Published in final edited form as:

Curr Med Chem. 2013 ; 20(29): 3664–3675.

Multimodality Imaging of RNA Interference

Tapas R. Nayak¹, Lazura K. Krasteva¹, and Weibo Cai^{1,2,3,*}

¹Department of Radiology, University of Wisconsin - Madison, WI, USA

²Department of Medical Physics, University of Wisconsin - Madison, WI, USA

³University of Wisconsin Carbone Cancer Center, University of Wisconsin - Madison, WI, USA

Abstract

The discovery of small interfering RNAs (siRNAs) and their potential to knock down virtually any gene of interest has ushered in a new era of RNA interference (RNAi). Clinical use of RNAi faces severe limitations due to inefficiency delivery of siRNA or short hairpin RNA (shRNA). Many molecular imaging techniques have been adopted in RNAi-related research for evaluation of siRNA/shRNA delivery, biodistribution, pharmacokinetics, and the therapeutic effect. In this review article, we summarize the current status of in vivo imaging of RNAi. The molecular imaging techniques that have been employed include bioluminescence/fluorescence imaging, magnetic resonance imaging/spectroscopy, positron emission tomography, single-photon emission computed tomography, and various combinations of these techniques. Further development of non-invasive imaging strategies for RNAi, not only focusing on the delivery of siRNA/shRNA but also the therapeutic efficacy, is critical for future clinical translation. Rigorous validation will be needed to confirm that biodistribution of the carrier is correlated with that of siRNA/shRNA, since imaging only detects the label (e.g. radioisotopes) but not the gene or carrier themselves. It is also essential to develop multimodality imaging approaches for realizing the full potential of therapeutic RNAi, as no single imaging modality may be sufficient to simultaneously monitor both the gene delivery and silencing effect of RNAi.

Keywords

RNA interference (RNAi); small interfering RNA (siRNA); gene therapy; molecular imaging; magnetic resonance imaging (MRI); positron emission tomography (PET); near-infrared fluorescence (NIRF); bioluminescence imaging (BLI)

INTRODUCTION

Gene therapy, which involves the use of DNA to supplement, alter, replace, or silence the function of genes within certain cells, has tremendous potential to treat a broad range of human diseases [1]. The discovery of small interfering RNAs (siRNAs) and their potential to knock down virtually any gene of choice has led to the emergence of a new class of gene therapeutics [2]. As double-stranded RNA molecules of 21-23 nucleotides in length, siRNAs contain 2 nucleotides overhanging on the 3' ends [3,4]. Once inside the cells, siRNAs can integrate into a multi-subunit protein complex known as the RNA-induced silencing complex (RISC) via an ATP dependent step, which in turn guides them to the target mRNA sequence [5]. The siRNA duplexes unwind, and a combination of endo- and exonucleases

*Requests for reprints: Departments of Radiology and Medical Physics, University of Wisconsin - Madison, Room 7137, 1111 Highland Ave, Madison, WI 53705-2275, USA. Fax: 1-608-265-0614; Tel: 1-608-262-1749; wcai@uwhealth.org.

CONFLICT OF INTEREST None.

facilitate cleavage and degradation of the complementary mRNA sequence [6]. Such sequence-specific suppression of mRNA translation, or RNA interference (RNAi), is currently being explored for the treatment of a variety of diseases. The phase I clinical trial of first siRNA-based therapeutics (bevasiranib) for treatment of age-related macular degeneration has been successful [7,8]. Bevasiranib was found to be well-tolerated with suitable pharmacokinetic properties. However, the use of “naked” siRNA is only possible via local or topical delivery routes such as in the eyes, respiratory system, and skin, since systemic administration would lead to rapid metabolism and elimination of siRNAs [9].

In addition to degradation by RNase, pharmacological applications of siRNAs also face a number of other limitations such as induction of type I interferon response which can lead to a global perturbation of gene expression [10,11], off-target mRNA degradation mediated by partial sequence hybridization [12], inefficient translocation across cell membranes, short blood circulation half-life, and low bioavailability [13]. To enable safe and efficient clinical use of siRNAs in a future, a variety of methodologies are under active investigation such as liposome encapsulation, nanoparticle formation with cationic lipids, or direct covalent attachment of naked or modified siRNA to polymeric carriers. Meanwhile, targeted delivery of siRNAs using specific ligands that can bind to cell surface receptors are also being explored. In order to optimize these novel treatment strategies, it is necessary to non-invasively assess and monitor not only siRNA delivery to tissues of interest, but also the therapeutic efficacy of gene silencing. Molecular imaging, a vibrant research field over the last decade [14,15], can be invaluable tools for these purposes.

Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and/or other living systems [16]. Generally speaking, molecular imaging includes the use of optical techniques such as bioluminescence imaging (BLI) and fluorescence imaging [17-19], molecular magnetic resonance imaging (MRI) [20], magnetic resonance spectroscopy (MRS), positron emission tomography (PET) [21-24], targeted ultrasound [25,26], and single-photon emission computed tomography (SPECT) [14,27]. In addition, many newly developed molecular imaging techniques are gaining popularity [28-31], and combination of molecular imaging and anatomical imaging (e.g. MRI and computed tomography [CT]) is commonly used to provide more detailed information [32-36]. Many molecular imaging techniques have been adopted in RNAi-related research, which typically requires flexible siRNA delivery systems that can stabilize the siRNA molecules and serve as an image-guided platform for enhanced delivery and target specificity [13]. In this review article, we summarize the current status of in vivo imaging of RNAi such as evaluation of siRNA delivery, biodistribution, pharmacokinetics, and the therapeutic effect.

IMAGING RNAI WITH BLI

BLI uses light-emitting enzymes, expressed by encoded bioluminescence reporters, which can serve as biological indicators [37]. Currently, the two most widely used bioluminescence reporters are those isolated from the North American firefly *Photinus pyralis* (Firefly luciferase or FLuc) and sea pansy *Renilla reniformis* (Renilla luciferase or RLuc) [38], which can be incorporated into laboratory animals either via a viral vector or by creating transgenic animals. FLuc and RLuc can catalyze the oxidation of D-luciferin or coelenterazine to yield oxyluciferin or coelenteramide and release photons, which serve as an indicator of enzyme activity. BLI is a highly sensitive tool for visualizing tumors, neoplastic development, metastatic spread, response to therapy, etc. in small animal models [39]. It can allow rapid and repeated quantitation of reporter gene expression and assessment of the effects of RNAi non-invasively in animal models. However, one major limitation of

BLI is that it is not clinically relevant, hence it is limited to preclinical research in cells and small animal models.

Unlike other molecular imaging techniques, optical imaging with BLI provides the option for two RNAi strategies in the form of siRNA or plasmid DNA encoding short hairpin RNA (shRNA). Although plasmid expressing shRNA must be delivered to the nucleus for shRNA production, the possibility of transcription amplification is an advantage. Unlike shRNA, synthetic siRNA can be delivered directly to the cytosol to combine with RISC. However, siRNAs are susceptible to intracellular metabolism. Overall, these two approaches possess different potency, as well as onset and duration of action, which has been compared using BLI through silencing of luciferase expression [40,41].

BLI was used for non-invasive assessment of shRNA-mediated silencing of P-glycoprotein, a product of the multidrug resistance 1 (MDR1) gene which is responsible for the failure of cancer chemotherapy [42]. Since coelenterazine, the substrate for RLuc, is also a known substrate for P-glycoprotein transport, BLI in mouse tumor models (transiently transfected with RLuc) successfully validated shRNA-mediated down-regulation of P-glycoprotein transport function. In the same study, another strategy for monitoring shRNA-mediated down-regulation of P-glycoprotein expression was explored using a MDR1-FLuc fusion construct [42]. The fusion plasmid along with a plasmid encoding shRNA against MDR1, scrambled shRNA, or an empty vector control was somatically transferred to mouse hepatocytes via hydrodynamic transfection. BLI after injection of D-luciferin revealed significant reduction of bioluminescence signal for MDR1-targeted RNAi, compared to the various control groups, due to down-regulation of P-glycoprotein-FLuc protein levels. Several years later, a similar study involving the reversal of MDR by shRNA-mediated RNAi was reported using RLuc-based BLI in a human colon cancer model [43].

