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Molecular Imaging: A blessing in disguise for tracking promising cell based therapeutics

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Review article

Molecular Imaging: A blessing in disguise for tracking promising cell based therapeutics

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Abstract

Imaging technologies allow assessing of complex structures and underlying dynamic biological processes that take place deep within the human body. A number of imaging modalities that exist today exploit the very depth of the energy spectrum. Light microscopy techniques ranging from confocal to multi-photon to super-resolution fluorescence microscopy, electron microscopy, fluorescence tomography, mass spectrometry imaging, and bioluminescence imaging are widely employed in today's medical research. Clinical imaging techniques such as X-ray computed tomography (CT), Positron Emission Tomography (PET), magnetic resonance imaging (MRI), ultrasound, and light-based methods [endoscopy and optical coherence tomography (OCT)] are frequently used as a part of clinical diagnostics. The integration of clinical and research modalities is vital to obtain critical information pertaining to therapeutic success of promising therapeutics, particularly in the emerging field of cell based therapies for various medical conditions.

Keywords: Imaging, Optical imaging, Stem cell therapies

Introduction

Recent developments in imaging technologies to detect molecular and biological processes have helped identify numerous human disorders and have also paved path to novel therapeutics. The advent of non-invasive super resolution imaging technologies *in vivo* is essential for understanding diseases and the development of new therapeutics. The major goal for this technology is to provide monitoring of various biological processes *in vivo* at the cellular and molecular level that include tumor regression or progression, gene expression; various drug and gene therapies.[1-3] Noninvasive molecular imaging technologies provide an excellent way to visualizing living cells *in vivo* and is vital to visualize the fate of cells *in vivo*.[2]

Stem cells have emerged as promising cellular delivery vehicles. Due to their inherent migratory properties, stem cells can be used for the delivery of therapeutic proteins in a variety of human cancers. Stem cells have remarkable potential in developmental biology, drug discovery, and regenerative medicine; imaging techniques are often employed to evaluate the purity, state of differentiation, number, and location of these cells. There are several sources from which stem cells can be obtained. Primarily, mesenchymal stem cells (MSCs), have been obtained from bone marrow of healthy adult donors or other tissue sources. Neural stem cells; induced pluripotent stem cells (iPSC) that are reprogrammed from adult fibroblasts by employing several transcription factors.[4,5] Imaging stem cells provides important data regarding the

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behavior and function of these cells including their location, protein expression levels, viability, percent viability, and differentiation status, as well as interactions between the cells and the adjacent tissue. Molecular imaging is a rapidly expanding field in biomedical research that allows us to quantify biological processes at sub-cellular levels within living organisms. Molecular imaging offers a novel and noninvasive approach to obtain a wide range of information using several probes targeted towards unique biomarkers expressed on cellular surfaces. Several developments have emerged in imaging methods that can be utilized to visualize various in vivo models of disease at a very high resolution. These techniques include magnetic resonance (MR) imaging, several radionuclide imaging techniques such as single photon emission tomography (SPECT), and positron emission tomography (PET). Alternatively, optical techniques for imaging that employ physical parameters of light interaction with tissues have also been demonstrated extensively. Optical imaging techniques rely on reflectance, absorption, fluorescence or bioluminescence as a source of contrast.[5] More recent optical imaging techniques, which are becoming more popular include near fluorescence-mediated tomography, intra-vital microscopy and infrared fluorescence (NIRF) imaging. Numerous such technologies have been recently developed to track the fate of cell-based therapies in experimental models and this have greater implications in developing novel therapeutic strategies for various conditions.[5]

Imaging technologies to track fate of cell based therapeutics

Optical imaging techniques allow the direct observation of cellular and molecular processes both in culture and in experimental models of disease. These techniques rely on the capture of optical photons and can be divided into fluorescence imaging and bioluminescence imaging. Advantages of optical imaging include the sensitivity and the possibility to image multiple labels with different characteristics at the same time. However, a major downside to optical imaging is a lack of penetration depth (especially with visible wavelengths), due to light scattering by the tissue.

