuvajev et al., 1995, 1996, 1998, 1999a; Morin et al., 1997; Gambhir et al., 1998, 1999, 2000; Haubner et al., 2000; Hospers et al., 2000]. Saito et al. [1982] were the first to propose, that the HSV-1-*tk* gene might be used as a marker gene for the early detection of herpes encephalitis by using specific radiolabeled nucleoside analogues as marker substrates [Saito et al., 1984]. However, only with the development of gene therapy strategies based on HSV-1-*tk* as suicide gene, the HSV-1-*tk* gene became attractive as PET marker gene to non-invasively follow gene therapy. Tjuvajev et al. [1995] could demonstrate for the first time, that in retrovirally transduced, stably TK-expressing rat RG2 glioma clones the accumulation rate of the specific HSV-1-TK marker substrate, 2′-fluoro-2′-deoxy-1-ß-D-arabinofuranosyl-5-iodo-uracil (FIAU), correlated with the level of *tk-mRNA* expression. The quantitative determination of TK expression by PET is based on the same principles as the measurement of local cerebral glucose utilization as described by Sokoloff et al. [1977] by measuring the accumulation rates of specific viral thymidine kinase substrates, such as [^{124}I]FIAU [Tjuvajev et al., 1998; Blasberg and Tjuvajev, 1999; Brust et al., 2001; Jacobs et al., 2001a,b,c], and the acyclic guanosine derivatives 8-[^{18}F]fluoro-ganciclovir ([^{18}F]FGCV; [Gambhir et al., 1998; Gambhir et al., 1999]), 8-[^{18}F]fluoro-penciclovir ([^{18}F]FPCV; [Gambhir et al., 2000; Iyer et al., 2001a]), 9-[(3-[^{18}F]fluoro-1-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG; [Hospers et al., 2000; de Vries et al., 2000; Brust et al., 2001; Hustinx et al., 2001]), and 9-[4-[^{18}F]fluoro-3-(hydroxymethyl)butyl]guanine ([^{18}F]FHBG; [Alauddin and Conti, 1998; Alauddin et al., 2001; Yaghoubi et al., 2001a]) as marker substrates by wild-type or mutated HSV-1-*tk* genes as marker genes [Black et al., 1996; Gambhir et al., 2000]. Most importantly, various levels of TK-expression could be non-invasively distinguished in vivo by PET after retroviral [Tjuvajev et al., 1996, 1998; Hospers et al., 2000; Iyer et al., 2001], adenoviral [Gambhir et al., 1998; Tjuvajev et al., 1999], and herpes viral [Jacobs et al., 2001b] vector-mediated *tk*-gene transfer into liver or subcutaneous glioma models in rodents (Figs. 2, 3), into the cardiac muscle of rats [Bengel et al., 2000; Wu et al., 2002a], or even into gliomas of patients [Jacobs et al., 2001c]. By incorporating the *tk*-gene as PET marker gene into a gene

therapy vector can thus enable a non-invasive detection of the location, level, and duration of vector-mediated *tk*-gene expression. These parameters determine the transduced "tissue-dose" of vector-mediated therapeutic gene expression which then can be correlated to the induced therapeutic response [Jacobs et al., 2001c]. A comparison of accumulation rates of [^{124}I]FIAU, [^{18}F]FHPG, and [^{18}F]FHBG in HSV-1-*tk* transduced tumors in experimental models [Brust et al., 2001; Tjuvajev et al., 2002] indicates that [^{124}I]FIAU and [^{18}F]FHBG might be the marker substrates of choice for clinical application [Alauddin et al., 2001; Jacobs et al., 2001c; Yaghoubi et al., 2001a; Tjuvajev et al., 2002].

Another marker gene/marker substrate combination was implemented using PET to quantify the level of adenovirus vector-mediated expression of a wild-type [MacLaren et al., 1999; Ogawa et al., 2000; Umegaki et al., 2002] or mutated [Liang et al., 2001] dopamine-2-receptor (D2R) by using the specific D2-receptor binding compounds [^{11}C]raclopride [Ogawa et al., 2000; Umegaki et al., 2002] and 3-(2′-[^{18}F]fluoroethyl)-spiperone [MacLaren et al., 1999; Liang et al., 2001]. After injection into the rat striatum a kinetic analysis revealed the maximum level of D2R expression 2–3 days after vector application declining to basal levels at day 16 [Ogawa et al., 2000; Umegaki et al., 2002] indicating that various levels of D2R expression can be differentiated by PET. By placing the *d2r* gene and a mutated HSV-1-*tk* gene under transcriptional control of a bi-directional, tetracycline-responsive element, Sun et al. [2001] could demonstrate that various levels of PET marker gene expression can be differentiated by PET in cell lines stably expressing these constructs depending on the state of induction. These data indicate that PET can monitor time-dependent variations of gene expression mediated by inducible promoters.