BLI of shRNA therapy inhibiting expression of the prolyl hydroxylase-2 (PHD2) protein has been investigated [44]. In general, hypoxia resulting from myocardial ischemia leads to upregulation of hypoxia inducible factor-1 alpha (HIF-1 α), which in turn activates several downstream angiogenic genes for improving cardiac function. However, the biological half-life of HIF-1 α is ~ 5 minutes, since it is quickly degraded by PHD2. In this report, a PHD2-targeting shRNA plasmid, incorporated with a FLuc reporter gene driven by a hypoxia response element, was injected intramyocardially into mice following ligation of the left anterior descending artery [44]. Significant improvement in angiogenesis and contractility was observed following shRNA-mediated PHD2 inhibition, which was successfully detected by BLI, demonstrating the potential of this technique for monitoring the efficacy of novel cardiovascular gene therapies.

BLI has also been used to evaluate novel antiviral agents in the form of shRNAs, which inhibit the hepatitis C virus (HCV) core protein [45]. A plasmid encoding FLuc with or without the upstream HCV core gene was hydrodynamically co-injected with two different shRNAs clones into the liver of C57BL/6 mice. The inhibitory effects of both shRNAs were successfully monitored by BLI, as the loss of FLuc activity coincided with degradation of the HCV core protein. Similar approaches were also used to evaluate tumor growth and metastasis, in which real time BLI enabled non-invasive assessment of shRNA-mediated inhibition of metastasis associated colon cancer-1 [46] and transforming growth factor β -activated kinase-I (Fig. (1)) [47].

In addition to the above shRNA-based studies, BLI has been evaluated and validated for detection of siRNA-mediated therapeutic effects, based on their direct inhibition of luciferase expression [48-50]. In addition, the potential of BLI has been investigated in a variety of tumor models such as prostate cancer [51], colorectal carcinoma [52], lung cancer

[53], pancreatic cancer [54], osteosarcoma [55], glioblastoma [56], and acute lymphoblastic leukemia [57]. Several non-viral methods for in vivo delivery of siRNA have also been investigated and optimized using BLI. For example, liposome-encapsulated siRNAs (targeting human integrin α_v) were found to inhibit the growth of luciferase-transfected PC-3 tumors, which was associated with decreased integrin α_v expression and increased apoptosis of tumor cells [58]. In other reports, the delivery of luciferase siRNA using a polymerized polyglycerol-based dendritic carrier [56], or lipid nanoparticles containing cationic lipids, polyethylene glycol (PEG), and cholesterol [59], was successfully measured by non-invasive BLI.

IMAGING RNAI WITH FLUORESCENCE

Fluorescence imaging can use either endogenous reporters or exogenous contrast agents [14,17,18,60-62]. Early studies on imaging of RNAi have utilized transfected fluorescent reporters such as the green fluorescent protein (GFP) or red fluorescent protein (RFP), which is not applicable for clinical investigation. Therefore, recent reports have used exogenously administered near-infrared fluorescent (NIRF) agents such as Cy5.5, which is more suitable for in vivo imaging applications because of low autofluorescence and good tissue penetration of light in the 700-900 nm range. Different from GFR or RFP, these agents can be chemically conjugated to targeting molecules or delivery vehicles for image-guided therapy.

In vivo fluorescence imaging following knockdown of enhanced GFP (eGFP) expression by siRNA was used to evaluate the changes in tumor size, which corresponded with changes in fluorescence signal intensity over time [63,64]. In another report, silencing of GFP expression by intramuscular co-injection of GFP plasmid DNA and siRNA against GFP into mice was documented by fluorescence imaging [65]. Similarly, downregulation of RFP expression by intratumorally injected siRNA was examined in a subcutaneous melanoma model [66]. Recently, melanoma models with dual fluorescent reporters have been evaluated, in which human A375 melanoma cells expressing the tandem tomato fluorescent protein (tdTFP) containing a siRNA target site and eGFP were used [67]. Intratumoral injection of siRNA specific to the integrated siRNA target site resulted in significant silencing of tdTFP compared to eGFP, as indicated by in vivo fluorescence imaging. In a transgenic mouse model with GFP expression confined to the epidermis, intravital fluorescence imaging was performed after intradermal injection of siRNAs targeting GFP [68]. Marked reduction of GFP in the treated areas was observed with a dual axes confocal fluorescence microscope.

Fluorescently labeled vascular endothelial growth factor (VEGF) siRNAs were formulated as micelles, which were composed of polyethyleneimine (PEI)-based nanoscale polyelectrolyte complex (PEC) core surrounded by a PEG shell [69]. Biodistribution and RNAi-mediated tumor growth suppression was monitored with in vivo fluorescence imaging, after intravenous administration of the micelles into a subcutaneous PC-3 prostate cancer model in mice. Predominant accumulation of siRNA-containing micelles in the tumor and significant suppression of tumor growth were observed, when compared to other siRNA formulations such as naked siRNA and siRNA/PEI. Similarly, targeted suppression of the glyceraldehyde 3-phosphate (GADPH) gene in rat hippocampus was successfully monitored by fluorescence imaging, using gold nanorods conjugated with fluorescently labeled siRNA [70].

In an interesting report, a new strategy for in vivo fluorescence imaging of siRNA delivery and therapy was developed using a tumor model [71]. Fluorescence imaging of the tumor tissue was made possible by RNAi-mediated downregulation of ferrochelatase, which led to

accumulation of protoporphyrin IX, a fluorescent metabolite of heme synthesis (Fig. (2)). Such a concept of inherent fluorescence associated with silencing of certain metabolic pathway can be more advantageous than the abovementioned strategies, since it may negate the requirement of exogenously labeled fluorescent molecules or the delivery of plasmids encoded with fluorescent reporters.

IMAGING RNAI WITH MRI

MRI can provide exquisite three-dimensional (3D) images of the organs and tissues, which exhibits good contrast between different soft tissues of the body, making it highly useful for imaging of the brain, muscle, heart, and tumors. Although MRI has another major advantage of not using ionizing radiation, the key disadvantage is that it suffers from very low detection sensitivity. Recently, a multifunctional delivery system based on PEG-grafted PEI was reported, which was functionalized with a neuroblastoma cell-specific disialoganglioside (GD2) single chain antibody fragment [72]. This nanocarrier was further complexed with superparamagnetic iron oxide nanoparticles (SPIONs) and siRNAs against the Bcl-2 gene, a key apoptosis inhibitor that is overexpressed in many tumors. In vitro and in vivo MRI demonstrated effective delivery of siRNAs to the tumor, which was confirmed by significant suppression of tumor growth and reduced Bcl-2 expression in tumors. Subsequently, similar nanocarriers were also functionalized with CD44v6 single chain variable fragment (scFv_{CD44v6}) for targeted delivery of siRNA to human gastric carcinoma tumors in mice [73,74].

MRI visible siRNA vectors have been explored for imaging of pancreatic islet transplantation in mice, which contained magnetic iron oxide nanoparticles and siRNA targeting either caspase-3 [75] or β_2 microglobulin (B2M) [76]. While the siRNA targeting caspase-3 inhibited apoptosis of insulinoma cells and human islets, the siRNA targeting B2M helped downregulate the major histocompatibility complex I (MHC class I) protein to overcome immune rejection after transplantation. Human islets labelled with these MRI visible vectors were implanted under the left kidney capsule of mice, whereas islets labelled with the parental nanoparticles were transplanted under the right kidney of the same animals as the control. In both studies, in vivo MRI showed no difference in graft volume reduction within the first week, but significantly higher graft volume in the test groups than the control groups after 2 weeks [75,76]. No significant changes in T2 relaxation times between test and control groups was found after analysis of a T2 map, suggesting relative stability of the delivery vectors in test and control kidneys during the study period. Overall, the data observed from both studies demonstrated the feasibility of simultaneous non-invasive MRI monitoring and protection of transplanted human pancreatic islets in mice using MRI visible siRNA vectors.

