1. **Dual-modality in vivo monitoring of subventricular zone stem cell migration and metabolism.**


   Rat subventricular zone (SVZ) stem cells were labeled with superparamagnetic iron oxide particles (SPIO) to follow their fate and migratory potential with magnetic resonance imaging (MRI) and positron emission tomography (PET). Labeled cells were transplanted into either the right rostral migratory stream (RMS) or striatum of normal adult Sprague-Dawley rats and serially followed for 3 months. Minimal migration of the cells implanted into the striatum was observed after 3 weeks whereas SVZ cells implanted into the RMS migrated toward the olfactory bulb at 1 week post-transplantation. PET studies of glucose metabolism using (18)F-FDG demonstrated enhanced glucose utilization in the striatum of transplanted animals. PET studies conducted 3 months after transplantation showed elevated accumulation of (11)C-raclopride (dopamine receptor type 2) and (11)C-CFT (dopamine transporter) binding in the striatal grafts. Implanted SVZ cells did not induce significant inflammation as identified by PET using (11)C-PK11195, a ligand detecting activated microglia. Histological analysis identified viable SPIO-labeled cells (some of which were nestin-positive) 7 weeks post-transplantation, suggesting a prolonged presence of undifferentiated neural stem cells within transplants. In addition, double immunostaining for neuronal and astrocytic markers (NeuN and GFAP) indicated that differentiation into neuronal and astrocytic phenotypes also occurred. Thus, combining MRI and PET enables monitoring of cell migration and metabolism non-invasively in vivo for extended periods of time. Copyright (c) 2007 John Wiley & Sons, Ltd.

2. **In vivo imaging in experimental preclinical tumor research-A review.**


   The multiparametric molecular cell and tissue analysis in vitro and in vivo is characterized by rapid progress in the field of image generation technologies, sensor biotechnology, and computational modeling. Fascinating new potentials in unraveling the detailed functions of single cells, organs, and whole organisms are presently emerging and permit the close monitoring i.e. tumor development or basic cell development processes with an unprecedented multiplicity of promising investigative possibilities. To answer basic questions of in vivo tumor development and progression fluorescence based imaging techniques provide new insights into molecular pathways and targets. Genetic reporter systems (eGFP, DsRED) are available and high sensitive detection systems are on hand. These techniques could be used for in vitro assays and quantified e.g. by microscopy and CCD based readouts. The introduction of novel fluorescent dyes emitting in the near infrared range (NIR) combined with the development of
sensitive detector systems and monochromatic powerful NIR-lasers for the first time permits the quantification and imaging of fluorescence and/or bioluminescence in deeper tissues. Laser based techniques particularly in the NIR-range (like two-photon microscopy) offer superb signal to noise ratios, and thus the potential to detect molecular targets in vivo. In combination with flat panel volumetric computed tomography (fpVCT), questions dealing e.g. with tumor size, tumor growth, and angiogenesis/vascularization could be answered noninvasively using the same animal. The resolution of down to 150 mum/each direction can be achieved using fpVCT. It is demonstrated by many groups that submillimeter resolutions can be achieved in small animal imaging at high sensitivity and molecular specificity. Since the resolution in preclinical small animal imaging is down to approximately 10 mum by the use of microCT and to subcellular resolutions using (approximately 1 mum) microscope based systems, the advances of different techniques can now be combined to "multimodal" preclinical imaging and the possibilities for in vivo intravital cytometry now become within one's reach. (c) 2007 International Society for Analytical Cytology.

3. Towards the use of nanoparticles in cancer therapy and imaging.
Pauwels E K, Erba P.
Nanosystems have unique physical and biological properties. Various systems have been designed for medical use and offer promising ways of delivering drugs at high concentrations at sites of interest, e.g., cancer lesions, while reducing systemic side effects. Nanotechnology has also opened ways for the development of contrast agents and radiopharmaceuticals for specific targeting, which is presently changing research strategies in the field of magnetic resonance imaging, ultrasound imaging and nuclear medicine applications. This article aims to overview how "nanomedicine" is presently influencing drug design for diagnostic and therapeutic purposes. (c) 2007 Prous Science. All rights reserved.