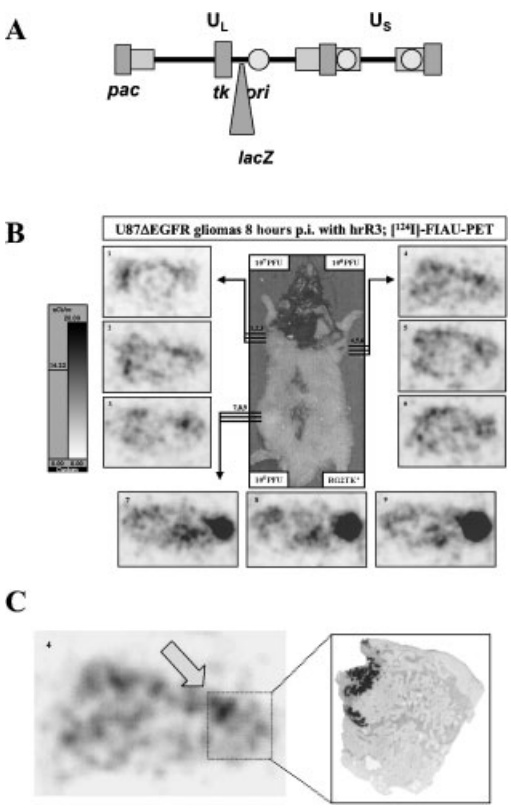

Fig. 2. Imaging HSV-1 vector mediated gene expression in vivo. The scheme of the replication-conditional HSV-1 vector hrR3 is shown in (**A**). The HSV-1 genome is a linear, double-stranded DNA of ~152 kb which encodes more than 80 genes. The genome is composed of unique long (U_L) and unique short (U_S) segments, which are flanked by inverted repeats. *Pac* signals are contained in the *a* sequences located at the junction between the long and short segment and at both termini. The HSV-1 genome contains two origins of DNA replication, ori_S and ori_L. Approximately half of the genes are essential for virus replication in cell culture. The other half encode accessory functions, which contribute to the virus life cycle in specific tissues or cell types, for example, post-mitotic neurons. The genes known to be dispensable for growth in cultured cells may be important for both optimal lytic replication and replication in vivo, contributing to pathogenesis, host range, latency, or spread in neurons. The hrR3 vector contains a lac-insertional mutation at the ribonucleotide reductase (RR) gene locus restricting replication of this vector to proliferating tumor cells with complementary RR activity. Both, *tk* and *lacZ* genes are under control of timely coordinated immediate early gene promoters. In (**B**), *direct* [^{124}I]FIAU–PET imaging of hrR3 vector-mediated HSV-1-*tk* gene expression in a nude rat bearing s.c. human U87dEGFR gliomas. Each animal had three s.c. U87dEGFR gliomas and one RG2TK+ positive control tumor. Each of the U87dEGFR tumors were injected with different doses of hrR3 (10^6–10^8 plaque forming units; pfu). The injection tract was along a cranio-caudal axis of the animal in the dorsomedial part of the tumor. [^{124}I]FIAU was administered 8 h after virus injection, and PET imaging was performed 48 h later. Transaxial PET images demonstrate regions of HSV-1-*tk* related radioactivity primarily around injection sites. A RG2-TK+ tumor demonstrated a 55–98-fold higher level of HSV-1-*tk* gene expression. Radioactivity concentration in tumors was assessed from the PET images and background activity (non-tumor tissue) subtracted. In (**C**), *indirect* localization of *lacZ* gene expression by [^{124}I]FIAU–PET is demonstrated by coregistration of corresponding [^{124}I]FIAU–PET images and X-Gal stained tissue sections. These comparisons demonstrate the spatial relationship between HSV-1-TK-related radioactivity (arrow) and *lacZ* gene expression (right) in the same tumor region, indicating that non-invasive localization of any proportionally coexpressed gene is possible by HSV-1-TK/[^{124}I]FIAU–PET [adapted from Jacobs et al., 2001b with permission].