MRI AND OPTICAL IMAGING OF RNAI

Due to the low sensitivity of MRI, it is often combined with the more sensitive optical imaging techniques which can provide complementary information. In a pioneering study, dual-modality MRI/optical imaging of RNAi was achieved using a multifunctional probe based on magnetic iron oxide nanoparticles, which was labeled with Cy5.5 and conjugated to a synthetic siRNA duplex targeting GFP and survivin [77,78]. Intracellular delivery of the agent was facilitated by surface modification with a membrane translocating polyarginine peptide, and in vivo tumor targeting was successfully visualized with both MRI and optical imaging (Fig. (3)) [77]. Since this agent does not contain a targeting ligand, tumor accumulation was primarily attributed to the enhanced permeability and retention effect due to the hyperpermeable tumor vasculature. Overall, this study demonstrated the feasibility of

using MRI and optical imaging to simultaneously monitor siRNA delivery and subsequent therapeutic effect in vivo.

In a follow-up study, another agent which has similar imaging capabilities along with specific delivery of siRNA to human breast tumors through the use of a tumor specific ligand was developed [79]. A peptide that specifically binds to uMUC-1, which was found in over 90% of human breast adenocarcinomas, and a synthetic siRNA that targets a tumor specific anti-apoptotic gene BIRC5 was used in this study [79]. In vivo MRI of mice bearing breast tumors revealed a significant decrease in tumor T2 relaxation time after injection of the agent, suggesting preferential tumor uptake and retention in vivo, which was confirmed by NIRF imaging carried out immediately after the MRI scans. The in vivo findings were further validated by ex vivo imaging of excised tumors.

Direct loading of a MRI contrast agent and fluorescently labeled siRNA targeting cyclooxygenase-2 (COX-2) into PEGylated polycationic liposomes was investigated for imaging of siRNA delivery and gene silencing in tumor-bearing mice [80]. The incorporation of a MRI contrast agent allows for non-invasive imaging of siRNA delivery, whereas PEGylation of the cationic liposomes can prevent recognition by macrophages and prolong the circulation half-life. Both Magnevist (a gadolinium-based MRI contrast agent) and Feridex (a SPION-based MRI contrast agent) were evaluated in vitro, which suggested loss of siRNA-mediated gene silencing when Magnevist was used. Therefore, Feridex was used for in vivo studies, which exhibited successful intratumoral delivery of siRNA in the MDA-MB-231 human breast cancer xenograft model, as evidenced by both MRI and optical imaging.

In another report, siRNA delivery by Gd-containing PEGylated cationic liposome-based nanoparticles was studied, which entrapped either fluorescently labelled anti-Survivin siRNA or a non-silencing negative control [81]. In vivo MRI demonstrated accumulation of the nanoparticles in subcutaneous tumors after intravenous injection, as indicated by an increase in T1-weighted signal intensity, which was confirmed by ex vivo fluorescence microscopy and a decrease in survivin expression based on Western blotting. The favourable biodistribution of the nanoparticles with prominent tumor uptake, efficient delivery of anti-survivin siRNA, as well as a decrease in survivin expression and tumor growth strongly suggested theranostic potential for these multifunctional nanoparticles, which can be utilized for co-entrapment of different therapeutic agents in the future. However, the moderate stability of the nanoparticles only allows a four day window for their therapeutic applications, which mandates more research effort in the future to develop more stable nanoparticles for potential clinical translation.

In a proof-of-principle study, a nanoplex carrying MRI and optical labels was reported for simultaneous delivery of a functional prodrug enzyme bacterial cytosine deaminase (bCD) and a siRNA targeting choline kinase- α (chk- α), an enzyme significantly upregulated in breast cancer cells [82]. In the MDA-MB-231 human breast cancer xenograft model, efficient delivery of the nanoplex to the tumor was achieved based on in vivo MRI and optical imaging. In addition, non-invasive detection of siRNA-mediated downregulation of chk- α and conversion of 5-fluorocytosine (5-FC) to cytotoxic 5-fluorouracil (5-FU) was carried out using ^1H and ^{19}F MRS. The combination of siRNA-mediated gene silencing and prodrug enzyme-activated treatment resulted in a significant delay in tumor growth when compared to either treatment alone. This intriguing study demonstrated not only successful multimodal therapy but also multimodal molecular imaging associated with therapeutic RNAi.

IMAGING RNAI WITH PET

PET is a non-invasive nuclear medicine imaging technique which utilizes positron-emitting radioisotopes to obtain 3D images [14,15,83]. Since it is quantitative, non-invasive, sensitive, and clinically relevant, it is the ideal imaging technique for precise measurement of the pharmacokinetics and biodistribution of siRNA delivery vehicles, which is not only critical but also indispensable for the development of RNAi-based medicine. Although a wide variety of PET isotopes have been investigated in preclinical and clinical studies [15,21,24,84-89], only ^{64}Cu and ^{18}F have been used for RNAi-based research to date.

In an early report, transferrin (Tf) targeted nanoparticles formed by cyclodextrin-containing polycations were used to load luciferase-targeting siRNA, which was conjugated to 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) at the 5' end for ^{64}Cu -labeling [90]. PET imaging was carried out to monitor the pharmacokinetics, whole-body biodistribution, and tumor localization in mice bearing luciferase-expressing Neuro2A subcutaneous tumors. In addition, BLI was performed to measure the knockdown of luciferase expression by the siRNA. The nanoparticles were formulated with or without Tf targeting ligands to determine the effect of cell-specific targeting on both biodistribution and its function in vivo, which showed similar biodistribution and tumor localization for the targeted nanoparticles and non-targeted nanoparticles based on PET imaging. However, BLI showed a reduction of tumor luciferase activity by ~50% at 1 day after injection of Tf targeted nanoparticles, when compared with the non-targeted nanoparticles (Fig. (4)). It was suggested that such disparity was due to the different distribution in the intracellular and extracellular tumor space for the targeted and non-targeted nanoparticles, where the presence of targeting ligands could enhance intercellular localization and subsequent gene silencing within the tumor cells.

Similarly, a polymer-based system called “dynamic polyconjugates (DPCs)” was investigated for the delivery of siRNA to hepatocytes, which was also labeled with ^{64}Cu via the DOTA chelator [91]. Investigation of whole-body biodistribution using PET/CT hybrid imaging suggested good siRNA delivery efficiency (~70%) to the liver. In comparison, free ^{64}Cu and ^{64}Cu -labeled siRNA could only result in 40% and 10% of the injected dose to the liver, respectively. No measurement of the therapeutic efficacy was reported in this study.

In addition to ^{64}Cu -labeled siRNA, ^{18}F -labeled siRNA has also been investigated. For example, PET imaging was carried out to study the biodistribution and pharmacokinetics of different chemically modified siRNAs (e.g. replacement of the 2'-OH group of certain nucleotides in an siRNA sequence by an F atom or a methoxy group) in comparison to unmodified naked siRNA, which were all labelled with ^{18}F via the synthon ^{18}F -FPyBrA and studied in rodents after intravenous injection [92]. In another study, N-succinimidyl 4- ^{18}F -fluorobenzoate (^{18}F -SFB) was used to label siRNA for PET imaging [93]. A differential biodistribution pattern in mice was observed for ^{18}F -labeled siRNA (which showed rapid clearance from the blood stream and renal excretion) and cationic liposome/ ^{18}F -labeled siRNA complexes (which were found to accumulate in the lungs). Subsequent NIRF imaging using fluorescently labeled siRNA and cationic liposome/siRNA complexes gave similar findings as were observed with PET.

Recently, another ^{18}F -labeled siRNA was studied to determine the delivery efficacy of cholesterol conjugated siRNA (siRNA-C) to B16F10 melanoma tumors, using RGD peptide conjugated PEGylated polycationic liposomes as the carrier [94]. Biodistribution as determined by PET suggested higher tumor accumulation of the PEGylated polycationic liposomes containing ^{18}F -siRNA-C when compared with the various control groups.

Interestingly, the accumulation in the liver, heart, and kidneys was similar across different groups. Typically, ^{18}F -labeling was time-consuming with low yield, which remains one of the major hurdles for broad clinical applications of ^{18}F -based agents. To tackle this challenge, various new methods for facile and efficient ^{18}F -labeling were reported [95-98], which may greatly facilitate future research of RNAi using ^{18}F as the radiolabel for measurement of pharmacokinetics and biodistribution of various siRNA delivery vehicles.

