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Application of magnetic resonance methods to studies of gene therapy

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1. Introduction

Disease in an individual usually arises from a combination of genetic status and environmental factors. The genetic status is considered to be immutable and therapeutic interventions have been to modify environmental factors so as to alleviate symptoms. For example, patients with familial hypercholesterolemia due to a heterozygous mutation in the low density lipoprotein receptor gene are advised to give up smoking and treated with statins to decrease cholesterol synthesis. Such lifestyle changes, pharmacological and possibly surgical interventions, do not always address the underlying genetic cause of disease. It was only until relatively recently that the possibility of modifying genetic status has been considered for curative treatment of diseases, of which the underlying cause is predominately genetic in nature. Advances in genetics and cell biology have brought about this paradigm shift in disease treatment.

Gene therapy is defined as the genetic modification of cells to produce a therapeutic effect [1]. There are two approaches to gene therapy, either involving the *in vivo* genetic modification of cells or the *ex vivo* genetic modification of patient cells and then its subsequent re-administration back to the patient (Fig. 1). For patients with monogenic genetic disorders, gene therapy can replace the defective copy of the gene involved with a normal copy, e.g., replacement of the cystic fibrosis transmembrane regulator gene in respiratory epithelium of patients with cystic fibrosis [2]. However, many diseases tend to be polygenic in origin, and gene therapy is being employed towards production of a therapeutic effect rather than to replace defective genes. For cancer therapy, tumour cell killing can be achieved by transfer of genes (i.e., transduction or transfection) that express enzymes capable of catalyzing the formation of cytotoxic metabolites from administered specific prodrugs into tumour cells, resulting in selective killing of transfected cells, e.g., introduction of herpes simplex virus thymidine kinase gene (HSV-tk) and ganciclovir for the cytotoxic treatment of cancer [3]. In neurological diseases such as Alzheimer's disease, an *ex vivo* gene therapy approach has been used to introduce the nerve growth factor gene into the basal forebrain to rescue degenerating cholinergic neurons in a phase I clinical trial [4].

Gene therapy involves three main interacting components: a therapeutic gene or similar (e.g., RNA molecule or synthetic oligonucleotide), a vehicle (gene therapy vector) to deliver the therapeutic gene to cells and a device to deliver the gene–gene therapy vector to the target organ.

As many terms used in this review may be unfamiliar to the reader, these are briefly explained in the glossary.

2. Therapeutic genes

The genetic information for most forms of life is in the form of DNA (deoxyribonucleic acid). DNA is a polymer of deoxyribonucleotide units, each deoxynucleotide consisting of a nitrogenous base, deoxyribose and a phosphate group. The nitrogenous bases are: adenine (A), thymine (T), cytosine (C) and guanine (G). The purine bases, A and G, readily form hydrogen bonds with the pyrimidine bases, T and C, respectively. Thus, two strands of DNA will pair if their sequences are complementary, to form a twisted ladder-like structure, i.e., a double stranded helix (Fig. 2). The helix is then wound around proteins, histones, which maintain structure and regulate gene expression, to form a chromosome. The collection of chromosomes in a cell is referred to as the genome, e.g., the human genome contains 46 chromosomes.

Genomic DNA sequences for many organisms – from microbes to plants to humans are now available [5] leading to the identification of thousands of genes. These genes, carried on chromosomes, are the basic physical and functional units of heredity. A single gene typically consists of several thousand base pairs (bp) with a total of 20–25K

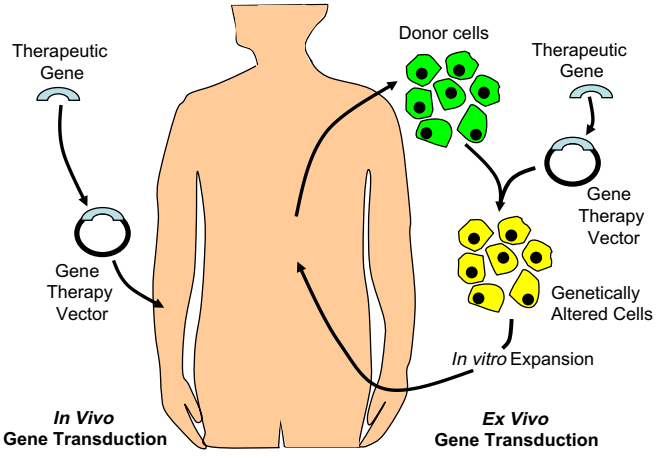

Fig. 1. *In vivo* and *ex vivo* approaches to gene therapy.

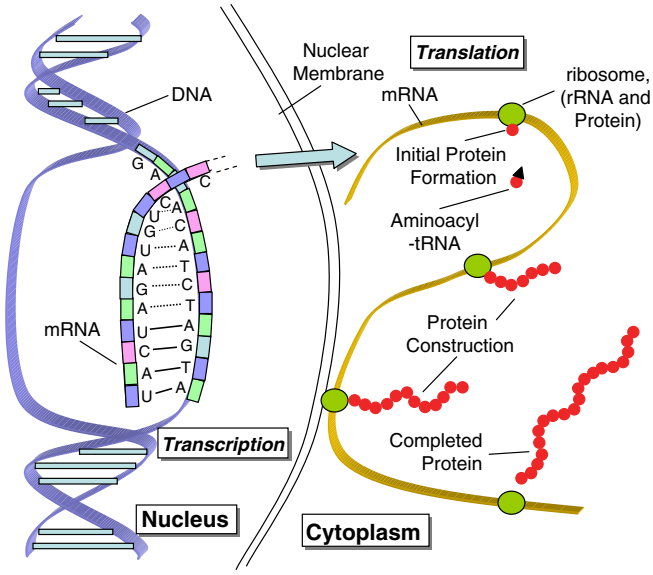

Fig. 2. Deoxyribonucleic acid (DNA) and gene expression.

genes being found in the human genome [6]. When a gene is active or 'expressed', the DNA strands separate and messenger ribonucleic acid (mRNA) is formed from the DNA template in a process known as transcription (Fig. 2). Transcription is catalyzed by RNA polymerase and is initiated at a DNA sequence referred to as the promoter. Ribonucleic acid (RNA) is comparable to DNA but consists of nucleotides in which the sugar is ribose, and the pyrimidine base, T, is replaced by another pyrimidine base, uracil (U), the latter lacking a methyl group. Transcription involves the 'reading' of the DNA strand in the 5′–3′ direction such that purine and pyrimidines pair between the DNA template and the growing mRNA strand. The mRNA strand consisting of a sequence of bases determined by the DNA template may then be processed prior to translation to proteins. Translation (Fig. 2) involves the use of other RNA molecules, ribosomal RNA (rRNA) and transfer RNA (tRNA). The genetic code is a triplet code, the sequence of three bases in mRNA, the codon, specifies one amino acid, the codon being translated by tRNA. Protein synthesis occurs in ribosomes which are complex assemblies of rRNA and a range of proteins.