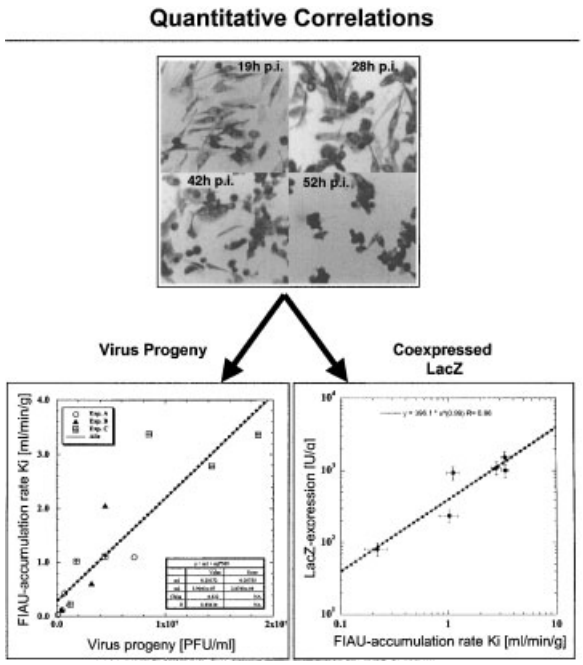

Fig. 3. Indirect quantification of other biological properties of the hrR3 vector by the radiotracer assay used for imaging. Proliferating rat 9L gliosarcoma cells were infected with hrR3 with a multiplicity of infection of 1.5. Time-dependent changes in *lacZ* gene expression and development of cytopathic changes can be observed and quantified. At each time-point after infection, the level of HSV-1-*tk* gene expression, viral yield, and *lacZ* gene expression was quantified. Correlations demonstrate, that the radiotracer assay is not only a direct measure for HSV-1-TK activity but serves also as *indirect* measure of viral progeny and proportionally coexpressed *lacZ* gene activity. The data presented in Figures 2C and 3 are the basis for the non-invasive localization and quantification of any gene of interest, which is proportionally coexpressed with a PET marker gene [adapted from Jacobs et al., 2001b with permission].

Most excitingly, non-invasive assessment of signal transduction pathways has been explored by using PET. The first non-invasive characterization of a signal transduction pathway has been successfully performed by Doubrovin et al. [2001]. A *tkgfp* dual reporter gene [Jacobs et al., 1999a] was used to monitor transcriptional activation of p53-dependent genes. Human U87 glioma and SaOS-2 osteosarcoma cells were retrovirally transduced with a *cis*-p53/TKGFP reporter system, in which the *tkgfp* marker gene was placed under control of an artificial *cis*-acting p53-specific enhancer. In rat xenografts the DNA damage-induced up-regulation of p53 transcriptional activity correlated with the expression of p53-dependent downstream genes in U87 (wild-type p53), but not in SaOS-2 osteosarcoma (p53−/−), cells and with the level of p53-dependent TKGFP expression as assessed by [^{124}I]FIAU–PET. These data indicate that PET is sufficiently sensitive to image the transcriptional regulation of genes in certain signal transduction pathways. This molecular imaging strategy shall enable non-invasive assessment of the activity of signal transduction pathways, of the expression of different endogenous genes, and of novel therapeutic strategies in vivo [Doubrovin et al., 2001].

Furthermore, a non-invasive method for imaging T-cell activity in vivo has been developed in order to study the role of specific genes and signal transduction pathways in the course of normal and pathologic immune response and to elucidate the temporal dynamics and immune regulation at different stages of disease [Ponomarev et al., 2001]. T-cell receptor (TCR)-dependent nuclear factor of activated T cells (NFAT)-mediated activation of T cells was non-invasively quantified by optical fluorescence imaging and PET. Again, the *tkgfp* dual reporter gene [Jacobs et al., 1999a] was used to monitor NFAT-mediated transcriptional activation in human Jurkat cells. Transduced Jurkat cells expressing the *tkgfp* gene under transcriptional control of an artificial *cis*-acting NFAT-specific enhancer were used to establish subcutaneous infiltrates in nude mice. Non-invasive imaging of T-cell activation was achieved after systemic administration of anti-human CD3 and CD28 antibodies to induce the NFAT pathway. PET imaging of TCR-induced NFAT-dependent transcriptional activity may be useful in the assessment of T cell responses, T-cell-based adoptive therapies, vaccination strategies, and immunosuppressive drugs [Ponomarev et al., 2001].