In addition to monitoring the efficiency of siRNA delivery, PET has also been used to measure the therapeutic effect of RNAi [99]. In this early report, siRNA duplexes against CXCR4 (a gene which is involved in tumor metastasis) were investigated to block breast cancer metastasis both in vitro and in vivo. Using ^{18}F -FDG, the clinical “gold standard” PET tracer for measuring the therapeutic response in cancer patients, the in vivo effect of CXCR4 siRNAs on inhibition of breast cancer metastasis was confirmed by PET. ^{18}F -FDG uptake in the metastatic tumors was found to be significantly higher (indicating higher tumor metabolic activity) in the control group when compared to mice subjected to siRNA treatment.

IMAGING RNAI WITH SPECT

SPECT is an imaging technique that uses gamma-emitting radioisotopes such as ^{111}In , ^{123}I , ^{131}I , $^{99\text{m}}\text{Tc}$, etc. [15,100-102]. Generally speaking, SPECT is at least an order of magnitude less sensitive than PET [14,103]. In one report, the feasibility of in vivo tracking of siRNA delivery in nude mice bearing human kidney cancer xenografts was investigated with $^{99\text{m}}\text{Tc}$ -labeled 18-nucleotide oligoribonucleotides [104]. After intravenous injection, SPECT imaging was carried out for non-invasive and longitudinal monitoring of in vivo delivery of these small RNAs.

Subsequently, SPECT imaging was also used in a series of studies to monitor the biodistribution, pharmacokinetics, and stability of siRNA in different formulations upon various routes of administration, which was radiolabeled with gamma emitters such as ^{111}In or $^{99\text{m}}\text{Tc}$ [105-107]. In a recent study, amine-modified dicer-substrate siRNAs were radiolabeled with ^{111}In via DTPA and subsequently formulated with dextran nanogels for in vivo studies after retroorbital administration into mice [108]. SPECT imaging suggested renal accumulation following a delayed transition from kidney to bladder of the nanogels were compared to free siRNA.

CONCLUSION AND FUTURE PERSPECTIVES

This review provides a comprehensive summary of the various molecular imaging techniques that have been explored for in vivo imaging of RNAi, and representative examples are listed in Table 1. Many of these studies have been conducted to assess the feasibility of using imaging techniques to evaluate the delivery, biodistribution, and pharmacokinetics of siRNA/shRNA and/or the delivery vehicles. In addition, several reports have also utilized molecular imaging to monitor the gene silencing efficiency of delivered siRNA molecules for the treatment of various pathological disorders. Further development of these non-invasive imaging strategies for RNAi, not only focusing on the delivery of siRNA/shRNA but also the therapeutic efficacy, is critical for future clinical translation.

Optical imaging techniques such as BLI and fluorescence imaging, utilizing transfected reporter genes, used to be only imaging tools available for evaluation of therapeutic RNAi. However, these techniques are not applicable (e.g. BLI) or broadly useful (e.g. fluorescence) in clinical settings [14]. The use of more clinically relevant imaging modalities (e.g. PET and MRI), with biocompatible or biodegradable exogenously administered contrast agents

for labelling of the delivery vehicles, hold significant potential in the advancement of RNAi therapeutics [109].

Therapeutic application of RNAi requires safe and efficient delivery of siRNA/shRNA to the target cells. However, efficient delivery of naked siRNA is very challenging due to several factors such as RNase degradation, inefficient translocation across cellular membrane, and short circulation half-life. Therefore, a wide variety of delivery vehicles are currently under development/optimization for robust and site-specific delivery of siRNA/shRNA, with a few already entering clinical investigation [110,111]. Future incorporation of molecular imaging techniques to monitor the delivery efficiency or the therapeutic effect will dramatically facilitate the translation of RNAi into a powerful therapeutic modality in the clinic.

Over the last 20 years, molecular imaging with PET and SPECT has evolved significantly, and a wide variety of radiotracers that can bind to disease-related targets have been tested in the clinic [36,112-117]. These probes can be highly useful in the future for measuring the therapeutic effect of RNAi, aside from using imaging approaches to determine the delivery and pharmacokinetics after radiolabeling the genes or carriers. Rigorous validation will be needed to confirm that biodistribution of the carrier is correlated with that of siRNA/shRNA, since imaging only detects the label (e.g. radioisotopes) but not the gene or carrier themselves. Lastly, a single imaging modality may not be sufficient to simultaneously monitor both the gene delivery and silencing effect of RNAi. Therefore, it is essential to develop multimodality imaging approaches for realizing the full potential of therapeutic RNAi.

Acknowledgments

The authors are grateful for financial support from the University of Wisconsin Carbone Cancer Center and the Department of Defense (W81XWH-11-1-0644).

REFERENCES

- [1]. Verma IM, Somia N. Gene therapy -- promises, problems and prospects. *Nature*. 1997; 389:239–242. [PubMed: 9305836]
- [2]. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*. 1998; 391:806–811. [PubMed: 9486653]
- [3]. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature*. 2000; 404:293–296. [PubMed: 10749213]
- [4]. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature*. 2001; 409:363–366. [PubMed: 11201747]
- [5]. Nykanen A, Haley B, Zamore PD. ATP requirements and small interfering RNA structure in the RNA interference pathway. *Cell*. 2001; 107:309–321. [PubMed: 11701122]
- [6]. Martinez J, Patkaniowska A, Urlaub H, Luhrmann R, Tuschl T. Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. *Cell*. 2002; 110:563–574. [PubMed: 12230974]
- [7]. Tansey, B. Promising eye drug from S.F. firm / Macular degeneration treatment interferes with RNA messages. In *San Francisco Chronicle*; San Francisco: 2006.
- [8]. Shen J, Samul R, Silva RL, Akiyama H, Liu H, Saishin Y, Hackett SF, Zinnen S, Kossen K, Fosnaugh K, Vargeese C, Gomez A, Bouhana K, Aitchison R, Pavco P, Campochiaro PA. Suppression of ocular neovascularization with siRNA targeting VEGF receptor 1. *Gene Ther*. 2006; 13:225–234. [PubMed: 16195704]
- [9]. Vaishnav AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, Meyers R, de Fougerolles T, Maraganore J. A status report on RNAi therapeutics. *Silence*. 2010; 1:14. [PubMed: 20615220]