The draft human genome was published in 2001 [5], and estimated to contain 30–40K genes. Relatively recently, the number of genes in the human genome has been revised to contain only 20–25K genes [7]. Such a modest number of genes, despite the complex nature of the human body, suggest that it is not the genes themselves that gives rise to the possibility of complex interactions within the body but the regulation of these genes. Indeed, only a subset of these genes is expressed at any instant in time. Gene expression is the production of the specific protein or RNA from its gene. When 'expressed' or 'switched on', genes are either transcribed into mRNA and then translated into proteins, or transcribed into types of RNA such as tRNA or rRNA and not translated to proteins (as described above). Many phenomena, including cellular development, maturation and proliferation can be attributed to differential gene expression. Thus, knowledge of the genetic sequence *per se* is insufficient to determine the role of genes in health and disease. The subset of genes expressed confers unique properties to each cell type, and the subset may vary in response to certain stimuli and changing cellular requirements. Gene expression is complex and highly regulated, such that genes can be switched on or off with varying levels of expression, allowing the cell to respond dynamically to environmental stimuli and/or changing cell requirements. The challenge in the post-genomic era is to explore the functions of candidate genes to shed light on the role of specific gene dysfunctions in the pathophysiology of disease. Understanding such gene–pathophysiological interactions raises the possibility of identification of therapeutic genes for the treatment of disease either arising from inherited or acquired genetic defects as well as developments in the field of pharmacogenetics.

3. Gene therapy vectors

Gene therapy requires the introduction of genes into cells and the vehicle that packages the genes for transfer, denoted a vector, can be either non-viral or viral based. Table 1 compares the properties of some commonly used gene therapy vectors. Vectors should protect genes from degradation before and after cell entry, facilitate entry of genes into target cells, and ensure gene transcription following cell entry. Also, the ideal gene therapy vector should be easily produced in a pure form in high volumes, and be sufficiently large to carry the therapeutic gene insert, and efficiently and stably transduce non-proliferating cells without production of cytotoxic effects, inflammatory and immune responses.

Non-viral based methods include the use of naked DNA [8], oligodeoxynucleotides [9] and lipoplexes and polyplexes [8]. The simplest form of non-viral gene transfer is the administration of naked DNA plasmid (a circular unit of double stranded DNA that replicates inside the cell, independent of chromosomal DNA). However, its low transfection efficiency *in vivo* limits its use in gene therapy protocols. Synthetic oligonucleotides such as antisense or siRNA (short interfering RNA or silencing) have been proposed in gene therapy to inactivate genes involved in the disease process but these are still relatively early days for such emerging technologies. Lipoplexes and polyplexes are synthetic vectors that have been developed for

Table 1
Comparison of commonly employed gene therapy vectors

	Retrovirus	Adenovirus	AAV	Herpesvirus	Plasmid-liposomal
Gene insert size	6–7 (bp)	7.5 (bp)	2–4.5 (bp)	10–100 (bp)	Unlimited
Genome	ssRNA	dsDNA	ssDNA	dsDNA	RNA/DNA
Site	Genome	Episome	Episome/genome	Episome	Episome
Efficiency	Low	Moderate	Variable	Moderate	Very low
Cell proliferation	Required	Not required	Not required	Not required	Not required
Expression	Permanent	Transient	Permanent/transient	Transient	Transient
Major advantages	Genomic expression	Relatively high transduction efficiency	Relatively high transduction efficiency, +/− genomic expression	Tropism	Safe, cheap
Major disadvantages	Insertional mutagenesis	Inflammatory and immune response	Insertional mutagenesis, difficult to produce	Difficult to produce	Poor efficiency

non-viral gene transfer [8]. Plasmid DNA covered with lipids in an organised structure such as a micelle or liposome are called lipoplexes, and have been used in several clinical trials for disease treatment, including for cancer [10]. Polyplexes are complexes of polymer and DNA and polymers can include cationic polymers. Again, the drawback of lipoplexes and polyplexes is their relative short duration of gene expression [8] and hence, the consideration of viral based therapy vectors. All viruses attack their hosts and introduce their genetic material into the host cells as part of their replication cycle, resulting in additional copies of the virus and increased number of cells becoming infected by the virus. Thus, if genetically modified to remove disease causing genes, viruses can be further modified to carry therapeutic genes for gene therapy. Commonly employed viral gene therapy vectors include: adenovirus, retrovirus and adeno-associated virus (AAV) and herpesvirus (Table 1). Viral vectors also have their disadvantages, mainly difficulties in large scale production and host immunogenicity.

Further to the consideration of the choice of gene therapy vector, is the requirement of actual gene expression in the target organ. Selective, tissue- or organ-restricted gene targetting is desirable to enhance the safety and efficiency of gene transfer *in vivo*. Targetting is dependent on the gene vector, the genes themselves and mode of delivery of the gene therapy vector (see below). Certain gene therapy vectors exhibit a preference towards certain organs, e.g., the herpes simplex virus (HSV) is neurotropic, and this may determine selection of the gene therapy vector. However, modification of gene therapy vectors is the objective of ongoing research, either to alter their natural tropism or to increase targeting to specific organs. If we again consider HSV, although neurotropic, HSV has many properties ideal for a gene therapy vector that can benefit non-neural gene therapies. AAV is another gene vector that has many favourable gene therapy vector characteristics but limited by its relative non-specificity, and attempts are ongoing to enable targeting to selected organs.

Selectivity of gene transfer can be programmed via surface moieties on the gene therapy vector. Tissue targeting is based on the addition of a ligand to the surface of the gene therapy vector to achieve specific binding to the desired tissues or cells [11–13]. Tissue specificity can also be achieved by the use of tissue selective promoters which will only initiate transcription in specific tissues, e.g., the prostate-specific antigen promoter, for prostate specificity [14]. The promoter for heat shock proteins (HSPs) activate gene expression in response to hyperthermia [15] and the HSP70 promoter and local hyperthermia has been proposed to control gene expression. Similarly, promoters containing hypoxia response elements which induce gene activation in the presence of hypoxia has been proposed [16]. Such selective induction of gene expression in gene therapy have been recently reviewed elsewhere [17–20].

As yet, the ideal gene therapy vector does not exist and much development is required for determining the suitability and development of individual gene therapy vectors in preclinical and clinical trials.

4. Gene delivery devices

Gene vectors carrying genes to be transduced may be administered systemically but a more common approach is to deliver the vector locally, circumventing the requirement for targeting of gene therapy vectors. This can be performed by direct injection into the target organ following surgical exposure or percutaneously (injection to target organ via needle puncture of the skin), or by catheter-based delivery systems [21]. Direct delivery of gene therapy vectors will aid locating of the vectors to the target organ at concentrations high enough for therapy.

5. Role of MR methods in gene therapy studies

The observation of a nuclear magnetic resonance (NMR) signal in condensed phases was made independently by Bloch et al. [22] and Purcell et al. [23]. Subsequently, NMR has played a major role in the non-destructive analysis of compounds in a range of applications. However, NMR was not applied to biological systems until Odeblad et al. [24] who made relaxation time measurements of protons of living cells and excised animal tissue. In the 1960s and 1970s, much work was published on relaxation, diffusion, and chemical exchange of water in a range of cells and tissues. In the 1970s, *in vivo* NMR was begun by the groups of Richards and Radda in Oxford [25] such that it is now possible to non-invasively assess *in vivo* the metabolism of various tissues in both animals and humans, selected reviews include [26–29]. In view of its use in the clinic, the term NMR has been replaced with magnetic resonance spectroscopy (MRS) to overcome the negative connotations implied in the term ‘nuclear’, and MRS will be used henceforth.

