

## REVIEW

# Molecular imaging and cancer gene therapy

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Gene therapy is known as one of the most advanced approaches for therapeutic prospects ranging from tackling genetic diseases to combating cancer. In this approach, different viral and nonviral vector systems such as retrovirus, lentivirus, plasmid and transposon have been designed and employed. These vector systems are designed to target different therapeutic genes in various tissues and cells such as tumor cells. Therefore, detection of the vectors containing therapeutic genes and monitoring of response to the treatment are the main issues that are commonly faced by researchers. Imaging techniques have been critical in guiding physicians in the more accurate and precise diagnosis and monitoring of cancer patients in different phases of malignancies. Imaging techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are non-invasive and powerful tools for monitoring of the distribution of transgene expression over time and assessing patients who have received therapeutic genes. Here, we discuss most recent advances in cancer gene therapy and molecular approaches as well as imaging techniques that are utilized to detect cancer gene therapeutics and to monitor the patients' response to these therapies worldwide, particularly in Iranian Academic Medical Centers and Hospitals.

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## INTRODUCTION

Cancer is a huge health concern worldwide, and therefore several molecular therapeutic approaches have been recently developed,<sup>1–6</sup> yet with many difficulties and progressing issues.<sup>7</sup> Many new therapeutic attempts to combat cancer have been made so far, many of which focus on gene therapeutic strategies.<sup>5,8,9</sup> It has been recently shown that cellular gene therapies may be considered as a new and promising platform for cancer therapy. However, gene and cell therapies have been associated with some limitations.<sup>10,11</sup> One of these limitations is the difficulty in tracking therapeutic genes and cells in patients' body as well as monitoring of patients' response to the treatment. In this regard, molecular imaging could be a useful tool for tracking and monitoring of cell and gene therapies for cancer and following up of the treatment outcome.<sup>10–12</sup> In a more general way, these techniques enable us to monitor biological processes at cellular and subcellular levels in living organisms and might lead to various applications in other human diseases besides cancer.<sup>12</sup> The role of imaging in cancer therapy, particularly in cancer gene therapy, has also raised some controversies, much of which are coming from technical concerns. Imaging may bridge the gap between cell and/or gene therapies and health outcomes by elucidating the mechanisms of action through longitudinal monitoring.<sup>10,11</sup>

Imaging-guided delivery of gene-targeting therapeutics represents a robust tool in the fight against cancer through gene

transfer or RNA interference.<sup>1</sup> An imaging-driven approach should allow, for example, the translating RNA interference cancer therapy into the clinical practice, as the technique could address many issues related to administration route, systemic blood circulation and cellular or tissue barriers, besides safety and ethical concerns.<sup>1</sup> Although cancer in Iran and Arabic countries still has very low survival rate,<sup>2,13</sup> gene therapy may represent an innovative and promising therapeutic strategy for cancer treatment, though technical concerns regarding its feasibility remain to be addressed.<sup>14</sup>

## IMAGING IN GENE TARGETING AND THERAPY: WHEAT AND CHAFFS

Cancer is one of the main public health problems worldwide.<sup>12</sup> Various studies assessed different dimensions of this disease. To date, through deepening understanding of molecular/cellular pathways involved in different cancers, researchers have designed many therapies and therapeutic regimens for cancer, although cancer yet remains as a main problem in numerous communities.<sup>10–12</sup> Among various current anticancer therapies, gene therapy has emerged as a new platform for the cancer treatment.<sup>10–12</sup> Gene therapy enables researchers to introduce a new therapeutic gene in receipt cells by specific vector systems such as plasmid, transposon and lentivirus. One of the major challenges in these systems is how to detect these vectors in

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**Table 1.** Some advantages and disadvantages in various imaging techniques

<i>Imaging technique</i>	<i>Advantage (s)</i>	<i>Disadvantage (s)</i>
PET	High sensitivity High penetration depth	High costs
SPECT	Different radioisotopes High penetration depth Low costs Labeling simultaneously	Influence on biomolecule Limitation in spatial resolution
MRI	High spatial resolution Extracting physiological, molecular and anatomical information	Time consuming High costs
Computed tomography	High spatial resolution	Limitation in biocompatible contrasts Radiation exposure Limited imaging in soft tissue
Ultrasound	Clinical friendly High spatial and temporal resolution Low costs	Limitation in bone and lung
Optical	High temporal resolution Non-invasive functional imaging High sensitivity Low costs	Low penetration of tissue Non-quantification

Abbreviations: MRI, magnetic resonance imaging; PET, positron emission tomography; SPECT, single-photon emission computed tomography.