- [10]. Sledz CA, Holko M, de Veer MJ, Silverman RH, Williams BR. Activation of the interferon system by short-interfering RNAs. *Nat. Cell Biol.* 2003; 5:834–839. [PubMed: 12942087]
- [11]. Robbins MA, Rossi JJ. Sensing the danger in RNA. *Nat. Med.* 2005; 11:250–251. [PubMed: 15746933]
- [12]. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, Li B, Cavet G, Linsley PS. Expression profiling reveals off-target gene regulation by RNAi. *Nat. Biotechnol.* 2003; 21:635–637. [PubMed: 12754523]
- [13]. Ifediba MA, Moore A. In vivo imaging of the systemic delivery of small interfering RNA. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 2012; 4:428–437. [PubMed: 22228711]
- [14]. James ML, Gambhir SS. A molecular imaging primer: modalities, imaging agents, and applications. *Physiol. Rev.* 2012; 92:897–965. [PubMed: 22535898]
- [15]. Nolting DD, Nickels ML, Guo N, Pham W. Molecular imaging probe development: a chemistry perspective. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:273–306. [PubMed: 22943038]
- [16]. Mankoff DA. A definition of molecular imaging. *J. Nucl. Med.* 2007; 48:18N, 21N.
- [17]. Huang X, Lee S, Chen X. Design of “smart” probes for optical imaging of apoptosis. *Am. J. Nucl. Med. Mol. Imaging.* 2011; 1:3–17. [PubMed: 22514789]
- [18]. Wu Y, Zhang W, Li J, Zhang Y. Optical imaging of tumor microenvironment. *Am. J. Nucl. Med. Mol. Imaging.* 2013; 3:1–15. [PubMed: 23342297]
- [19]. Contag CH, Bachmann MH. Advances in in vivo bioluminescence imaging of gene expression. *Annu. Rev. Biomed. Eng.* 2002; 4:235–260. [PubMed: 12117758]
- [20]. Sosnovik DE, Weissleder R. Emerging concepts in molecular MRI. *Curr. Opin. Biotechnol.* 2007; 18:4–10. [PubMed: 17126545]
- [21]. Alauddin MM. Positron emission tomography (PET) imaging with ^{18}F -based radiotracers. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:55–76. [PubMed: 23133802]
- [22]. Cai W, Hong H. Peptoid and Positron Emission Tomography: an Appealing Combination. *Am. J. Nucl. Med. Mol. Imaging.* 2011; 1:76–79. [PubMed: 22022661]
- [23]. Eary JF, Hawkins DS, Rodler ET, Conrad EU 3rd. ^{18}F -FDG PET in sarcoma treatment response imaging. *Am. J. Nucl. Med. Mol. Imaging.* 2011; 1:47–53. [PubMed: 23133794]
- [24]. Grassi I, Nanni C, Allegri V, Morigi JJ, Montini GC, Castellucci P, Fanti S. The clinical use of PET with ^{11}C -acetate. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:33–47. [PubMed: 23133801]
- [25]. Liang HD, Blomley MJ. The role of ultrasound in molecular imaging. *Br. J. Radiol.* 2003; 76(Spec No 2):S140–150. [PubMed: 15572336]
- [26]. Dayton PA, Rychak JJ. Molecular ultrasound imaging using microbubble contrast agents. *Front. Biosci.* 2007; 12:5124–5142. [PubMed: 17569635]
- [27]. Bhargava P, He G, Samarghandi A, Delpassand ES. Pictorial review of SPECT/CT imaging applications in clinical nuclear medicine. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:221–231. [PubMed: 23133813]
- [28]. Thorek D, Robertson R, Bacchus WA, Hahn J, Rothberg J, Beattie BJ, Grimm J. Cerenkov imaging - a new modality for molecular imaging. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:163–173. [PubMed: 23133811]
- [29]. Yigit MV, Medarova Z. In vivo and ex vivo applications of gold nanoparticles for biomedical SERS imaging. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:232–241. [PubMed: 23133814]
- [30]. Yao J, Wang LV. Photoacoustic tomography: fundamentals, advances and prospects. *Contrast Media Mol. Imaging.* 2011; 6:332–345. [PubMed: 22025335]
- [31]. Wang LV, Hu S. Photoacoustic tomography: in vivo imaging from organelles to organs. *Science.* 2012; 335:1458–1462. [PubMed: 22442475]
- [32]. Aparici CM, Carlson D, Nguyen N, Hawkins RA, Seo Y. Combined SPECT and Multidetector CT for Prostate Cancer Evaluations. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:48–54. [PubMed: 22267999]
- [33]. Balyasnikova S, Lofgren J, de Nijs R, Zamogilnaya Y, Hojgaard L, Fischer BM. PET/MR in oncology: an introduction with focus on MR and future perspectives for hybrid imaging. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:458–474. [PubMed: 23145362]

- [34]. Cai W, Hong H. In a “nutshell”: intrinsically radio-labeled quantum dots. *Am. J. Nucl. Med. Mol. Imaging*. 2012; 2:136–140. [PubMed: 23133808]
- [35]. Tejawani A, Lavaf A, Parikh K, Mokhtar B, Swamy U, Emmolo J, Guirguis A, Ashamalla H. The role of PET/CT in decreasing inter-observer variability in treatment planning and evaluation of response for cervical cancer. *Am. J. Nucl. Med. Mol. Imaging*. 2012; 2:307–313. [PubMed: 23133818]
- [36]. Zhang L, Chang RC, Chu LW, Mak HK. Current neuroimaging techniques in Alzheimer’s disease and applications in animal models. *Am. J. Nucl. Med. Mol. Imaging*. 2012; 2:386–404. [PubMed: 23133824]
- [37]. McCaffrey A, Kay MA, Contag CH. Advancing molecular therapies through in vivo bioluminescent imaging. *Mol. Imaging*. 2003; 2:75–86. [PubMed: 12964305]
- [38]. Kelkar M, De A. Bioluminescence based in vivo screening technologies. *Curr. Opin. Pharmacol.* 2012; 12:592–600. [PubMed: 22954534]
- [39]. Klerk CP, Overmeer RM, Niers TM, Versteeg HH, Richel DJ, Buckle T, Van Noorden CJ, van Telling O. Validity of bioluminescence measurements for noninvasive in vivo imaging of tumor load in small animals. *Biotechniques*. 2007; 43:7–13. 30. [PubMed: 17936938]
- [40]. McCaffrey AP, Meuse L, Pham TT, Conklin DS, Hannon GJ, Kay MA. RNA interference in adult mice. *Nature*. 2002; 418:38–39. [PubMed: 12097900]
- [41]. McAnuff MA, Rettig GR, Rice KG. Potency of siRNA versus shRNA mediated knockdown in vivo. *J. Pharm. Sci.* 2007; 96:2922–2930. [PubMed: 17518360]
- [42]. Pichler A, Zelcer N, Prior JL, Kuil AJ, Piwnicka-Worms D. In vivo RNA interference-mediated ablation of MDR1 P-glycoprotein. *Clin. Cancer Res.* 2005; 11:4487–4494. [PubMed: 15958634]
- [43]. Jeon YH, Bae SA, Lee YJ, Lee YL, Lee SW, Yoon GS, Ahn BC, Ha JH, Lee J. Evaluation of the reversal of multidrug resistance by MDR1 ribonucleic acid interference in a human colon cancer model using a Renilla luciferase reporter gene and coelenterazine. *Mol. Imaging*. 2010; 9:343–350. [PubMed: 21084030]
- [44]. Huang M, Chan DA, Jia F, Xie X, Li Z, Hoyt G, Robbins RC, Chen X, Giaccia AJ, Wu JC. Short hairpin RNA interference therapy for ischemic heart disease. *Circulation*. 2008; 118:S226–233. [PubMed: 18824759]
- [45]. Du J, Zhao F, Zhou Y, Yan H, Duan XG, Liang SQ, Wang YL, Fu QX, Wang XH, Peng JC, Zhan LS. Bioluminescence imaging allows monitoring hepatitis C virus core protein inhibitors in mice. *PLoS One*. 2010; 5:e14043. [PubMed: 21124971]
- [46]. Pichorner A, Sack U, Kobelt D, Kelch I, Arlt F, Smith J, Walther W, Schlag PM, Stein U. In vivo imaging of colorectal cancer growth and metastasis by targeting MACC1 with shRNA in xenografted mice. *Clin. Exp. Metastasis*. 2012; 29:573–583. [PubMed: 22484916]
- [47]. Ray DM, Myers PH, Painter JT, Hoenerhoff MJ, Olden K, Roberts JD. Inhibition of transforming growth factor-beta-activated kinase-1 blocks cancer cell adhesion, invasion, and metastasis. *Br. J. Cancer*. 2012; 107:129–136. [PubMed: 22644295]
- [48]. Bartlett DW, Davis ME. Insights into the kinetics of siRNA-mediated gene silencing from live-cell and live-animal bioluminescent imaging. *Nucleic Acids Res.* 2006; 34:322–333. [PubMed: 16410612]
- [49]. Eefting D, Grimbergen JM, de Vries MR, van Weel V, Kaijzel EL, Que I, Moon RT, Lowik CW, van Bockel JH, Quax PH. Prolonged in vivo gene silencing by electroporation-mediated plasmid delivery of small interfering RNA. *Hum. Gene Ther.* 2007; 18:861–869. [PubMed: 17850190]
- [50]. Huang B, Mao CP, Peng S, Hung CF, Wu TC. RNA interference-mediated in vivo silencing of fas ligand as a strategy for the enhancement of DNA vaccine potency. *Hum. Gene Ther.* 2008; 19:763–773. [PubMed: 18627219]
- [51]. Pulukuri SM, Rao JS. Small interfering RNA directed reversal of urokinase plasminogen activator demethylation inhibits prostate tumor growth and metastasis. *Cancer Res.* 2007; 67:6637–6646. [PubMed: 17638874]
- [52]. Gray MJ, Van Buren G, Dallas NA, Xia L, Wang X, Yang AD, Somcio RJ, Lin YG, Lim S, Fan F, Mangala LS, Arumugam T, Logsdon CD, Lopez-Berestein G, Sood AK, Ellis LM. Therapeutic targeting of neuropilin-2 on colorectal carcinoma cells implanted in the murine liver. *J. Natl. Cancer Inst.* 2008; 100:109–120. [PubMed: 18182619]