Markus Loening A, Wu A M, Sam Gambhir S.
Nat Methods. 2007; (Jul 8).
The use of R. reniformis luciferase (RLuc) as a reporter gene in small-animal imaging has been hampered by its 481 nm peaked emission spectrum, as blue wavelengths are strongly attenuated in biological tissues. To overcome this, we generated variants of RLuc with bathochromic (red) shifts of up to 66 nm (547 nm peak) that also had greater stability and higher light emission than native RLuc.
5. **Bright monomeric red fluorescent protein with an extended fluorescence lifetime.**
   
   
   Merzlyak E M, Goedhart J, Shcherbo D, et al.
   

Fluorescent proteins have become extremely popular tools for in vivo imaging and especially for the study of localization, motility and interaction of proteins in living cells. Here we report TagRFP, a monomeric red fluorescent protein, which is characterized by high brightness, complete chromophore maturation, prolonged fluorescence lifetime and high pH-stability. These properties make TagRFP an excellent tag for protein localization studies and fluorescence resonance energy transfer (FRET) applications.

6. **Studying the biodistribution of positron emission tomography reporter probes in mice.**
   
   
   Yaghoubi S S, Berger F,Gambhir S S.
   
   *Nat Protoc.* 2007;2:1752-1755. Positron emission tomography (PET) reporter probes (PRPs) are used to detect PET reporter gene (PRG) expression in living subjects. This article details protocols for analyzing the biodistribution of a PRP used to detect herpes simplex virus 1 thymidine kinase (HSV1-tk) or mutant HSV1-sr39tk PRG expression. However, the methods described are generalizable to other beta- or gamma/positron-emitting probes. Accumulation of PRPs in animal tissues can be determined by counting PRP activity of isolated tissues, whereas digital whole-body autoradiography (DWBA) provides high-resolution images of PRP biodistribution in 5- to 45-mum tissue slices of killed research animals at a single time point. Biodistribution assay results may be obtained in less than a week after beginning the assay, and DWBA image acquisitions can take up to 3 months depending on the probe's radioisotope.

7. **In vivo tracking of stem cells in brain and spinal cord injury.**
   
   
   Sykova E,Jendelova P.
   
   *Prog Brain Res.* 2007;161C:367-383. Cellular magnetic resonance (MR) imaging is a rapidly growing field that aims to visualize and track cells in living organisms. Superparamagnetic iron oxide (SPIO) nanoparticles offer a sufficient signal for T2 weighted MR images. We followed the fate of embryonic stem cells (ESCs) and bone marrow mesenchymal stem cells (MSCs) labeled with iron oxide nanoparticles (Endorem((R))) and human CD34(+) cells labeled with magnetic MicroBeads (Miltenyi) in rats with a cortical or spinal cord lesion, models of stroke and spinal cord injury (SCI), respectively. Cells were either grafted intracerebrally, contralaterally to a cortical photochemical lesion, or injected intravenously. During the first post-transplantation week, grafted MSCs
or ESCs migrated to the lesion site in the cortex as well as in the spinal cord and were visible in the lesion on MR images as a hypointensive signal, persisting for more than 30 days. In rats with an SCI, we found an increase in functional recovery after the implantation of MSCs or a freshly prepared mononuclear fraction of bone marrow cells (BMCs) or after an injection of granulocyte colony stimulating factor (G-CSF). Morphometric measurements in the center of the lesions showed an increase in white matter volume in cell-treated animals. Prussian blue staining confirmed a large number of iron-positive cells, and the lesions were considerably smaller than in control animals. Additionally, we implanted hydrogels based on poly-hydroxypropylmethacrylamide (HPMA) seeded with nanoparticle-labeled MSCs into hemisected rat spinal cords. Hydrogels seeded with MSCs were visible on MR images as hypointense areas, and subsequent Prussian blue histological staining confirmed positively stained cells within the hydrogels. To obtain better results with cell labeling, new polycation-bound iron oxide superparamagnetic nanoparticles (PC-SPIO) were developed. In comparison with Endorem, PC-SPIO demonstrated a more efficient intracellular uptake into MSCs, with no decrease in cell viability. Our studies demonstrate that magnetic resonance imaging (MRI) of grafted adult as well as ESCs labeled with iron oxide nanoparticles is a useful method for evaluating cellular migration toward a lesion site.