To further elucidate the potential role of radionuclide molecular imaging strategies in the molecular characterization of disease models, repetitive microPET imaging was used to demonstrate that regulation of endogenous albumin gene expression can be non-invasively assessed in transgenic mice in which the HSV-1-*tk* marker gene is driven by the albumin enhancer/promoter to target HSV-1-*tk* gene expression to be restricted to hepatocytes [Green et al., 2002]. These AL-HSV-1-tk-transgenic mice (137-7) were originally generated to develop an inducible model of hepatic disease, which can be utilized to repopulate the liver with donor cells [Braun et al., 2000].

In summary, PET imaging can be used to directly localize and quantify the expression of endogenous genes and exogenous marker genes as well as to study signal transduction pathways and transgenic animals.

Radionuclide Imaging of Cell Trafficking

Molecular imaging aims towards the in vivo characterization and measurement of biological processes at the cellular and molecular level. Therefore, non-invasive trafficking of cells in disease pathogenesis over time is another exciting and evolving field in molecular imaging. A non-invasive imaging technique for cells is of special importance as quantitation and kinetic analysis of cell distribution in organs is time-consuming and unreliable. In general, any kind of cell which moves from one location to another within the body may be investigated, for example, stem cells or neural progenitor cells migrating from intact brain across hemispheres towards gliomas [Aboody et al., 2000] or stroke [Snyder and Macklis, 1995]; T-cells or other specific immune cells; or tumor cells [Hardy et al., 2001].

Copper-64-pyruvaldehyde-bis(N^4-methylthiosemicarbazone)(^{64}Cu-PTSM) has been used to radiolabel C6 rat glioma cells and lymphocytes without affecting cell viability and proliferation rate and enabled trafficking of C6-cells into lungs and liver and of lymphocytes into spleen after tail-vein-injection in mice [Adonai et al., 2002]. PTSM mediates transmembrane transport of ^{64}Cu into cells. The process by which ^{64}Cu is retained in cells is governed by the reduction of the stable ^{64}Cu(II)–PTSM complex to a labile ^{64}Cu(I)–PTSM complex, trapping the dissociated ^{64}Cu(I) ion in the cell because of charge [Adonai et al., 2002].

Moreover, systemic distribution and tumor localization of adoptively transferred lymphocytes were investigated in mice using ^{111}In-oxine-labeled, primed T lymphocytes directed against the tumor [Melder et al., 2002]. Interestingly, no significant differences were observed between primed and control naïve T lymphocytes with respect to systemic distribution of cells in normal organs and with respect to kinetics of lymphocyte localization to the tumor apart from delayed clearance of primed lymphocytes from the lungs [Melder et al., 2002]. These studies indicate that detailed analyses of homing of genetically engineered T-cells is possible over time in the same experimental animal.

MR Imaging of Gene Expression

ß-Galactosidase is the most commonly used histochemical marker gene, its expression can be detected by a colorimetric staining assay, in which the cleavage of an indicator substrate yields an opaque blue precipitate. Louie et al. [2000] developed an interesting assay based on the use of ß-galactosidase for in vivo imaging of gene expression by MRI by designing and synthesizing a contrast agent that is enzymatically processed by ß-gal. The contrast agent (1-(2-(ß-galactopyranosyloxy)propyl)-4,7,10-tris-(carboxymethyl)-1,4,7,10-tetraazacyclododecane)gadolinium(III) consists of (i) a chelator with high-affinity binding to gadolinium that occupies eight of the nine coordination sites of gadolinium, and (ii) a galactopyranose residue positioned to block the remaining coordination site on the gadolinium ion from water. In this water-inaccessible conformation, the contrast agent is "inactive" and does not strongly modulate T1. However, ß-galactosidase enzymatically cleaves the galactopyranose from the chelate, freeing a coordination site, and causing the irreversible transition of the contrast agent to an "active" state. This method facilitated MR imaging of ß-galactosidase expression to cellular resolution [Louie et al., 2000] indicating that in vivo mapping of gene expression in transgenic animals will be possible by MRI.

MRI of gene expression has also been employed in the context of an engineered transferrin receptor which mediates the incorporation of transferrin-tagged, superparamagnetic monocrystalline iron oxide nanoparticles (MION) into cells [Moore et al., 1998, 2001; Weissleder et al., 2000]. Localization of gene expression is possible at high resolution (50–100 μm), however, quantification of gene expression is difficult as uptake of MIONs does not follow a linear proportionality to transferrin receptor expression, and the induced signal changes on MRI are not linear proportional to the MION load of transduced cells.