- [53]. Kawata E, Ashihara E, Kimura S, Takenaka K, Sato K, Tanaka R, Yokota A, Kamitsuji Y, Takeuchi M, Kuroda J, Tanaka F, Yoshikawa T, Maekawa T. Administration of PLK-1 small interfering RNA with atelocollagen prevents the growth of liver metastases of lung cancer. *Mol. Cancer Ther.* 2008; 7:2904–2912. [PubMed: 18790771]
- [54]. Ramachandran V, Arumugam T, Wang H, Logsdon CD. Anterior gradient 2 is expressed and secreted during the development of pancreatic cancer and promotes cancer cell survival. *Cancer Res.* 2008; 68:7811–7818. [PubMed: 18829536]
- [55]. Rousseau J, Escriou V, Perrot P, Picarda G, Charrier C, Scherman D, Heymann D, Redini F, Trichet V. Advantages of bioluminescence imaging to follow siRNA or chemotherapeutic treatments in osteosarcoma preclinical models. *Cancer Gene Ther.* 2010; 17:387–397. [PubMed: 20075983]
- [56]. Ofek P, Fischer W, Calderon M, Haag R, Satchi-Fainaro R. In vivo delivery of small interfering RNA to tumors and their vasculature by novel dendritic nanocarriers. *FASEB J.* 2010; 24:3122–3134. [PubMed: 20385622]
- [57]. Fazzina R, Lombardini L, Mezzanotte L, Roda A, Hrelia P, Pession A, Tonelli R. Generation and characterization of bioluminescent xenograft mouse models of MLL-related acute leukemias and in vivo evaluation of luciferase-targeting siRNA nanoparticles. *Int. J. Oncol.* 2012; 41:621–628. [PubMed: 22665135]
- [58]. Bisanz K, Yu J, Edlund M, Spohn B, Hung MC, Chung LW, Hsieh CL. Targeting ECM-integrin interaction with liposome-encapsulated small interfering RNAs inhibits the growth of human prostate cancer in a bone xenograft imaging model. *Mol. Ther.* 2005; 12:634–643. [PubMed: 16039164]
- [59]. Tao W, Davide JP, Cai M, Zhang GJ, South VJ, Matter A, Ng B, Zhang Y, Sepp-Lorenzino L. Noninvasive imaging of lipid nanoparticle-mediated systemic delivery of small-interfering RNA to the liver. *Mol. Ther.* 2010; 18:1657–1666. [PubMed: 20628357]
- [60]. Cai W, Zhang Y, Kamp TJ. Imaging of Induced Pluripotent Stem Cells: From Cellular Reprogramming to Transplantation. *Am. J. Nucl. Med. Mol. Imaging.* 2011; 1:18–28. [PubMed: 21841970]
- [61]. Chin PT, Beekman CA, Buckle T, Josephson L, van Leeuwen FW. Multispectral visualization of surgical safety-margins using fluorescent marker seeds. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:151–162. [PubMed: 23133810]
- [62]. Gaikwad SM, Ray P. Non-invasive imaging of PI3K/Akt/mTOR signalling in cancer. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:418–431. [PubMed: 23145359]
- [63]. Golzio M, Mazzolini L, Ledoux A, Paganin A, Izard M, Hellaudais L, Bieth A, Pillaire MJ, Cazaux C, Hoffmann JS, Couderc B, Teissie J. In vivo gene silencing in solid tumors by targeted electrically mediated siRNA delivery. *Gene Ther.* 2007; 14:752–759. [PubMed: 17344906]
- [64]. Paganin-Gioanni A, Bellard E, Couderc B, Teissie J, Golzio M. Tracking in vitro and in vivo siRNA electrotransfer in tumor cells. *J. RNAi Gene Silencing.* 2008; 4:281–288. [PubMed: 19771237]
- [65]. Golzio M, Teissie J. Optical in vivo imaging of electrically mediated delivery of siRNA into muscle for gene function analysis. *Methods Mol. Biol.* 2008; 423:279–287. [PubMed: 18370206]
- [66]. Suh MS, Shim G, Lee HY, Han SE, Yu YH, Choi Y, Kim K, Kwon IC, Weon KY, Kim YB, Oh YK. Anionic amino acid-derived cationic lipid for siRNA delivery. *J. Control. Release.* 2009; 140:268–276. [PubMed: 19567256]
- [67]. Hickerson RP, Gonzalez-Gonzalez E, Vlassov AV, Li M, Lara MF, Contag CH, Kaspar RL. Intravital Fluorescence Imaging of Small Interfering RNA-Mediated Gene Repression in a Dual Reporter Melanoma Xenograft Model. *Nucleic Acid Ther.* 2012; 22:438–443. [PubMed: 23098239]
- [68]. Gonzalez-Gonzalez E, Ra H, Hickerson RP, Wang Q, Piyawattanametha W, Mandella MJ, Kino GS, Leake D, Avilion AA, Solgaard O, Doyle TC, Contag CH, Kaspar RL. siRNA silencing of keratinocyte-specific GFP expression in a transgenic mouse skin model. *Gene Ther.* 2009; 16:963–972. [PubMed: 19474811]

- [69]. Kim SH, Jeong JH, Lee SH, Kim SW, Park TG. Local and systemic delivery of VEGF siRNA using polyelectrolyte complex micelles for effective treatment of cancer. *J. Control. Release*. 2008; 129:107–116. [PubMed: 18486981]
- [70]. Bonoio AC, Bergey EJ, Ding H, Hu R, Kumar R, Yong KT, Prasad PN, Mahajan S, Picchione KE, Bhattacharjee A, Ignatowski TA. Gold nanorod–siRNA induces efficient in vivo gene silencing in the rat hippocampus. *Nanomedicine (Lond)*. 2011; 6:617–630. [PubMed: 21718174]
- [71]. Wan K, Ebert B, Voigt J, Wang Q, Dai Y, Haag R, Kemmner W. In vivo tumor imaging using a novel RNAi-based detection mechanism. *Nanomedicine*. 2012; 8:393–398. [PubMed: 22349098]
- [72]. Shen M, Gong F, Pang P, Zhu K, Meng X, Wu C, Wang J, Shan H, Shuai X. An MRI-visible non-viral vector for targeted Bcl-2 siRNA delivery to neuroblastoma. *Int. J. Nanomedicine*. 2012; 7:3319–3332. [PubMed: 22802690]
- [73]. Chen Y, Wang W, Lian G, Qian C, Wang L, Zeng L, Liao C, Liang B, Huang B, Huang K, Shuai X. Development of an MRI-visible nonviral vector for siRNA delivery targeting gastric cancer. *Int. J. Nanomedicine*. 2012; 7:359–368. [PubMed: 22848158]
- [74]. Chen Y, Lian G, Liao C, Wang W, Zeng L, Qian C, Huang K, Shuai X. Characterization of polyethylene glycol-grafted polyethylenimine and superparamagnetic iron oxide nanoparticles (PEG-g-PEI-SPION) as an MRI-visible vector for siRNA delivery in gastric cancer in vitro and in vivo. *J. Gastroenterol*. 2012 ePub.
- [75]. Wang P, Yigit MV, Medarova Z, Wei L, Dai G, Schuetz C, Moore A. Combined small interfering RNA therapy and in vivo magnetic resonance imaging in islet transplantation. *Diabetes*. 2011; 60:565–571. [PubMed: 21270267]
- [76]. Wang P, Yigit MV, Ran C, Ross A, Wei L, Dai G, Medarova Z, Moore A. A theranostic small interfering RNA nanoprobe protects pancreatic islet grafts from adoptively transferred immune rejection. *Diabetes*. 2012; 61:3247–3254. [PubMed: 22923469]
- [77]. Medarova Z, Pham W, Farrar C, Petkova V, Moore A. In vivo imaging of siRNA delivery and silencing in tumors. *Nat. Med*. 2007; 13:372–377. [PubMed: 17322898]
- [78]. Medarova Z, Kumar M, Ng SW, Moore A. Development and application of a dual-purpose nanoparticle platform for delivery and imaging of siRNA in tumors. *Methods Mol. Biol*. 2009; 555:1–13. [PubMed: 19495684]
- [79]. Kumar M, Yigit M, Dai G, Moore A, Medarova Z. Image-guided breast tumor therapy using a small interfering RNA nanodrug. *Cancer Res*. 2010; 70:7553–7561. [PubMed: 20702603]
- [80]. Mikhaylova M, Stasinopoulos I, Kato Y, Artemov D, Bhujwalla ZM. Imaging of cationic multifunctional liposome-mediated delivery of COX-2 siRNA. *Cancer Gene Ther*. 2009; 16:217–226. [PubMed: 18927599]
- [81]. Kenny GD, Kamaly N, Kalber TL, Brody LP, Sahuri M, Shamsaei E, Miller AD, Bell JD. Novel multifunctional nanoparticle mediates siRNA tumour delivery, visualisation and therapeutic tumour reduction in vivo. *J. Control. Release*. 2011; 149:111–116. [PubMed: 20888381]
- [82]. Li C, Penet MF, Wildes F, Takagi T, Chen Z, Winnard PT, Artemov D, Bhujwalla ZM. Nanoplex delivery of siRNA and prodrug enzyme for multimodality image-guided molecular pathway targeted cancer therapy. *ACS Nano*. 2010; 4:6707–6716. [PubMed: 20958072]
- [83]. Phelps ME, Hoffman EJ, Mullani NA, Ter-Pogossian MM. Application of annihilation coincidence detection to transaxial reconstruction tomography. *J. Nucl. Med*. 1975; 16:210–224. [PubMed: 1113170]
- [84]. Hao G, Singh AN, Liu W, Sun X. PET with non-standard nuclides. *Curr. Top. Med. Chem*. 2010; 10:1096–1112. [PubMed: 20388114]
- [85]. Wong AW, Ormsby E, Zhang H, Seo JW, Mahakian LM, Caskey CF, Ferrara KW. A comparison of image contrast with ⁶⁴Cu-labeled long circulating liposomes and ¹⁸F-FDG in a murine model of mammary carcinoma. *Am. J. Nucl. Med. Mol. Imaging*. 2013; 3:32–43. [PubMed: 23342299]
- [86]. Yang Z, Xiong C, Zhang R, Zhu H, Li C. Synthesis and evaluation of ⁶⁸Ga-labeled DOTA-2-deoxy-D-glucosamine as a potential radiotracer in muPET imaging. *Am. J. Nucl. Med. Mol. Imaging*. 2012; 2:499–507. [PubMed: 23145365]
- [87]. Zhang Y, Hong H, Severin GW, Engle JW, Yang Y, Goel S, Nathanson AJ, Liu G, Nickles RJ, Leigh BR, Barnhart TE, Cai W. ImmunoPET and near-infrared fluorescence imaging of CD105