MRS *in vivo* allows the determination of various biochemical species within a defined volume or voxel. A spectrum can also be obtained from a number of voxels in an image, known as spectroscopic imaging [30]. Spectroscopic imaging allows determination of the biochemical profiles of a number of selected voxels.

Magnetic resonance imaging (MRI) is also based on the same NMR phenomenon as MRS. However, it was not until the development of spatial encoding by Lauterbur [31] that MRI ‘came of age’. Previous to this, MR had been one-dimensional, lacking spatial information as the origin of the MR signal from the sample was unknown. By application of magnetic field gradients in three dimensions and computerized axial tomography-scan back projection (i.e., projection–reconstruction), Lauterbur [31] was able to obtain the first MRI images of two tubes of water. From these early days, MRI has been developed to be a major non-invasive imaging tool for measurement of anatomical

and functional parameters in a range of physiopathological processes in man and animals.

MRI is a powerful clinical tool, able to delineate normal and disease tissue arising predominantly from differences in T_1 (spin–lattice or longitudinal) and T_2 (spin–spin or transverse) relaxation characteristics. However, the overlap between the relaxation times of normal and disease tissues are sufficiently large so as to decrease specificity of the technique, and hence, the need to use contrast agents. Contrast agents attenuate T_1 and T_2 relaxation times to differing extents. Those that operate predominantly via T_1 relaxation usually lead to an increase in the MRI signal (positive contrast) whereas those operating via T_2, usually result in a decrease in the MRI signal (negative contrast). Basic parameters of MRI acquisition, repetition time (TR) and echo time (TE), 'weight' the MRI experiment to preferentially detect differences in T_1 or T_2 relaxation times of tissues, respectively (Fig. 3).

Positive contrast agents tend to be low molecular weight compounds, commonly lanthanide chelates such as gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA, Fig. 4). The lanthanide ion interacts with surrounding water protons so as to enhance their T_1 relaxation, leading to increased signal intensity (positive enhancement) in T_1-weighted MRI images. Free lanthanide ions are toxic [32] and hence, the requirement for the free ion to be chelated for use in biological systems. The use of T_1-weighted MRI sequences emphasises differences between the T_1 relaxation rates between the protons.

Gadolinium chelates (>50,000 molecules) have been incorporated into the outer layer of nanoparticles and filled

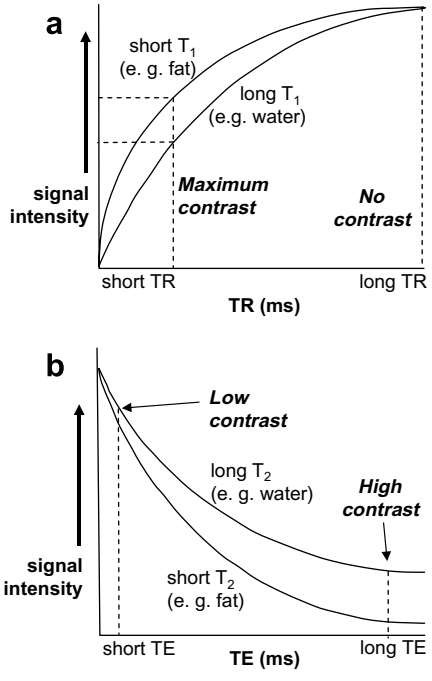

Fig. 3. Differences in (a) spin–lattice, T_1 and (b) spin–spin, T_2 relaxation times enable generation of contrast by changing repetition time (TR) and echo time (TE), respectively.

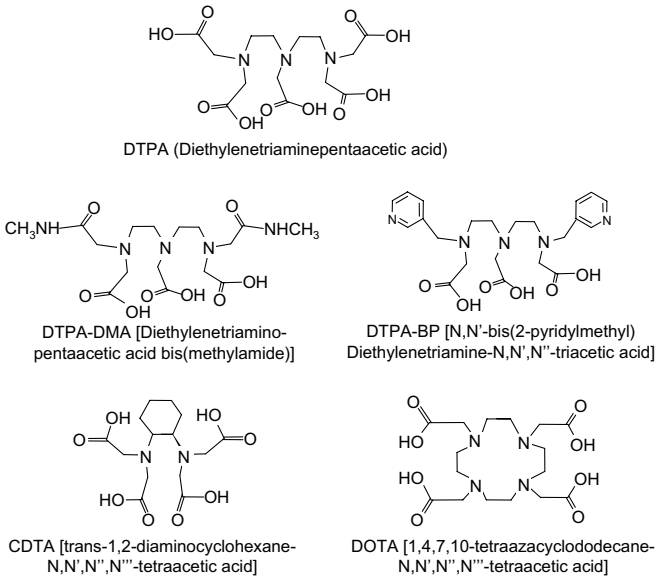

Fig. 4. Structures of commonly employed gadolinium chelates for MRI.

with perfluorocarbons (PFC) to form PFC nanoparticles. Such particles may be imaged by both T_1-weighted MRI and ^{19}F MRI, the latter due to the presence of PFCs [33]. PFCs are a special group of negative contrast agents, having no signal by ^{1}H MRI but of course provide a ^{19}F MR signal. The ^{19}F nucleus has 80% the sensitivity of the ^{1}H nucleus and has the advantage of no endogenous background signal. The use of ^{19}F MRI allows qualitative and quantitative imaging of PFC nanoparticles [34], contributing to their advantage in molecular and cellular imaging.

Negative contrast agents induce a loss in MR signal, i.e., results in negative enhancement in the MRI image, especially when T_2-weighted MRI sequences are employed. The most commonly used T_2 relaxation agents are based on monocrystalline iron oxide nanoparticles (MIONs): superparamagnetic iron oxide (SPIO), very small superparamagnetic iron oxide (VSPIO) or ultrasmall superparamagnetic iron oxide (USPIO), differing from each other according to size (Fig. 5). These particles usually consist of a crystalline iron oxide core surrounded by a polymer coating. The coating may be carbohydrate in

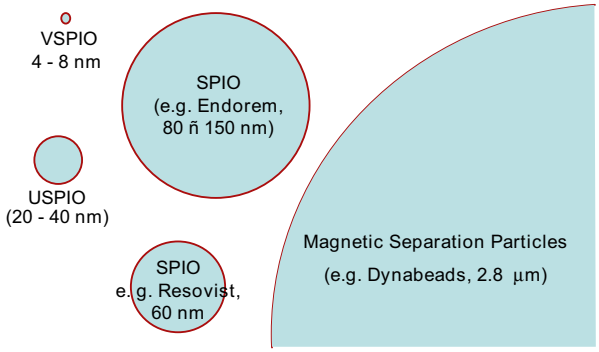

Fig. 5. Monocrystalline iron oxide nanoparticles (MIONs) employed in MRI.

nature, e.g., dextran, or consist of synthetic polymers, polycations or polyamines, determining the pharmacokinetic properties of the contrast agent. MIONS significantly decreases the values of T_2 of the surrounding water protons but also significantly affect local magnetic susceptibility and so also decrease values of T_2^*. T_2^* results from a sum of T_2 relaxation and local susceptibility effects, and so MIONs are also readily imaged by gradient-echo based MRI methods.