8. Molecular Imaging of Bone Marrow Mononuclear Cell Homing and Engraftment in Ischemic Myocardium.
Bone marrow mononuclear cell (BMMC) therapy shows promise as a treatment for ischemic heart disease. However, the ability to monitor long-term cell fate remains limited. We hypothesize molecular imaging can be used to track stem cell homing and survival after myocardial ischemia-reperfusion (I/R) injury. We first harvested donor BMMCs from adult male L2G85 transgenic mice constitutively expressing both firefly luciferase (Fluc) and enhanced green fluorescence protein (eGFP) reporter gene. FACS analysis revealed approximately 0.07% of the population to consist of classical hematopoietic stem cells (lin-, thy-int, c-kit+, Sca-1+). Afterwards, adult female FVB recipients (n=38) were randomized to sham surgery or acute I/R injury. Animals in the sham (n=16) and I/R (n=22) groups received 5x10(6) of the L2G85-derived BMMCs via tail vein injection. Bioluminescence imaging (BLI) was used to track cell migration and survival in vivo for 4 weeks. BLI showed preferential homing of BMMCs to hearts with I/R injury compared to sham hearts within the first week following cell injection. Ex vivo analysis of explanted hearts by histology confirmed BLI imaging results, and quantitative RT-PCR (for the male Sry gene) further demonstrated higher number of BMMCs in hearts with I/R injury compared to the sham group. Functional evaluation by echocardiography demonstrated a trend towards improved left ventricular fractional shortening in animals receiving BMMCs.
Taken together, these data demonstrate that molecular imaging can be used to successfully track BMMC therapy in murine models of heart disease. Specifically, we demonstrate that systemically delivered BMMCs preferentially home to and are retained by injured myocardium.

9. Magnetic resonance tracking of magnetically labeled autologous bone marrow CD34+ cells transplanted into the spinal cord via lumbar puncture technique in patients with chronic spinal cord injury: CD34+ cells' migration into the injured site.


The purpose of this study was to demonstrate the possibility of delivering autologous bone marrow precursor cells into the spinal cord via lumbar puncture technique (LP) in patients with spinal cord injury (SCI). Magnetic resonance imaging provides a noninvasive method for studying the fate of transplanted cells in vivo. Considering these propositions, we studied magnetic resonance tracking of autologous bone marrow CD34(+) cells labeled with magnetic nanoparticles delivered into the spinal cord via LP in patients with SCI. Sixteen patients with chronic SCI were enrolled and divided into two groups; one group got their own labeled-CD34(+) cells injected into the spinal cord via LP (n = 10); the others received an injection, but it contained magnetic beads without stem cells (controls, n = 6). CD34(+) cells were magnetically labeled with magnetic beads coated with a monoclonal antibody specific for the CD34 cell membrane antigen. Magnetic resonance images were obtained by a standard turbospin echo-T2 weighted sequences before and 20 and 35 days after post-transplantation. The median number of CD34(+) cells injected via LP was 0.7 x 10(6) (range 0.45 to 1.22 x 10(6)). Magnetically labeled CD34(+) cells were visible at the lesion site as hypointense signals in five patients of the labeled-CD34(+) group 20 and 35 days after transplantation; these signals were not visible in any patient of the control group. We suggested for the first time that autologous bone marrow CD34(+) cells labeled with magnetic nanoparticles delivered into the spinal cord via LP technique migrated into the injured site in patients with chronic SCI.

10. Correlations between Diffusion Tensor Imaging (DTI) and Magnetic Resonance Spectroscopy (1H MRS) in schizophrenic patients and normal controls.