- expression using a monoclonal antibody dual-labeled with ^{89}Zr and IRDye 800CW. *Am. J. Transl. Res.* 2012; 4:333–346. [PubMed: 22937210]
- [88]. Hong H, Zhang Y, Engle JW, Nayak TR, Theuer CP, Nickles RJ, Barnhart TE, Cai W. In vivo targeting and positron emission tomography imaging of tumor vasculature with ^{66}Ga -labeled nano-graphene. *Biomaterials.* 2012; 33:4147–4156. [PubMed: 22386918]
- [89]. Hong H, Zhang Y, Orbay H, Valdovinos HF, Nayak TR, Bean J, Theuer CP, Barnhart TE, Cai W. Positron Emission Tomography Imaging of Tumor Angiogenesis with a $^{61/64}\text{Cu}$ -Labeled F(ab')_2 Antibody Fragment. *Mol. Pharm.* 2013; 10:709–716. [PubMed: 23316869]
- [90]. Bartlett DW, Su H, Hildebrandt IJ, Weber WA, Davis ME. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:15549–15554. [PubMed: 17875985]
- [91]. Mudd SR, Trubetskoy VS, Blokhin AV, Weichert JP, Wolff JA. Hybrid PET/CT for noninvasive pharmacokinetic evaluation of dynamic PolyConjugates, a synthetic siRNA delivery system. *Bioconjug. Chem.* 2010; 21:1183–1189. [PubMed: 20552976]
- [92]. Viel T, Boisgard R, Kuhnast B, Jegou B, Siquier-Pernet K, Hinnen F, Dolle F, Tavittian B. Molecular imaging study on in vivo distribution and pharmacokinetics of modified small interfering RNAs (siRNAs). *Oligonucleotides.* 2008; 18:201–212. [PubMed: 18729822]
- [93]. Hatanaka K, Asai T, Koide H, Kenjo E, Tsuzuku T, Harada N, Tsukada H, Oku N. Development of double-stranded siRNA labeling method using positron emitter and its in vivo trafficking analyzed by positron emission tomography. *Bioconjug. Chem.* 2010; 21:756–763. [PubMed: 20210335]
- [94]. Kenjo E, Asai T, Yonenaga N, Ando H, Ishii T, Hatanaka K, Shimizu K, Urita Y, Dewa T, Nango M, Tsukada H, Oku N. Systemic Delivery of Small Interfering RNA by Use of Targeted Polycation Liposomes for Cancer Therapy. *Biol. Pharm. Bull.* 2013; 36:287–291. [PubMed: 23370357]
- [95]. Li Y, Guo J, Tang S, Lang L, Chen X, Perrin DM. One-step and one-pot-two-step radiosynthesis of cyclo-RGD- ^{18}F -aryltrifluoroborate conjugates for functional imaging. *Am. J. Nucl. Med. Mol. Imaging.* 2013; 3:44–56. [PubMed: 23342300]
- [96]. Li Y, Liu Z, Harwig CW, Pourghasian M, Lau J, Lin KS, Schaffer P, Benard F, Perrin DM. ^{18}F -click labeling of a bombesin antagonist with an alkyne- ^{18}F - ArBF_3^- : in vivo PET imaging of tumors expressing the GRP-receptor. *Am. J. Nucl. Med. Mol. Imaging.* 2013; 3:57–70. [PubMed: 23342301]
- [97]. Liu S, Park R, Conti PS, Li Z. “Kit like” ^{18}F labeling method for synthesis of RGD peptide-based PET probes. *Am. J. Nucl. Med. Mol. Imaging.* 2013; 3:97–101. [PubMed: 23342304]
- [98]. Li Z, Lin TP, Liu S, Huang CW, Hudnall TW, Gabbai FP, Conti PS. Rapid aqueous [^{18}F]-labeling of a bodipy dye for positron emission tomography/fluorescence dual modality imaging. *Chem. Commun. (Camb).* 2011; 47:9324–9326. [PubMed: 21779546]
- [99]. Liang Z, Yoon Y, Votaw J, Goodman MM, Williams L, Shim H. Silencing of CXCR4 blocks breast cancer metastasis. *Cancer Res.* 2005; 65:967–971. [PubMed: 15705897]
- [100]. Buckle T, van Berg NS, Kuil J, Bunschoten A, Oldenburg J, Borowsky AD, Wesseling J, Masada R, Oishi S, Fujii N, van Leeuwen FW. Non-invasive longitudinal imaging of tumor progression using an $^{111}\text{indium}$ labeled CXCR4 peptide antagonist. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:99–109. [PubMed: 23133805]
- [101]. Ogasawara Y, Ogasawara K, Suzuki T, Yamashita T, Kuroda H, Chida K, Fujiwara S, Aso K, Kobayashi M, Yoshida K, Terasaki K, Ogawa A. Preoperative ^{123}I -iomazenil SPECT imaging predicts cerebral hyperperfusion following endarterectomy for unilateral cervical internal carotid artery stenosis. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:77–87. [PubMed: 23133803]
- [102]. Zhao R, Wang J, Deng J, Yang W, Wang J. Efficacy of $^{99\text{m}}\text{Tc}$ -EDDA/HYNIC-TOC SPECT/CT scintigraphy in Graves' ophthalmopathy. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:242–247. [PubMed: 23133815]
- [103]. Temma T, Saji H. Radiolabelled probes for imaging of atherosclerotic plaques. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:432–447. [PubMed: 23145360]