MR Imaging of Cell Trafficking

Due to its high spatial resolution, MR imaging will be the technology of choice for studying cell kinetics in vivo. The migration of 3×10^7 superparamagnetically labeled T-cells through the spleen of a mouse during a 24-h period has

been investigated recently with MR using spin-echo pulse sequence at 4.7 T [Dodd et al., 2001]. T-cells were loaded with iron oxide nanoparticles derivatized with a peptide sequence from the transactivator protein (Tat) of HIV-1. Homing of labeled T-cells into the spleen could be non-invasively observed by a decrease in MRI signal intensity within 1 h after systemic administration of cells indicating that biodistribution of labeled cells by MRI is possible [Dodd et al., 2001]. The same method was successfully used for cell trafficking studies of hematopoietic and neural progenitor cells indicating that non-invasive analysis of specific stem cell and organ interaction becomes possible which is critical for advancing the therapeutic use of stem cells [Lewin et al., 2000].

Optical Imaging of Gene Expression

The firefly and *Renilla* luciferase genes have been used as marker genes with D-luciferin and coelenterazine as specific marker substrates, respectively, which can be detected by use of a cooled charged couple detector (CCD) camera [Bhaumik and Gambhir, 2002]. The level of marker gene expression is expressed as relative light units per minute (RLU/min) and has been assessed so far for the determination of adenoviral-mediated luciferase gene transduction into rat myocardium [Wu et al., 2002a] and skeletal muscle of mice [Wu et al., 2001]. Most importantly, this imaging system has been used for the non-invasive quantitation of growth and therapy-induced changes in tumor burden in the intracranial rat 9L-gliosarcoma model [Rehemtulla et al., 2000, 2002]. These data indicate that this imaging system is a rapid and easy screening method for the non-invasive determination of therapy response, where more sophisticated and expensive methods such as PET and MRI can follow for a more detailed analysis.

This imaging system has been further developed to non-invasively study protein–protein interactions in vivo by a modification of the yeast two-hybrid system adapted for mammalian cells [Ray et al., 2002]. In short, the NF-κB promoter was used to drive expression of two fusion proteins (VP16-MyoD and GAL4-ID). Tumor necrosis factor-induced activation of the NF-κB promoter led to expression of both fusion proteins with subsequent interaction of MyoD with ID leading to GAL4-mediated binding to a GAL4-binding site with subsequent VP16-mediated activated transcription of the luciferase gene [Ray et al., 2002]. Induction of this paradigm could be imaged in vivo indicating that this system may have important implications in the non-invasive localization and assessment of protein–protein interactions in living subjects.

Most importantly, the in vivo detection of luciferase enzyme as a transcriptional reporter facilitated rapid screening for both the presence and function of transgenes in transgenic animals [Zhang et al., 2001].

A further optical imaging method was developed for the non-invasive assessment of protease expression by near-infrared fluorescence imaging (NIRFI) using synthetic protease-sensing probes consisting of biocompatible autoquenched near-infrared fluorochromes that are released from long-circulating synthetic graft copolymers by protease-specific cleavage [Weissleder et al., 1999; Tung et al., 2000]. By using this method, the level of Cathepsin-B expression could be non-invasively assessed in mice bearing experimental breast cancer, suggesting that Cathepsin-B may be used as a new marker gene for NIRF optical imaging, molecular profiling, and screening [Mahmood et al., 1999; Bremer et al., 2002; Marten et al., 2002]. Similarly, assessment of matrix metalloproteinase-2 (MMP-2) activity by NIRFI was developed by implementing an optical contrast agent that was highly activatable by means of MMP-2-induced conversion [Bremer et al., 2002]. This method allowed a non-invasive assessment of successful MMP-2 inhibition in a therapeutic model [Bremer et al., 2001] indicating that this method might serve surrogate biomarkers to study the effects of MMP inhibitors in vivo.

Optical Imaging of Cell Trafficking

Bioluminescence imaging has also been used for cell trafficking studies, for example, to monitor quantitatively the growth and regression of labeled human cervical carcinoma cells engrafted into immunodeficient mice in response to both, chemotherapy and immunotherapy, using human T-cell-derived effector cells [Sweeney et al., 1999]. In the absence of therapy, animals showed progressive increase in signal intensity over time. Cisplatin treatment as well as immunotherapy dramatically reduced signals at high effector-to-target cell ratios, and significant decreases were observed with lower ratios. These results indicate, that

non-invasive bioluminescence imaging allows sensitive, quantitative, real-time spatio-temporal analysis of the dynamics of neoplastic cell growth, and facilitates the rapid assessment of effective treatment strategies [Sweeney et al., 1999; Hardy et al., 2001].