ABSTRACT: BACKGROUND: Evidence suggests that white matter integrity may play an underlying pathophysiological role in schizophrenia. N-acetylaspartate (NAA), as measured by Magnetic Resonance Spectroscopy (MRS), is a neuronal marker and is decreased in white matter lesions and regions of axonal loss. It
has also been found to be reduced in the prefrontal and temporal regions in patients with schizophrenia. Diffusion Tensor Imaging (DTI) allows one to measure the orientations of axonal tracts as well as the coherence of axonal bundles. DTI is thus sensitive to demyelination and other structural abnormalities. DTI has also shown abnormalities in these regions. METHODS: MRS and DTI were obtained on 42 healthy subjects and 40 subjects with schizophrenia. The data was analyzed using regions of interests in the Dorso-Lateral Prefrontal white matter, Medial Temporal white matter and Occipital white matter using both imaging modalities. RESULTS: NAA was significantly reduced in the patient population in the Medial Temporal regions. DTI anisotropy indices were also reduced in the same Medial Temporal regions. NAA and DTI-anisotropy indices were also correlated in the left medial temporal region. CONCLUSION: Our results implicate defects in the medial temporal white matter in patients with schizophrenia. Moreover, MRS and DTI are complementary modalities for the study of white matter disruptions in patients with schizophrenia.

11. Functional imaging of cancer with emphasis on molecular techniques.
A multitude of noninvasive, quantitative, functional imaging techniques are currently in use to study tumor physiology, to probe tumor molecular processes, and to study tumor molecules and metabolites in vitro and in vivo using computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography (US), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and optical imaging (OI). Such techniques can be used in conjunction with structural imaging techniques to detect, diagnose, characterize, or monitor tumors before and after therapeutic intervention. These can also be used to study tumor gene expression, to track cells and therapeutic drugs, to optimize individualized treatment planning for patients with tumors, and to foster new oncologic drug development. In this article, we review the rich variety of functional imaging techniques that are available for these purposes, which are becoming increasingly important for optimal individualized patient treatment in this day and age of "personalized medicine."

BACKGROUND: The ability to image vascular inflammatory responses may allow early diagnosis and treatment of atherosclerosis. We hypothesized that molecular imaging of vascular cell adhesion molecule-1 (VCAM-1) expression
with contrast-enhanced ultrasound (CEU) could be used for this purpose.

METHODS AND RESULTS: Attachment of VCAM-1-targeted and control microbubbles to cultured endothelial cells was assessed in a flow chamber at variable shear stress (0.5 to 12.0 dynes/cm²). Microbubble attachment to aortic plaque was determined by en face microscopy of the thoracic aorta 10 minutes after intravenous injection in wild-type or apolipoprotein E-deficient mice on either chow or hypercholesterolemic diet. CEU molecular imaging of the thoracic aorta 10 minutes after intravenous microbubble injection was performed for the same animal groups. VCAM-1-targeted but not control microbubbles attached to cultured endothelial cells, although firm attachment at the highest shear rates (8 to 12 dynes/cm²) occurred only in pulsatile flow conditions. Aortic attachment of microbubbles and targeted CEU signal was very low in control wild-type mice on chow diet. Aortic attachment of microbubbles and CEU signal for VCAM-1-targeted microbubbles differed between treatment groups according to extent of VCAM-1-positive plaque formation (median CEU videointensity, 1.8 [95% CI, 1.2 to 1.7], 3.7 [95% CI, 2.9 to 7.3], 6.8 [95% CI, 3.9 to 7.6], and 11.2 [95% CI, 8.5 to 16.0] for wild-type mice on chow and hypercholesterolemic diet and for apolipoprotein E-deficient mice on chow and hypercholesterolemic diet, respectively; P<0.001). CONCLUSIONS: CEU molecular imaging of VCAM-1 is capable of rapidly quantifying vascular inflammatory changes that occur in different stages of atherosclerosis. This method may be potentially useful for early risk stratification according to inflammatory phenotype.


Aaron J, Nitin N, Travis K, et al.