**Table 2.** Main imaging techniques and their application

<i>Imaging technique</i>	<i>Main type of cancer</i>	<i>Markers</i>	<i>References</i>
<i>X-ray-based approaches</i>			
Flat panel CR	Bone		43
Digital radiography, TAC	Lung		44
Multislice CT system	Bone		45
<i>Magnetic resonance imaging (MRI)</i>			
Diffusion waived imaging	Breast cancer		46
MR elastography	Neuroendocrine and hepatic metastasis		47
MR perfusion imaging	Breast	A	48
MR spectroscopy	Breast	B	49
MR-guided focused ultrasound	HCC		50
MR-guided galvanotherapy	Prostate		51
<i>Sonography-ultrasound</i>			
Miniaturization of ultrasound systems	Prostate		53–55
Acoustic radiation force impulse imaging	Hepatic malignancies		
High-intensity-focused ultrasound	Prostate		
<i>Non-ionizing electromagnetic imaging</i>			
Photo- and thermoacoustic imaging	Breast		56–58
Electrical impedance tomography			
Near-infrared optical tomography			
<i>Nuclear medicine imaging</i>			
PET	Colon	C	59,60
SPECT		D	

Abbreviations: CR, computed radiography; CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography; SPECT, single-photon emission computed tomography. Markers: (A) cell death, necrosis, tissue damage and modification; (B) angiogenesis; (C) metabolism; (D) receptor recycling and expression.

preclinical and clinical studies. Imaging is home to a wide array of clinical diagnostic and therapeutic technologies.<sup>5,8,9</sup> In regenerative medicine, imaging not only is greatly useful in unveiling damaged tissues but also in monitoring of safety and efficacy of a defined targeted therapy (Table 1).<sup>15</sup> Table 2 lists the main imaging techniques used in cancer diagnosis and therapy.

Positron emission tomography (PET) with I-124-labeled 2'-fluoro-2'-deoxy-1b-D-arabino-furanosyl-5-iodo-uracil (<sup>124</sup>I-FIAU)—a specific marker substrate for gene expression of HSV-1-tk—has been

recently used to detect the location, magnitude and extent of vector-mediated HSV-1-tk gene expression in a phase I/II clinical trial of gene therapy for recurrent glioblastoma.<sup>16</sup> This approach has yet raised some critical issues in identification of the exogenous gene expression introduced into the patients with glioma.<sup>17</sup> The [<sup>124</sup>I]-FIAU substrate does not succeed in penetrating intact blood-brain barrier (BBB); however, it appears that this substrate can penetrate in disrupted areas of BBB (for example, glioblastomas). Accordingly, to detect localization of HSV-1-tk gene expression in