- [104]. Liu N, Ding H, Vanderheyden JL, Zhu Z, Zhang Y. Radiolabeling small RNA with technetium-99m for visualizing cellular delivery and mouse biodistribution. *Nucl. Med. Biol.* 2007; 34:399–404. [PubMed: 17499729]
- [105]. Merkel OM, Librizzi D, Pfestroff A, Schurra T, Behe M, Kissel T. In vivo SPECT and real-time gamma camera imaging of biodistribution and pharmacokinetics of siRNA delivery using an optimized radiolabeling and purification procedure. *Bioconjug. Chem.* 2009; 20:174–182. [PubMed: 19093855]
- [106]. Merkel OM, Librizzi D, Pfestroff A, Schurra T, Buyens K, Sanders NN, De Smedt SC, Behe M, Kissel T. Stability of siRNA polyplexes from poly(ethylenimine) and poly(ethylenimine)-g-poly(ethylene glycol) under in vivo conditions: effects on pharmacokinetics and biodistribution measured by Fluorescence Fluctuation Spectroscopy and Single Photon Emission Computed Tomography (SPECT) imaging. *J. Control. Release.* 2009; 138:148–159. [PubMed: 19463870]
- [107]. Merkel OM, Beyerle A, Librizzi D, Pfestroff A, Behr TM, Sproat B, Barth PJ, Kissel T. Nonviral siRNA delivery to the lung: investigation of PEG-PEI polyplexes and their in vivo performance. *Mol. Pharm.* 2009; 6:1246–1260. [PubMed: 19606864]
- [108]. Naeye B, Deschout H, Caveliers V, Descamps B, Braeckmans K, Vanhove C, Demeester J, Lahoutte T, De Smedt SC, Raemdonck K. In vivo disassembly of IV administered siRNA matrix nanoparticles at the renal filtration barrier. *Biomaterials.* 2013; 34:2350–2358. [PubMed: 23261216]
- [109]. Hong H, Zhang Y, Cai W. In vivo imaging of RNA interference. *J. Nucl. Med.* 2010; 51:169–172. [PubMed: 20080892]
- [110]. Davis ME, Zuckerman JE, Choi CH, Seligson D, Tolcher A, Alabi CA, Yen Y, Heidel JD, Ribas A. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature.* 2010; 464:1067–1070. [PubMed: 20305636]
- [111]. Taberero J, Shapiro GI, Lorusso PM, Cervantes A, Schwartz GK, Weiss GJ, Paz-Ares L, Cho DC, Infante JR, Alsina M, Gounder MM, Falzone R, Harrop J, Seila White AC, Toudjarska I, Bumcrot D, Meyers RE, Hinkle G, Svrikapa N, Hutabarat RM, Clausen VA, Cehelsky J, Nochur SV, Gamba-Vitalo C, Vaishnav AK, Sah DW, Gollob JA, Burris HA 3rd. First-in-Man Trial of an RNA Interference Therapeutic Targeting VEGF and KSP in Cancer Patients with Liver Involvement. *Cancer Discov.* 2013 ePub.
- [112]. Fakhri GE. Ready for prime time? Dual tracer PET and SPECT imaging. *Am. J. Nucl. Med. Mol. Imaging.* 2012; 2:415–417. [PubMed: 23145358]
- [113]. Mason NS, Lopresti BJ, Ruszkiewicz J, Dong X, Joyce S, Leef G, Sen M, Wahed AS, Mathis CA, Grandis JR, Thomas SM. Utility of 3'-[(18)F]fluoro-3'-deoxythymidine as a PET tracer to monitor response to gene therapy in a xenograft model of head and neck carcinoma. *Am. J. Nucl. Med. Mol. Imaging.* 2013; 3:16–31. [PubMed: 23342298]
- [114]. Cai W, Niu G, Chen X. Imaging of integrins as biomarkers for tumor angiogenesis. *Curr. Pharm. Des.* 2008; 14:2943–2973. [PubMed: 18991712]
- [115]. Zhang Y, Hong H, Cai W. PET tracers based on Zirconium-89. *Curr. Radiopharm.* 2011; 4:131–139. [PubMed: 22191652]
- [116]. Cai W, Chen X. Multimodality molecular imaging of tumor angiogenesis. *J. Nucl. Med.* 2008; 49(Suppl 2):113S–128S. [PubMed: 18523069]
- [117]. Cai W, Niu G, Chen X. Multimodality imaging of the HER-kinase axis in cancer. *Eur. J. Nucl. Med. Mol. Imaging.* 2008; 35:186–208. [PubMed: 17846765]

Figure 1.

Mice bearing tumors that express TAK1 shRNA are less likely to develop lung metastases.

A. Serial bioluminescence images taken after tumour removal. **B.** The percentage of metastasis-free mice was shown in a Kaplan-Meier plot. There were 23 mice in the control shRNA and 21 mice in the TAK1 shRNA group, with a P-value of 0.0044 indicating highly significant difference between the two groups. Adapted with permission from [47].

Figure 2.

In vivo fluorescence imaging of RNA interference. MDA-MB-435 melanoma tumor-bearing mice were treated with various formulations and the fluorescence signal from endogenous protoporphyrin IX was measured over time. Circles indicate the tumors. ALA: 5-aminolevulinic acid; FECH: Ferrochelatase, the target for the siRNA used. Adapted with permission from [71].

Figure 3.

A multifunctional nanoparticle for RNA interference and in vivo dual-modality imaging. **A.** Schematic illustration of the multifunctional agent consisting of a magnetic nanoparticle labeled with a near-infrared fluorescent (NIRF) dye Cy5.5, membrane translocation peptides (MPAP), and siRNA molecules targeting the green fluorescent protein (siGFP). **B.** In vivo MRI of mice bearing subcutaneous LS174T human colorectal adenocarcinoma (arrows) before and after treatment. A high-intensity NIRF signal in the tumor confirmed the delivery of the nanoparticle. Adapted with permission from [77].

Figure 4.

Multimodality in vivo imaging of ⁶⁴Cu-DOTA-siRNA delivered by targeted (Tf) and non-targeted (PEG) nanoparticles with PET and BLI. **A.** Fused PET/CT images of mice at 1, 10, and 60 min after injection of ⁶⁴Cu-DOTA-siRNA. **B.** Blood clearance and tumor localization of Tf-targeted and non-targeted nanoparticles. **C.** Fused PET/CT images showing tumor-associated (arrow) activity at 1 day after injection of targeted or non-targeted nanoparticles containing ⁶⁴Cu-DOTA-siRNA. **D.** BLI of the same mice shown in **C** before injection and 1 day after injection. **E.** Relative changes in luciferase expression at 1 day after injection of Tf-targeted or non-targeted nanoparticles containing ⁶⁴Cu-DOTA-siRNA. Error bars indicate standard error. Adapted with permission from [90].

Table 1

Representative examples of in vivo imaging of RNA interference.

Imaging Modality	Image Tag	Target gene	Disease	Reference
BLI	Luciferase	MDR1	Colon cancer	[42,43]
		PHD2	Myocardial ischemia	[44]
		HCV core protein	Hepatitis C	[45]
		MACC1	Colorectal cancer	[46]
		TAK1	Breast cancer	[47]
		uPA	Prostate cancer	[51]
		NRP2	Colorectal carcinoma	[52]
		PLK-1	Lung cancer	[53]
		AGR2	Pancreatic cancer	[54]
		RANKL	Osteosarcoma	[55]
Fluorescence	GFP/RFP	GFP/RFP	Various cancer types	[63,64,66,67]
Fluorescence	Cy5.5	VEGF	Prostate cancer	[69]
Fluorescence	Cy3	GADPH	Neuronal disorder	[70]
Fluorescence	Inherent PpIX	Ferrochelatase	Melanoma	[71]
MRI	SPION	Bcl-2	Neuroblastoma	[72]
MRI	SPION	CD44v6	Gastric carcinoma	[73,74]
MRI	Iron oxide nanoparticle	Caspase-3/B2M	Diabetes	[75,76]
MRI/Optical	Iron oxide nanoparticle/Cy5.5	GFP/survivin	Colorectal cancer	[77]
MRI/Optical	Iron oxide nanoparticle/Cy5.5	BIRC5	Breast cancer	[79]
MRI/Optical	Feridex/DY647	COX-2	Breast cancer	[80]
MRI/Optical	Gd/Rhodamine/AlexaFluor 488	Survivin	Ovarian cancer	[81]
MRI/MRS/Optical	Gd/Rhodamine	Chk- α	Breast cancer	[82]
PET/BLI	⁶⁴ Cu/Luciferase	Luciferase	Neuroblastoma	[90]
PET	¹⁸ F	c-myc, MDM2, VEGF	Melanoma	[94]
SPECT	¹¹¹ In	Luciferase	/	[105]
	¹¹¹ In	GFP	/	[108]