Another source of contrast aside from employment of contrast agents such as MIONs and gadolinium chelates are chemical exchange saturation transfer (CEST) agents [35]. CEST agents have exchangeable protons e.g., –NH and –OH, resonating at specific chemical shifts distinguishable from that of bulk water. Application of a radiofrequency pulse of appropriate frequency and power leads to saturation of the exchangeable protons, resulting in transfer of their magnetization to bulk water and attenuation of the water signal. CEST agents can allow contrast to be switched 'on' and 'off' by simply changing the MR experiment. Whilst several agents can produce a CEST effect [36], the closeness of the chemical shifts of the exchangeable protons and bulk water renders specific saturation of the former difficult, such that the attenuation of the water signal may arise from either magnetization transfer or direct saturation. To overcome this limitation, paramagnetic CEST agents (PARACEST) may be employed, such agents are based on paramagnetic chelates with exchangeable protons. The presence of paramagnetic ions shifts the chemical shift of the bound water away from bulk water, allowing distinct saturation of the exchangeable protons [37].

Positive enhancement is the usual preferred option as it allows quantification of signal, not readily possible for negative enhancement due to generation of signal voids. However, T_2 relaxation agents such as MIONS have the advantage that detection of low concentrations in the nanomolar–micromolar range is possible as compared to the gadolinium chelates, for which millimolar concentrations are required. Hence, the preferred use of MIONs compared to gadolinium chelates in molecular and cellular imaging. Molecular imaging may be defined as the '*in vivo characterization and measurement of biological processes at the cellular and molecular level*'. Cellular imaging may be defined as the '*non-invasive and repeated imaging of targeted cell populations and cellular processes*'. Often, for both molecular and cellular imaging, sensitivity is an issue. However, use of MIONs of 10–50 nm diameter, may produce substantially enhance T_1 relaxation, such that T_1 rather T_2 relaxation predominates [38]. Furthermore, an advantage of using MIONs is that certain formulations, e.g., Endorem™ have already been approved for clinical use and partly explain the popularity of MION usage in molecular and cellular imaging.

Due to the popularity of the use of MIONs for molecular imaging, and especially cellular labelling [39], there is a drive towards the ability to visualise MIONs by positive contrast in the MRI image. A number of methods have been proposed including the off-resonance [40] and 'white marker' [41] methods. More recently, we have proposed the use of MRI based on ultra-short echo time (UTE) methods [42] to detect MIONs [43]. Positive contrast paves the way towards the possibility of quantification of MION enhancement.

Another common problem faced with the use of MIONs for imaging is the difficulty experienced in determining whether the signal voids arise from MIONs, from bulk susceptibility differences, (as occurs at air–tissue interfaces), or from haemorrhage. So far, of the MRI methods currently available, only the diagonal SPRITE (single point ramped imaging with T_1 enhancement) methodology based on UTE techniques appears to be capable of discerning the source of the signal voids in the MRI image [43].

Both MRS and MRI are non-destructive methodologies and ideal tools for the non-invasive imaging of gene expression *in vivo*, especially due to their ready translation to the clinic. The role of MR in gene therapy studies can be broadly defined as:

(i) to monitor the delivery of gene therapy vectors to target sites;
(ii) to target and enhance gene therapy;
(iii) to image expression of the therapeutic gene;
(iv) to monitor body response to gene therapy.

This article details the principles of MR-based strategies for application to gene therapy protocols.

6. Imaging delivery and targeting of gene therapy vectors and cells

As mentioned earlier, tissue- and organ-selectivity of gene therapy is essential to minimise toxicity and to increase gene transfer to target organs *in vivo*. Toward developing such gene therapy protocols, it is a prerequisite that the delivery of gene therapy vectors to the target organ has to be monitored. This can be achieved by 'tagging' the gene therapy vectors in such a way so as to be detectable by MR through the association of the gene therapy vector with a MR probe. Räty et al. [44] recently developed an avidin displaying baculovirus which enables coating of the virus with biotinylated MIONs for imaging by MRI. Whilst Allen et al. [45] used the protein cage of Cowpea chlorotic mottle virus (CCMV) to conjugate >100 gadolinium chelate molecules to create a method for tracking viral vectors as well as using viral protein cages as a new platform for MR contrast agents. Similarly, Anderson et al. [46] conjugated >500 gadolinium chelate molecules to MS2 capsids to enable MRI tracking of such viral particles. For liposomal vectors, gadolinium chelates can be conjugated to lipids in the liposomal lipid layer for subsequent detection by MRI [47,48]. Such gadolinium chelate–lipid conjugates consequently integrate into the plasma membrane leading to an increase in MRI signal of the target tissue.

For *ex vivo* gene therapy approaches, genetically modified cells may be tagged with MR contrast agents for imaging *in vivo*. Extensive research has been performed in the area of cellular imaging and the reader is advised to refer to such texts [39,49–51].

Alternatively, both gene therapy vectors and cells can be tracked by imaging gene expression specific to them and strategies will be described below.

7. MR-guided and enhanced gene therapy

MRI has been proposed to have a role in monitoring and enhancing gene delivery. A limitation of gene therapy protocols currently available is the low efficiency of gene transfer at the target sites. For example, *in vivo* gene transfer in vasculature can be as low as 1% for non-viral vectors and <5% for viral vectors [51,52]. *In vitro* studies have suggested that controlled heating can enhance gene transfer by fracturing tissues by heat, increasing plasma membrane permeability and cell metabolism, and/or increased activity of heat-sensitive heat shock proteins [53,54]. In practice, heating needs to be generated locally at the target site rather than over the whole body and this can be achieved by the use of a small internal heating device such as a loopless MR attenna. Such a device allows generation of heat locally as well as MR thermal mapping to monitor and control heat distribution at the targeted vessels [55] and has been shown to enhance gene transfer [56]. Monitoring of gene delivery can be simply performed by addition of gadolinium chelate to the gene therapy dose [57].

Low frequency focussed ultrasound (FUS) has also been proposed for enhancing gene transfer as such ultrasound can induce cell membrane porosity [58] However low frequency FUS does not provide high resolution imaging and so MRI has been proposed to guide FUS application (MRI-FUS). Reports of enhancement of gene expression using a heat-sensitive promoter by MRI-FUS has been reported [59,60].