An effective cancer control strategy requires improved early detection methods, patient-specific drug selection, and the ability to assess response to targeted therapeutics. Recently, plasmon resonance coupling between closely spaced metal nanoparticles has been used to develop ultrasensitive bioanalytical assays in vitro. We demonstrate the first in vivo application of plasmon coupling for molecular imaging of carcinogenesis. We describe molecular-specific gold bioconjugates to image epidermal growth factor receptor (EGFR); these conjugates can be delivered topically and imaged noninvasively in real time. We show that labeling with gold bioconjugates gives information on the overexpression and nanoscale spatial relationship of EGF receptors in cell membranes, both of which are altered in neoplasia. EGFR-mediated aggregation of gold nanoparticles in neoplastic cells results in more than a 100-nm color shift and a contrast ratio of more than tenfold in images of normal and precancerous epithelium in vivo, dramatically increasing contrast beyond values reported previously for antibody-targeted fluorescent dyes.
14. **Fusion of Gaussia Luciferase to an Engineered Anti-carcinoembryonic Antigen (CEA) Antibody for In Vivo Optical Imaging.**


*Mol Imaging Biol.* 2007; (Jun 19).

The bioluminescent protein Gaussia luciferase (GLuc) was fused to an anti-carcinoembryonic antigen (CEA) antibody fragment, the diabody, for in vivo optical tumor imaging. A 15-amino acid N-terminal truncation (GLDelta15) resulted in a brighter protein. Fusions of the anti-CEA diabody to full-length GLuc and GLDelta15 retained high affinity for the antigen, emitted light, and exhibited excellent enzymatic stability. In vivo optical imaging of tumor-bearing mice demonstrated specific targeting of diabody-GLDelta15 to CEA-positive xenografts, with a tumor/background ratio of 3.8 +/- 0.4 at four hours after tail-vein injection, compared to antigen-negative tumors at 1.3 +/- 0.1 (p = 0.001). MicroPET imaging using (124)I-diabody-GLDelta15 demonstrated specific uptake in the CEA-positive tumor (2.6% ID [injected dose]/g) compared to the CEA-negative tumor (0.4% ID/g) at 21 hours. Although further optimization of this fusion protein may be needed to improve in vivo performance, the diabody-GLDelta15 is a promising optical imaging probe for tumor detection in vivo.

15. **Variation of the choline signal intensity in the dorsolateral prefrontal cortex of rats exposed to the forced swimming test as detected by in vivo(1)H MR spectroscopy.**


*J Neurosci Methods.* 2007; (May 24).

BACKGROUND: (1)H magnetic resonance spectroscopy (MRS) has documented an increased Cho/Cr ratio in the dorsolateral prefrontal cortex (DLPFC) in major depressive disorder (MDD). The aim of this study was to investigate neurochemical alterations in the left DLPFC, considered a main area of pathogenesis in depression, using rats exposed to the forced swimming test (FST). MATERIALS AND METHODS: Twenty-four male rats were used for the MRI and in vivo(1)H MRS studies. Rats exposed to the FST to induce a depressed mental status. Using in vivo(1)H MRS, the metabolite ratios of the rats with a depressed mental status and the controls, were measured and the values of the two groups were compared. RESULTS: The Cho/Cr and Cho/NAA ratios in the DLPFC of the rats with a depressed mental status were significantly higher than that in the controls. CONCLUSIONS: The present study demonstrates a significantly increased Cho/Cr ratio in the DLPFC of rats with depression compared with controls. This result may suggest an accelerated turnover of membrane without neuronal loss is occurring in the DLPFC of the rats with depression.
16. Multifunctional nanoparticles for combining ultrasonic tumor imaging and targeted chemotherapy.
Rapoport N, Gao Z, Kennedy A.
BACKGROUND: Drug delivery in polymeric micelles combined with tumor irradiation by ultrasound results in effective drug targeting, but this technique requires prior tumor imaging. A technology that combined ultrasound imaging with ultrasound-mediated nanoparticle-based targeted chemotherapy could therefore have important applications in cancer treatment. METHODS: Mixtures of drug-loaded polymeric micelles and perfluoropentane (PFP) nano/microbubbles stabilized by the same biodegradable block copolymer were prepared. Size distribution of nanoparticles was measured by dynamic light scattering. Cavitation activity (oscillation, growth, and collapse of microbubbles) under ultrasound was assessed based on the changes in micelle/microbubble volume ratios. The effect of the nano/microbubbles on the ultrasound-mediated cellular uptake of doxorubicin (Dox) in MDA MB231 breast tumors in vitro and in vivo (in mice bearing xenograft tumors) was determined by flow cytometry. Statistical tests were two-sided. RESULTS: Phase state and nanoparticle sizes were sensitive to the copolymer/perfluorocarbon volume ratio. At physiologic temperatures, nanodroplets converted into nano/microbubbles. Doxorubicin was localized in the microbubble walls formed by the block copolymer. Upon intravenous injection into mice, Dox-loaded micelles and nanobubbles extravasated selectively into the tumor interstitium, where the nanobubbles coalesced to produce microbubbles with a strong, durable ultrasound contrast. Doxorubicin was strongly retained in the microbubbles but released in response to therapeutic ultrasound. Microbubbles cavitated under the action of tumor-directed ultrasound, which enhanced intracellular Dox uptake by tumor cells in vitro to a statistically significant extent relative to that observed with unsonicated microbubbles (drug uptake ratio = 4.60; 95% confidence interval [CI] = 1.70 to 12.47; P = .017) and unsonicated micelles (drug uptake ratio = 7.97; 95% CI = 3.72 to 17.08; P = .0032) and resulted in tumor regression in the mouse model. CONCLUSIONS: Multifunctional nanoparticles that are tumor-targeted drug carriers, long-lasting ultrasound contrast agents, and enhancers of ultrasound-mediated drug delivery have been developed and deserve further exploration as cancer therapeutics.