**Table 3.** Cancer therapeutic approaches in Iranian Medical schools in recent years

Experimental system	Background	Objective	References
<i>Cell therapy</i>			
CSCs targeting	Target and/or modifying CSC markers	Gene target immunotherapy	61,62
MSCs	Stem cell research in cancer treatment	Cell EMT/MET transition	
<i>Receptor inhibitors</i>			
Tyrosine kinase inhibitors: c-kit inhibitors	Targeting c-kit as a novel gene therapy	Gene–protein molecular targeting	62
<i>Nanotechnology</i>			
Polymeric nanoparticles	Nanocarriers used for gene therapy	PLGA, PEIs	63
Graphene oxide nanocarriers for siRNAs	Polymeric nanoparticles, lipid-based carriers (liposomes/micelles), dendrimers, carbon nanotubes and gold nanoparticles (nanoshells/nanocages)	PL-PEG and CPPs	64
D3-loaded nanoparticles			65
<i>Gene therapy</i>			
siRNA/miRNA-interfering RNAs	siRNAs in ovarian cancer	Interfering RNA	5 66
Epigenetics	Epigenetics in multiple sclerosis	Perspective on the use of epigenetics	67
Transcription factors	BTB and CNC homology 1, basic leucine zipper transcription factor 1	BACH1 targeting as a new tool	68
Long non-coding RNAs	LncRNAs as targets	Targeting LncRNAs	69
LNA-modified oligonucleotides	LNA-anti-miR-21 (50 pmol $\mu\text{l}^{-1}$ )	Used in colorectal cancer	43
<i>In silico</i> analysis	G-quadruplexes in WT1 oncogene	Drug design	43

Abbreviations: CPP, cell-penetrating peptide; CSC, cancer stem cell; EMT, epithelial–mesenchymal transition; LNA, locked nucleic acid; LncRNAs, long non-coding RNAs; MET, mesenchymal-epithelial transition; MSC, mesenchymal stem cell; PEI, polyethyleneimine; PLGA, poly lactic-co-glycolic acid; PL-PEG, phospholipid-based amphiphilic polymer; siRNA, short interfering RNA.

the central nervous system by FIAU PET, washout of nonspecific radioactivity for several days was highly recommended.<sup>18</sup> PET technique, as either micro-PET or micro-computed tomography, is also currently included in gene therapy protocol for small animal models.<sup>19,20</sup> By emerging more sensitive and high-capacity nuclear-imaging methods, Zinn *et al.*<sup>21</sup> demonstrated that utilizing single-photon emission computed tomography (SPECT) methods such as gamma camera imaging provided a high-capacity method to image gene transfer to cancer cells. They showed that gamma camera imaging can detect the expression of the hSSTR2 reporter protein in human cancer cells (like A-427 non-small cell lung, SKOV3.ip1 ovarian, MDA-MB-468 breast and BxPC-3 pancreatic) by imaging an internalized [sup 99 m]Tc-labeled, hSSTR2-binding peptide. The approach was later developed as radiolabeled imaging, for example, by inducing tumor cells to express the human somatostatin receptor subtype 2 (hSSTR2)—a high-affinity receptor for radiolabeled somatostatin analogs.<sup>22</sup> Although through improvement of imaging technology, PET and SPECT methods have notably improved the ability to detect and to follow up gene transfer in tumor cells, some barriers including high cost, time-consuming nature, technique difficulty, reliance on radioactive reagents, immature reimbursement structure and the need for continuous training negatively influenced on their wide adoption in clinical practice.<sup>23,24</sup> Magnetic resonance imaging (MRI) represents a more available alternative.<sup>25,26</sup> In gene silencing using short interfering RNA (siRNA), the complexation of siRNA with polyethyleneimine prevents rapid degradation of siRNA in tumors and, more interestingly, enhances gene transfection efficiency.<sup>26,27</sup> Interestingly, PEGylated polyethyleneimine modified with gadolinium chelator (DOTA), which has been used as a template for synthesis of polyethyleneimine/gadolinium-related nanoparticles, is a well-known nanocarrier for gene silencing and has potential application in the MRI ambient.<sup>28</sup> The use of MRI in gene silencing and gene transfer has been adopted in recent years;<sup>29–31</sup> however,