8. Imaging gene expression

8.1. Introduction

The success or failure of gene therapy protocols is determined by whether the therapeutic gene is being expressed and performs its expected function. Conventional methods of measuring gene expression include: microarrays; proteomics; reverse-transcriptase-polymerase chain reaction; RNA interference, differential analysis and use of reporter genes in both immunohistochemistry and histochemical staining, and *in situ* hybridisation. These techniques involve measurement of the products of gene expression, either RNA or protein. Despite their success for determination of gene expression, the methods suffer significant limitations when used in biomedical applications. These methods are *in vitro*-based, either requiring death of the organism studied or invasive sampling, thereby limiting their use in time-course (and interventional) studies. For histological based methods of determining gene expression, chemical fixation of removed tissues is usually required. Observation of biological samples under such non-physiological conditions means generally the dynamics of cellular processes cannot be resolved. Also most importantly, it is very difficult to generate quantitative three-dimensional datasets using conventional microscopy. Although the use of genetically encoded fluorescent tags has revolutionized the application of microscopy in biology, some of the drawbacks remain. These include the problem of obtaining samples for analysis and the inability to survey many or all tissues simultaneously within an organism, replete with its myriad of assorted cell–tissue interactions. Performing studies in a whole body context, i.e., *in vivo* assessment, allows phenomena such as tolerance, complementation and redundancies in biological pathways to be studied [61] with respect to gene therapy. Hence, the necessity of non-invasive imaging modalities such as those based on MR to determine spatiotemporal expression of genes *in vivo* and providing information essential for developing gene therapy strategies.

8.2. Choice of imaging modality

When considering imaging modalities for assessing gene expression *in vivo*, they can be broadly based on MR techniques (mainly MRI), radionuclide (e.g., positron emission tomography, PET) or optical (e.g., bioluminescence) methods (Table 2). A major advantage of MRI is its potential for high resolution; resolution as high as 100 μm in animals is routinely obtained as compared to mm resolution for radionuclide and optical based methods. Furthermore, MRI simultaneously provides anatomical/physiological information. However, MR-based methods may be limited by their relative insensitivity compared to radionuclide and optical based methods. Relatively greater amounts of imaging probe must be present for detection and so, greater amounts of probe must be injected. Hence the drive towards co-registration of datasets, especially of MRI and PET, or MRI and bioluminescence, combining the high spatial resolution and anatomical information produced by MRI with the greater sensitivity of PET and bioluminescence. Other advantages of MRI include its ready translation to the clinic without the use of ionising radiation, whilst the former advantage is also true for PET, the use of ionising radiation for PET may be a concern, especially in time-course studies. As with MR-based methods, optical based methods do not require use of ionising radiation. Although easily accessible, cheap and directly translatable from optical microscopy methods, optical imaging is limited to use in biological research only as structures can be imaged only if less than a few mm deep, although sampling up to 10–15 cm deep is possible with the use of near-infrared wavelengths [63]. The relative greater sensitivity of radionuclide and optical imaging methods compared to those based on MR renders the

Table 2
Comparison of commonly employed non-invasive imaging modalities for the assessment of gene expression (modified from [62])

Imaging modality	Spatial resolution	Depth	Temporal resolution	Sensitivity	Examples of imaging probes (amount of probe used)	Quantitative
Magnetic resonance based methods (non-ionizing radiation)						
MRI	25–100 μm	No limit	Minutes–hours	10^{-3}–10^{-5} M	Gadolinium chelates, iron oxide nanoparticles (μg–mg)	++
Radionuclide based methods (ionizing radiation)						
PET	1–2 mm	No limit	Seconds–minutes	10^{-11}–10^{-12} M	^{18}F, ^{11}C, ^{124}I isotopes (ng)	+++
SPECT	1–2 mm	No limit	Minutes	10^{-10}–10^{-12} M	^{99m}Tc, ^{111}In isotopes (ng)	++
Optical based methods (non-ionizing radiation)						
BIL	3–5 mm[a]	1–3 cm	Seconds–minutes	10^{-15}–10^{-17} M[b]	Luciferin (μg–mg)	+–++
Fluorescence	2–3 mm	<1 cm	Seconds–minutes	10^{-9}–10^{-12} M[b]	Green fluorescence protein (μg–mg)	+–++

Abbreviations: PET, positron emission tomography; SPECT, single photon emission computerized tomography; BIL, bioluminescence.
[a] Resolution is ⩽depth.
[b] Not well characterized.

non-MR-based techniques more rapid to perform in a pre-clinical setting. Thus, the imaging modalities are complementary to each other and the choice of imaging modality is dependent on the questions asked and the specific issues being addressed regarding the models studied.

8.3. Basic strategy

All of the proposed methods for the assessment of gene expression by MR are based on detection of the protein product rather than the mRNA product. This is principally because more copies of proteins are produced than mRNA per cell, ~100–1,000,000 proteins are produced as compared to only 50–1000 copies of mRNA.

Basic strategies for imaging therapeutic gene expression by MR-based methods can be categorised into direct and indirect imaging methods (Fig. 6). Direct imaging methods involve detection of the therapeutic protein itself or after interaction with a MR probe. Usually, the therapeutic protein does not have endogenous contrast in the MRI image nor is it detectable by MRS, and so needs to interact with a specific MR probe to allow association of the probe with the cell expressing the protein and hence, MR detection. The protein can be either on the cell surface or a receptor, and interaction with a MR probe leads to its retention on the cell surface or internalisation into the cell (Fig. 7). Alternatively, the therapeutic protein can be inside the cell or more specifically an enzyme (Fig. 7). For the former, the protein can interact with an imaging probe and lead to

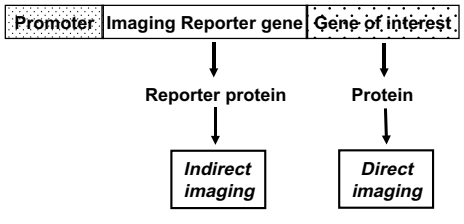

Fig. 6. Direct and indirect strategies for imaging therapeutic gene expression.

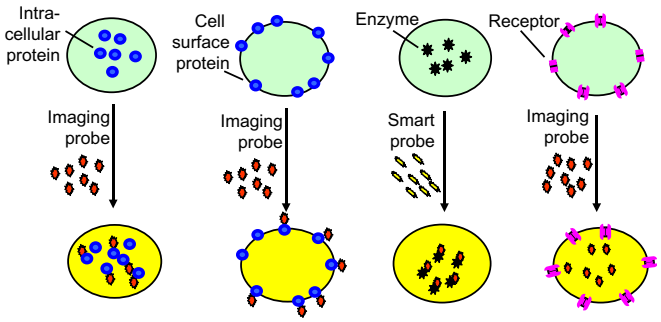

Fig. 7. Methodologies for imaging gene expression by MR-based methods.

retention of the probe within the cell and thus allowing the cell to be imaged. For the latter, a special imaging probe is used, more often referred to as a smart probe. It is termed a smart probe as it cannot be visualised until metabolised in a reaction catalyzed by the enzyme to provide a product that can be detected by MR methods.

More commonly, the therapeutic gene cannot be visualised and an indirect approach to imaging therapeutic gene expression is required, through the use of reporter gene technologies (Fig. 6). Reporter genes are routinely used by molecular biologists to study endogenous gene expression by fusing a reporter gene to the gene of interest and its promoter. As the therapeutic gene is being expressed, the reporter gene is also expressed. Conventionally, the reporter protein is monitored by immunohistochemistry or histochemical staining for the protein; *in situ* hybridisation with probes targeted for the reporter gene mRNA; or blood sampling in which the reporter protein is a protein secreted in the bloodstream. For imaging gene expression, the reporter protein is imaged as it 'reports' on the expression of the therapeutic gene. A fixed relationship between the expressions of the gene of interest and the imaging reporter gene (IRG) should exist and this is possible by the use of an internal ribosomal entry site (IRES). In this case, transcription of both genes (driven by the same promoter, i.e., genetic control element) leads to the production of a single mRNA molecule.