17. Optical imaging of the neonatal brain.
Austin T.
18. **Tumour-homing peptides: tools for targeting, imaging and destruction.**


   Enback J, Laakkonen P.


   Each normal organ and pathological condition contains organ- or disease-specific molecular tags on its vasculature that constitute a vascular 'zip code' system. Tissue-selective tumour metastasis may also depend on vascular addresses. We have used phage display peptide libraries to map disease-specific differences in the vasculature. By using this technology, we have isolated several peptides which are targeted specifically to tumour blood vessels, lymphatic vessels and/or tumour cells. Some of the tumour-homing peptides recognize common angiogenesis markers and are capable of binding to several types of tumour, whereas other peptides recognize tumour-type-specific differences. We have also shown that the vasculature of a pre-malignant lesion differs from that of a full-blown tumour and also from the vasculature of the corresponding normal organ. Our peptides have revealed molecules that act as novel biomarkers of this vascular heterogeneity. Interestingly, some of our homing peptides are able to penetrate the target cells. This internalization differs from that of the Tat, penetratins and other related peptides in that our peptides enter the cell in a cell-type-specific manner. These peptides appear to be able to concentrate in the target tissue, making them particularly efficient delivery vectors for the targeting of drugs, other therapeutic moieties and imaging agents.

19. **Targeted Quantum Dot Conjugates for siRNA Delivery.**


   Derfus A M, Chen A A, Min D H, et al.


   Treatment of human diseases such as cancer generally involves the sequential use of diagnostic tools and therapeutic modalities. Multifunctional platforms combining therapeutic and diagnostic imaging functions in a single vehicle promise to change this paradigm. In particular, nanoparticle-based multifunctional platforms offer the potential to improve the pharmacokinetics of drug formulations, while providing attachment sites for diagnostic imaging and disease targeting features. We have applied these principles to the delivery of small interfering RNA (siRNA) therapeutics, where systemic delivery is hampered by rapid excretion and nontargeted tissue distribution. Using a PEGylated quantum dot (QD) core as a scaffold, siRNA and tumor-homing peptides (F3) were conjugated to functional groups on the particle’s surface. We found that the homing peptide was required for targeted internalization by tumor cells, and that siRNA cargo could be coattached without affecting the function of the peptide. Using an EGFP model system, the role of conjugation chemistry was investigated, with siRNA attached to the particle by disulfide cross-linkers showing greater silencing efficiency than when attached by a nonreducible thioether linkage. Since each particle contains a limited number of attachment
sites, we further explored the tradeoff between number of F3 peptides and the number of siRNA per particle, leading to an optimized formulation. Delivery of these F3/siRNA-QDs to EGFP-transfected HeLa cells and release from their endosomal entrapment led to significant knockdown of EGFP signal. By designing the siRNA sequence against a therapeutic target (e.g., oncogene) instead of EGFP, this technology may be ultimately adapted to simultaneously treat and image metastatic cancer.