its application has been expanded into not only oncology but also several other fields. MRI is a much more widely used and accepted method than PET, SPECT and CT. Combined with new techniques such as cine-MRI and functional methods such as perfusion- and diffusion-weighted imaging, MRI may also be considered as an alternative tool to conventional radiological-based approaches such as radiography, computed tomography and PET/CT imaging in the evaluation of cancer patients.<sup>32</sup> Some molecules, such as creatine kinase reporter gene, act as sensitive bio-magnetic detectors in MRI for gene transfer, providing advantages for utilizing MRI in gene transfer.<sup>33</sup> CK is particularly abundant in the human muscle and the brain, but not in the liver; therefore, combined application of CK reporter gene and the phosphorus-31 magnetic resonance spectroscopy is particularly useful to detect and monitor any liver-directed gene transfer.<sup>33</sup> Emerging of new imaging approaches, such as quantitative three-dimensional oxygen imaging using electron paramagnetic resonance imaging and hyperpolarized <sup>13</sup>C metabolic MRI, might be additionally useful in monitoring and prediction of tumor response to anticancer gene therapy through tracing reporter gene-derived new metabolic reactions.<sup>34</sup> The evolution of new MRI probes may extend the application of this imaging technique to cancer gene therapy; for example, in prostate cancer, introduction of hyperpolarized <sup>13</sup>C-organic acids, such as lactic acid, pyruvic acid or even alanine and most recently hyperpolarized [<sup>13</sup>C]pyruvate, has enhanced sensitivity of conventional MRI.<sup>35,36</sup>

In a more general way, imaging in gene therapy has been widely adopted both in MRI with ancillary approaches to improve the MRI resolution and more recently in positron-emitting techniques. Further methods are currently being developed to monitor cancer gene therapy outcome. For example, in a spontaneous tumor-bearing animal model, intratumoral interleukin-12 electrogene therapy was monitored via contrast-enhanced ultrasound.<sup>37</sup> In another study, Aalinkeel *et al.*<sup>38</sup> utilized MRI and ultrasonography to monitor tumor perfusion following

cationic polylactide (CPLA) interleukin-8 siRNA nanocomplexes.<sup>38</sup> Various studies have reported that, imaging techniques such as PET scan, CT or ultrasound (for example, ultrasound-guided endoscopy), combined by other technologies, can be used for monitoring, management and differential diagnosis of tumors, particularly prostate cancer.<sup>39</sup>

The identification of various markers in response to different therapies could contribute to better treatment of cancer.<sup>40,70–74</sup> Many of these molecules and agents could be used as markers for assessment of response to treatment in various cancers. For example, in tumor cells, there is an increase in glycolysis rate, DNA synthesis and angiogenesis—the processes that could be utilized for monitoring of cancer patients. Various studies revealed that there are two classes of imaging techniques associated with gene therapy including biodistribution and transduction imaging. Transduction imaging methods could detect transgene-mediated protein production.<sup>10</sup> On the other hand, biodistribution-imaging methods could track gene delivery vectors.<sup>41</sup> The individual using the above-mentioned imaging techniques is associated with some limitations. For example, transduction pattern may show an inaccurate image of viral biodistribution so that a virus could enter into several cells, but its transgene of interest is not expressed in all transduced cells. Hence, one of the main issues that should be assessed is that of transgene expression and viral particle kinetics *in vivo*.<sup>42</sup> Imaging technologies use different forms of energy when interacting with different tissues. It has been shown that some techniques, including MRI and CT, are relied on energy–tissue interactions. On the other hand, other techniques such as SPECT and PET are relied on injection of reporter probes, as previously indicated. Imaging techniques are attempted to save time and costs through minimizing the utility of laboratory animals and time-consuming invasive techniques.<sup>10</sup> Table 3 summarizes some of the most recent advances in gene therapy and molecular approaches that are utilized to fight cancer in Iranian Academic Medical Centers and Hospitals. In order to drive chemopreventive nanocarriers or nanostructured siRNA or microRNA (miRNA) vehicles, imaging techniques should be performed.<sup>1</sup>

## CONCLUSION

What is the best and currently available imaging approach to monitor safety, efficacy and success of a gene therapy protocol? This still represents a major concern in many countries where advanced imaging approaches and equipment are currently introduced into preclinical and clinical practices. Important considerations in choosing the most appropriate technique include gene therapy protocol, tumor type and location, safety, availability, and more precise and accurate data acquisition. Technological progress in imaging techniques will help us not only to develop safe and efficient gene therapy protocols for clinical application but also enable us to monitor therapeutic effects in cancer patients who have received therapeutic genes.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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