The IRG used for reporting on therapeutic gene expression by MR methods needs to encode a protein that is MR detectable. This is comparable to the above situation regarding imaging of the therapeutic protein (Fig. 7). The protein rarely has endogenous contrast but can interact with imaging probes to be visualised (e.g., ferritin). As with direct imaging of the therapeutic protein, the reporter protein can be a protein on the cell surface, a cell surface receptor, or an enzyme (Fig. 7), and visualization is comparable to that for direct imaging of the therapeutic protein, involving the use of exogenous administration of a MR probe. Most importantly, the reporter gene must encode for a protein not normally expressed in cells or tissues to be studied, or be significantly expressed so as to be detectable above background.

The sensitivity of MR-based methods to assess gene expression is dependent on sufficient expression of the IRG or the therapeutic gene. The MR probes used needs to be specific and have high affinity to bind to the reporter or therapeutic protein. Obviously, the probe needs to interact specifically with the protein to be detected to prevent generation of artefacts. MR probes consisting of antibodies or antibody fragments (such as $F(ab)_2$, minibodies) conjugated to MR contrast agents are often employed so as to exploit the specificity of the antigen–antibody interaction. High affinity is also required of the ideal MR probe to provide a robust interaction between probe and protein. Other factors regarding the choice of MR probe include its distribution inside the body as it is essential that the MR probe can access and interact with the reporter protein. Thus, the pharmacokinetics and ability of the MR probe to penetrate barriers, such as extravasation from blood capillaries and crossing cell membranes (including the plasma membrane), needs to be considered for use in assessing gene expression by MR methods.

MRI- and MRS-based systems to assess gene expression currently available are explained in detail below.

8.4. MRI-based systems

Quite simply, direct imaging of therapeutic gene expression is possible by the use of MR contrast agents conjugated to antibodies to the therapeutic gene product to be imaged (Fig. 7). A prerequisite for this methodology is that the protein needs to be on the cell surface to allow recognition of and binding to the specific antibody. Although this strategy has not been used to assess gene expression in gene therapy studies, it has been used to image a range of endogenously expressed proteins for diagnostic and cell labelling applications. By conjugating MION to $F(ab)_2$ fragments of a highly specific and high affinity anti-human E-selectin antibody, E-selectin expression was imaged both *in vitro* [64] and *in vivo* [65]. Endothelial expression of integrin $\alpha_v\beta_3$, a marker of angiogenesis, was imaged by gadolinium labelled liposomes, targeted using biotinylated antibodies to integrin $\alpha_v\beta_3$ conjugated to the liposomal surface via avidin linker proteins [66]. Tumours can be imaged by using MR contrast agents conjugated to antibodies reactive to tumour specific antigens [67–75]. A similar method was used by Artemov et al., to image the expression of HER-2/*neu* receptors *in vivo*, employing a two step approach involving initial labelling of HER-2/neu expressing cancer cells with a biotinylated antibody to the receptor, and then administration of avidin conjugated to Gd-DTPA [76] or MION [77].

Cells have been labelled *ex vivo* by the use of magnetic antibodies to epitopes expressed on the cell surface for cell tracking *in vivo* by MRI [78,79]. The probe MION-46L-OX-26 was specifically internalised by transferrin receptors expressed on the surface of rat oligodendrocyte progenitor cells: MION-46L-OX-26 is an antibody to the transferrin receptor (TfR) that is conjugated to MION and is internalised following interaction with the TfR [78]. The CD11c epitope expressed on dendritic cells was employed to label such cells by the use of magnetic antibodies to CD11c [79].

As mentioned earlier, indirect imaging, or reporter based methodology is required for imaging gene expression. There are a number of reporter based systems that have been proposed to report on gene expression by MRI. Such systems are commonly based on the same principles as the use of reporter based systems in conventional methods of assessing gene expression.

A number of reporter systems proposed for imaging gene expression exploits cellular mechanisms of regulating and storing iron [80] as iron is capable of enhancing T_2 relaxation and so, cellular accumulation of iron is readily detectable by MRI. Iron is ferried in the blood circulation by association with transferrin (Tf), becoming halo-Tf. Halo-Tf carries two iron ions, and on binding to the TfR, the complex is internalised into an endosome. A feedback mechanism operates such that when sufficient amounts of iron are present in the cell, TfR expression is downregulated. Iron is released from endosomes and stored by ferritin which is non-toxic and provides a ready source of iron for the cell. Ferritin, a metalloprotein, is composed of 24 subunits of two types, the heavy subunit and the light subunit: different amounts of each subunit type give rise to isoferritins. Ferritin consists of an apoprotein shell surrounding a core of up to 4500 atoms of iron in the form of the mineral ferrihydrite although maximal relaxitivity can be observed with only 13–14 iron ions. Ferritin shortens both T_1 and T_2 relaxation times such that the amount of ferritin-bound iron in pathologies such as Alzheimer's disease can be measured by MRI [81].

8.4.1. Receptor-based reporter protein

Weissleder et al. first proposed the use of an engineered TfR (ETR) to report on transgene expression [82]. As with the ferritin-based methods, it is based on the increased transport of iron into the cell, leading to negative enhancement by T_2 weighted MRI. The method employs the use of a modified transferrin receptor, ETR, which is insensitive to changing levels of cellular iron. Thus, the negative feedback mechanism is non-operational and no down

regulation of TfR expression is observed despite increasing iron concentrations. Instead of depending on the usual iron carrier, halo-Tf, to transport iron, the method uses Tf-MION which increases the sensitivity of the technique as 2064 iron ions are internalised as compared too only two in the case of halo-Tf, for a single endocytotic event, thereby giving rise to the possibility of single cell detection. Recently, expression of ETR has been shown to correlate with therapeutic gene expression when both genes are in the same vector [83].

8.4.2. Direct imaging of reporter protein

Recently, two methods exploiting ferritin (without administration of a MR probe), to assess gene expression by MRI have been proposed [84,85]. In these approaches, the over-expression of ferritin by cells generate negative MRI contrast as a result of (i) increased ferritin storage of iron and (ii) increased entry of iron into the cells due to upregulation of TfR resulting from the lowered labile iron pool arising from increased iron sequestration by ferritin. The advantage of such methods is that no administration of an exogenous MR probe is required, i.e., they result in the generation of endogenous contrast.

Genove et al. [84] genetically modified adenovirae to carry either the genes for the light- or heavy-chain subunits of human ferritin. The administration of both types of genetically modified adenovirae to cells (in a 1:1 ratio) lead to negative enhancement in the MRI image of ferritin expressing cells, although iron supplementation was required as insufficient amounts of iron was present in the culturing media. Injection of such genetically modified adenovirae into the striatum of a mouse brain lead to the observation of negative enhancement in that area arising from ferritin gene transfer to cells in the area. Negative enhancement was observed for as long as 5 weeks post-inoculation.