Imaging the Expression of A Gene of Interest

As not all genes of interest (GOI) carry an enzymatic function, which could be used for an enzymatic radionuclide assay in vivo, the general attempt is to proportionally co-express any GOI with an imaging marker gene. Therefore, to be able to indirectly and quantitatively assess the expression of any GOI, gene co-expression strategies are being used. Apart from coordinately promoter-based co-expression ([Jacobs et al., 2001b]; Figs. 2, 3), strategies serving a proportional co-expression of a PET- or MRI-marker gene and a gene of interest make use of gene fusion [Jacobs et al., 1999a], of an internal ribosome entry site (IRES) derived from encephalomyocarditis virus (ECMV) [Tjuvajev et al., 1999b; Yu et al., 2000] or of the proteolytic 2A-element derived from picornaviruses [de Felipe et al., 1999; de Felipe and Izquierdo, 2000]. In addition, co-administration of two distinct but otherwise identical adenovirus vectors has been shown to result in proportional co-expression of PET marker genes expressed by these vectors [Yaghoubi et al., 2001b]. In single cell derived retrovirally transduced tumor clones, it could be demonstrated that PET imaging of HSV-1-TK expression could be used to monitor the topology and activity of the *lacZ*-gene as second gene under the transcriptional control of a single promoter within a bicistronic unit that includes an IRES [Tjuvajev et al., 1999b]. In a similar approach, the activity of proportionally coexpressed *Renilla* luciferase and a dopamine type 2 receptor could be quantified and localized by PET [Yu et al., 2000].

The applicability of this 'indirect' imaging method has been translated into a model of vector application in vivo employing recombinant HSV-1 vectors which are commonly used for virus therapy of gliomas [Jacobs et al., 1999b,c; Jacobs et al., 2001b]; (Figs. 2, 3). In the first (hrR3) and second (MGH-1) generation HSV-1 vectors, the PET marker gene HSV-1-*tk* and the marker gene *lacZ* are under transcriptional control of early gene, and hence, timely coordinated active promoters [Goldstein and Weller, 1988; Kramm et al., 1997]. The level and location of PET-based imaging of hrR3- and MGH-1-mediated TK-expression in vivo reflected indirectly the level and location of LacZ-expression and also the viral progeny of these vectors [Jacobs et al., 2001b]; (Fig. 2). However, the propensity of replication-conditional vectors to eventually disrupt cellular functions interferes with the enzymatic radiotracer assay so that the PET-imageable TK-expression mediated by these vectors identifies only the viable portion of infected tumor tissue. To circumvent HSV-1 vector induced toxicity interfering with the PET imaging of these vectors, helper virus-free HSV-1 amplicon vectors [Fraefel et al., 1996] are currently engineered and functionally characterized bearing transcriptionally linked GOI which shall serve proportional coexpression of three gene functions: (i) a marker gene for HSV-1 vector generation in culture (*gfp*); (ii) the HSV-1-*tk* as PET marker gene for assessment of HSV-1 vector-mediated gene expression in vivo; (iii) a therapeutic gene (*Escherichia coli* cytosine deaminase; *cd*) for suicide gene therapy of gliomas (Jacobs et al., submitted). Functional proportional coexpression of the PET marker gene HSV-1-*tk* and the linked therapeutic *E. coli cd*-gene could be observed irrespective of the location of both genes within the constructs. These HSV-1 amplicon vectors carrying the HSV-1-*tk* as PET marker gene and a linked therapeutic gene shall enable the indirect non-invasive localization of the distribution of therapeutic gene expression by PET and, hence, shall allow the correlation of the primary transduction efficiency of these vectors with their induced therapeutic response.

FUTURE DIRECTIONS

In the past years, several marker gene/marker substrate combinations as well as various imaging technologies have been developed; they have been adapted for imaging small animals; and they already have been applied in humans. In the forthcoming years, these imaging systems will be used to further characterize various animal models of human disease, and they will be implemented in the clinical application to get further insight into molecular disease pathogenesis and to speed up drug and new treatment development. Each of these imaging modalities

will serve complementary parameters to achieve a non-invasive quantitative assessment of gene expression, protein regulation, and function in vivo at high sensitivity and high spatial resolution.

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