Cohen et al. [85] also proposed a ferritin-based reporter system involving over-expression of the heavy subunit of murine ferritin. Their IRG was a fusion of the genes for the heavy subunit of murine ferritin, engineered green fluorescent protein and haemagglutinin, enabling correlation of MRI, optical and histochemical data, respectively. The expression of the fusion gene is under the control of tetracycline, this standard tetracycline-off gene control system allows gene expression to be controlled by the use of tetracyclines such that in their presence the gene is switched off [86]. Over-expression of ferritin lead to increased iron (and ferritin) content *in vitro* and negative enhancement in the MR image. Negative enhancement was also observed in the tumour formed from implantation of such cells subcutaneously in the flank of a mouse.

Both the ferritin-based reporter technologies mentioned above leads to an overall increase in cellular iron concentrations arising from increased storage by ferritin. The sequestration of iron by ferritin alleviates the possible toxicological (i.e., iron-catalyzed hydroxyl radical production via the Fenton reaction) consequences of increased levels of iron concentrations in the cell.

8.4.3. Enzyme based reporter protein

A commonly used reporter gene in assessing gene expression by conventional methods is lacZ, whose protein product is β-galactosidase. Gene expression can be assessed by assaying for enzyme activity by the use of chromogenic substrates such as 5-bromo-4-indolyl-β-D-galactopyranoside (X-gal) which forms an intense blue precipitate when hydrolyzed by β-galactosidase. These reporter genes can also be used to assess gene expression in tissue specimens by the use of immunohistochemistry and histochemical staining. Such methodology has been adapted to allow MRI of gene expression by the use of a MR probe instead of a chromogenic substance. The MR probe is often termed a 'smart' contrast agent as it is not MR detectable until metabolised by β-galactosidase to form a product that is detectable. This was proposed by Louie et al. [87] using a new smart contrast agent called EgadME, consisting of a chelated gadolinium caged by a galactopyranose residue. The chelator binds with high affinity to gadolinium, occupying eight of the nine coordination sites of gadolinium. A galactopyranose residue is positioned so as to block the access of a water molecule to the remaining coordination site on gadolinium. This water-inaccessible conformation is not MR active until the galactopyranose residue is cleaved by β-galactosidase, allowing water access to gadolinium and hence, increased signal intensity by T_1-weighted MRI. The use of EgadME to image gene expression *in vivo* was demonstrated in the *Xenopus laevis* embryo. At the two cell stage, both cells were injected with EgadME but only one cell received either the mRNA or the DNA coding for β-galactosidase. The embryo was allowed to grow before being imaged live by MRI and then chemically fixed and stained with X-gal for histochemical analysis. The lineage of the cells in the embryo is such that at the two cell stage, cells from one side are derived from one of the two cells, and cells from the other side, from the remaining cell. Thus, positive X-gal staining observed predominantly on one side of the embryo, correlated with high intensity regions of the MR image. Whilst imaging gene expression is possible with this methodology, the technology requires the cellular entry of the smart contrast agent. Louie et al. were able to gain cell entry of the contrast agent by microinjection into cells, which is unlikely to be possible for most applications. To facilitate the cell uptake of contrast agents, contrast agents may be modified by conjugation to membrane translocating peptides such as the tat peptide from human immunodeficiency virus [88–91].

Melanin, a biopolymer pigment, giving rise to colour in the skin, eyes and hair in humans and animals, has a high affinity for a variety of paramagnetic metal ions, including iron [92]. The scavenging properties of melanin to form metallomelanin explain the high signal intensity of melanotic melanomas by T_1-weighted MRI [93]. The enzyme, tyrosinase, catalyzes the rate-limiting-step in melanogenesis

and its gene has been proposed as an IRG [94]. Increased expression of tyrosinase leads to increased production of melanin [95] and thus, transfer of the tyrosinase gene into cells generates increased signal intensity in the T_1-weighted MR imaging [94]. Note that there is no requirement for exogenous administration of a MR probe for this reporting method.

Melanin, plays a major role in photo-protection but the process of melanogenesis produces toxic intermediates. In the body, this potential hazard is attenuated by the process being compartmentalized to special organelles (melanosomes) in melanocytes [95]. Thus, Alfke et al. [96] modified the methodology by using the tetracycline-off gene expression system to regulate tyrosinase expression. The inducible control of tyrosinase expression by tetracyclines prevents the continued production of melanin and so, potential cellular damaging effects of ongoing melanogenesis. Furthermore, melanin is a stable molecule and will persist after production and thus the methodology needs to be further modified such that a less stable end product is produced, allowing increased temporal resolution imaging of gene expression.

8.4.4. Epitope based reporter protein

As mentioned above, gene expression of a therapeutic gene can be directly assessed by using MR probes consisting of the specific antibody conjugated to magnetic particles. However, we proposed recently a modification to this strategy to enable indirect imaging of the therapeutic gene by coupling the therapeutic gene to an IRG that codes for an epitope or tag on the cell surface [97]. We employed a truncated form of the H2k antigen as a tag. The native H2k antigen is present on cells of certain murine strain, e.g., CBA mice. This modified form does not activate secondary messenger systems in the cell following epitope and antibody binding and hence, has minimal effects on cell metabolism and function. Hela cells (human cervical cancer cell line), transiently transfected with a plasmid carrying the truncated H2k gene, were shown to give rise to negative enhancement by T_2-weighted MRI, consistent with the lower T_2 values of the transfected cells.

Similar to our use of the truncated H2k antigen, Tannous et al. [98] suggested the surface expression of biotin as a tag for reporting gene expression. The biotin tag is routinely employed in molecular biology and immunohistochemical techniques due to the versatility of the biotin–streptavidin or biotin–avidin system [99]: the interaction between biotin and streptavidin or avidin being almost as strong as a covalent bond.

8.4.5. Chemical exchange saturation transfer reporter proteins

Frequency selective contrast results from the transfer of nuclear spin saturation from exchangeable protons of the CEST reporter protein to water protons (see above). Biologically exchangeable protons include the amide protons of lysine, and researchers have synthesised a novel CEST reporter protein that is rich in lysine residues, lysine-rich protein (LRP, [100]). This artificial reporter protein can provide 'on' and 'off' contrast by application of a radiofrequency pulse at the amide proton frequency. The technology provides the opportunity of simultaneous use of multiple contrast agents by frequency selective imaging, comparable to optical imaging methods in which optical agents with different wavelength sensitivities can be used.

8.5. MRS-based methods of assessing gene expression

MRS methods of assessing gene expression are based on the IRG product being an enzyme which catalyzes the conversion of substrate to a product with an unique chemical shift value, i.e., different from that from the substrate and endogenous metabolites in the tissue. Kinases, arginine and creatine kinases (AK, CK), have been proposed as possible reporter proteins [101,102], catalyzing the phosphorylation of arginine and creatine, respectively.

AK is not present in mammalian tissues and infection of skeletal muscle in mice with recombinant adenovirus carrying this gene from *Drosophila* leads to the phosphorylation of arginine to phosphoarginine (PArg). The formation of PArg was monitored by *in vivo* ^{31}P MRS, showing AK expression persisted for at least 8 months after injection [101]. Similarly, CK can be used to report on gene expression by the monitoring of phosphocreatine (PCr) formation [102,103]. CK can be readily used to report on gene expression in tissues not expressing endogenous CK, e.g., in liver and kidney. However, by measuring the increment in CK activity using magnetization transfer methods [104], CK can be used to act as a reporter in tissues expressing CK endogenously, e.g., skeletal muscle.

Cytosine deaminase (CD) is a microbial enzyme, absent in mammalian cells, that catalyzes the conversion of 5-fluorocytosine (5-FC) to the toxic metabolite, 5-fluorouracil (5-FU). 5-FU is an antimetabolite which is incorporated into DNA and RNA, forming fluorinated nucleotides that lead to cell death by cell cycle arrest and apoptosis. 5-FU is used in cancer chemotherapy but dosage is limited by its systemic toxicity which may be overcome by the transduction of CD only in cancer tissues and the use of 5-FC rather than 5-FU [105]. No fluorinated endogenous metabolites were detected by ^{19}F-MRS *in vivo*. Thus, CD can be used as a reporter protein as well as a therapeutic protein since 5-FC, 5-FU and fluorinated nucleotides have different ^{19}F chemical shifts which may be monitored by ^{19}F MRS [106]. Stegman et al. [107] demonstrated the use of *in vivo* ^{19}F MRS to non-invasively monitor the metabolism of 5-FC and to quantify CD expression in animals bearing subcutaneous tumours expressing CD.

β-Galactosidase is also being developed as a reporter protein for MRS. This requires development of MR probes which are substrates for this enzyme, generating a product with characteristic chemical shift(s). A prototype MR β-galactosidase sensitive molecule, 4-fluoro-2-nitrophenyl-β-D-galactopyranoside (PFONPG), has been reported

recently [108]. Employing ^{19}F MRS, the relative gene expression of cells transfected with the lacZ gene was determined by monitoring formation of the product aglycone, PFONP, from the PFONPG substrate, catalyzed by β-galactosidase. However, use of PFONPG is limited due to the toxicity of PFONP but this does raise the possibility of PFONPG use in cancer chemotherapy as induced expression of β-galactosidase by tumour cells can lead to PFONP formation in these cells and so killing of cancer cells. Compared to EgadMe as a MR probe, PFONPG does have the advantage that it readily penetrates the cell membrane for catalysis by β-galactosidase and is also a better substrate for the enzyme. However, specific spatial information may be limited due to a lack of trapping of the product PFONP within cells.

Polyphosphate (polyP) is a linear polymer of orthophosphate residues linked by high energy phosphoanhydride bonds found in all organisms but at lower levels in higher eukaryotes, and has been proposed as a reporter molecule for assessing gene expression [109]. In yeast, levels of polyP are controlled by the genes *VTC1* and *VMA2*, encoding the vacuolar transporter chaperon (Vtc) [110] complex and vacuolar H^+-ATPase (V-ATPase [111]), respectively. Quantification using ^{31}P MRS showed correlation of polyP levels with expression of *VTC1* or *VMA2*. However, VTC1 orthologs have not been identified in mammals and VMA2 orthologs cause genetic disease in humans [112,113] so an alternative exogenous MRI reporter is needed for use in mammalian systems. Preliminary unpublished results [109] suggest that the *Escherichia coli PPK1* gene is a possible candidate. The PPK1 gene encodes a polyP kinase and converts the terminal phosphate of ATP to polyP [114].

9. Monitoring of therapeutic response

An important aspect in the development of any therapeutic strategy is the need to objectively and reproducibly assess its clinical usefulness. In the case of gene therapy, it is essential that once the therapeutic gene has been transferred to the target cells/tissue and it has been shown (*in vivo* or *in vitro*) to be expressing its product at the required level and intensity, it is of the essence that its physiological and clinical effectiveness be routinely monitored. Clearly this cannot be readily carry our by invasive techniques such as tissue biopsies, hence the need for non-invasive techniques such as MRI and PET. One of the main advantages of MRI, over other imaging methods, is its ability to not only detect gene transfer and expression *in vivo*, but also to produce high resolution anatomical and functional images. MR imaging and spectroscopy are therefore unique approaches that allow researchers and clinicians to directly assess morphological, functional and metabolic changes directly and longitudinally throughout the lifetime of the patient, if necessary. In the field of cancer therapy, MRI and MRS can be used to determine temporal changes in tumour size, cellular dynamic, tissue perfusion, bioenergetics and metabolism associated with gene therapy [115]. Similarly, in gene therapy for cardiovascular diseases, MRI can be used to determine tissue perfusion and elasticity in order to assess the overall and regional recovery of tissue function. In neurological diseases and skeletal muscle disorders, a combination of tissue perfusion, morphological measurements, MR angiography, vascular remodelling and metabolic measurements would be invaluable tools for the long term assessment of the efficacy of gene therapy [116]. Indeed, this may be particularly important for rescuing dystrophic muscle in children with muscular dystrophy.

10. Summary and future perspectives

As well as revealing gene activity *in vivo*, MRI is likely to play a major role in the development and implementation of gene therapy, especially as this begins its slow, yet firm, translation from the pre-clinical setting, to its application to many forms of disease in humans. It is expected that in the next decade or so, the use of gene therapy, at least in monogenic disorders will become a reality, which in turn will make further demands on faster and more accurate *in vivo* methodologies for determining the long term consequences and benefits of such therapies. Also, imaging techniques such as MRI and PET may potentially greatly benefit from the gene targeting modalities being developed in gene therapy, making it a reality to be able to combine therapy with targeted contrast agents thus enhancing the specificity and capability of both MRI and MRS methodologies.

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Glossary

Antigen: A substance (usually a protein) which can trigger the release of antibodies as part of the body's immune response
DNA: Deoxyribonucleic acid, molecule that encodes genetic information
Episome: A genetic particle of certain cells that can exist either autonomously in the cytoplasm or as part of a chromosome
Epitope: The surface portion of an antigen capable of eliciting an immune response and of combining with a antibody
Gene: DNA sequence that is the basic physical and functional unit of heredity
Gene expression: Process by which the encoded genetic information is converted into RNA or protein
Gene product: RNA or protein resulting from gene expression
Genome: The complete set of genetic information of an organism
Immunohistochemistry: Antigen is identified by allowing reaction with its specific antibody and antibody is linked to a marker that can be detected
IRG: Imaging reporter gene
Molecular imaging: ***In vivo*** *characterization and measurement of biological processes at the cellular and molecular level*
Monogenic: Single gene involvement
Othologs: Genes in different species which evolved from a common ancestral gene
PET: Positron emission tomography
Pharmacogenetics: Study of the genetic response to drugs
Polygenic: Two or more genes involved
Promoter: DNA sequence to which RNA polymerase binds to initiate transcription
Protein: Polymer of amino acids in a specific sequence, and which performs cellular functions as well as a major constituent of cells
Reporter gene: Gene that encodes for a detectable protein
rRNA: Ribosomal RNA, part of the machinery involved translation
RNA: Ribonucleic acid; transcribed from DNA and may be translated for protein synthesis
Sequence: Order of nucleotides in DNA or RNA, or amino acids in proteins
Transduction: DNA transfer from one bacterium to another
Transfection: Transfer of exogenous DNA
Transgene: Foreign gene transferred into cells or tissues
Transcription: Production of RNA from DNA template
Translation: Production of proteins from mRNA template
tRNA: Transfer RNA; transfers individual amino acids to the growing protein chain