Fluorescence imaging

Fluorescence imaging uses fluorescent probes that, when illuminated with light of a certain wavelength, will absorb the energy and emit photons of longer wavelengths. External light of specific wavelengths is employed to excite fluorescent molecules in the target that emit photons of longer wavelength (and accordingly, of lower energy), which are subsequently registered by a detector. Optical visualization of fluorescence has been extensively employed for *in vitro* and *ex vivo* imaging of target molecules in cell biology. Currently, fluorescence-imaging techniques are being successfully expanded towards in vivo imaging.[6,7] *In vivo* fluorescence imaging techniques cover a wide range of resolution, sensitivity, and imaging depths. A study by Jang et al. reports the combination of a neuronal promoter based reporter system where in fluorescence imaging were utilized in live cells to visualize the enhanced differentiation of neuronal progenitor cells (NPCs) with overproduction of microRNA (miR)-124a *in vitro* and *in vivo*.[4] Various fluorescent probes have been developed to facilitate imaging in animal models of disease. These probes range from conventional small organic fluorophores to recent discovery of quantum dots that possess a high photo stability that allow for repeated visualizations.[5]

The discovery of fluorescence proteins, particularly the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* has changed perspectives of biological visualization. GFP achieves its native conformation and generates its chromophore without addition of any cofactors thus making it a universal fluorescent marker.[5] Shah et al. have engineered several different lentiviral vector constructs that express fluorescent and bioluminescent fusion proteins. These vectors were validated in human glioblastoma cells and adult stem cells.[8-10] The engineered tumor and stem cells were shown to express the fusion proteins at high intensity and the expression was preserved over repeated cell culture. Human stem cells were reported to retain their stemness following transduction and also their potential to differentiate into neuronal cells and astrocytes. These studies reveal that transduced stem cells retain allow a way to be tracked by optical imaging methods. [8-10]

Fluorescence imaging has several advantages: (i) it does not require the administration of a substrate that has to be enzymatically modified to emit photons. (ii) It allows for exploiting different fluorophores with different excitation and emission spectra to acquire multiple signals, monitoring different processes in the same experimental animal. (iii) The same fluorescent signal can be used for monitoring a specific molecular process in vivo and further validating it by fluorescence microscopy *ex vivo*.[6,7] However, fluorescence imaging requires the use of an excitation light that could be attenuated by the tissues with a consequent lower excitation efficiency of deeply located fluorochromes, and it is characterized by a significant amount of auto-fluorescence. Thus, the quantification of a fluorescent signal can be difficult.[6,7]

Bioluminescence imaging (BLI)

Bioluminescence imaging often referred as chemiluminescence uses a construct for luciferase production. Luciferase, an enzyme that catalyzes a chemical reaction involving the conversion of luciferin to oxyluciferin, which results in emitted light. The overall aim is to capture the emitted light from the experimental animal using a charge-coupled device. The emitted light intensity is presented with a pseudo-color scale of the bioluminescent images. The scaled representation of emitted light allows for attributing a gradient of differential emitted light. These images obtained are then overlaid light images of the experimental animal(s), which are captured during the imaging session. This allows to clearly differentiate the anatomical location of the emitted light thereby able to assess or track the parameters that are being followed over time.[3]

BLI offers numerous advantages in terms of the sensitivity and non-invasive nature of the technique and thereby is widely accepted in various animal models of oncology.[11] The incorporation of bioluminescent markers allows for a continuous tracking of the progression thereby serving as a checkpoint for both establishment of the disease in experimental models and also to track the effectiveness of a therapeutic agent or a test compound. BLI has widely been utilized for visualization of a variety of biological processes *in vivo*, owing to its sensitivity, ease of use, and lower operational expense.[11] Disadvantages include a limited penetration depth and poor spatial resolution, as a result of light scattering by the tissue.

BLI has emerged as the preferred imaging technique particularly in smaller laboratory animals such as rodents for optical tracking of cells. This technology enables simultaneous dual visualization of different luciferases that allows distinguishing between different processes being investigated.[12,13] Dual luciferase gene activities in the same animals contribute to reduction in experimental variations as a result of procedures performed or differences in animals. Wang *et al.*[14] reported a successful imaging of differentiation of stem cells to predict response of a novel stem cell therapy by utilizing the dual luciferase reporters: the Renilla Luciferase gene and the firefly luciferase gene.[12,13] Luciferases that are obtained from various species utilize differing substrates and emit lights at various wavelengths that allow for simultaneous bioluminescence imaging of transplanted neural stem cells *in vivo* in the brain.[12] Bioluminescence imaging of Fluc and Rluc provided the real-time monitor of tumor cells and hUC-MSCs administered simultaneously in breast cancer therapy.[15]

Magnetic resonance imaging (MRI) MRI is a noninvasive imaging technique that provides insights into the anatomy, function, and metabolism of tissues in living subjects without the use of any ionizing radiation. Nowadays, MRI technology is moving beyond anatomical or structural imaging. It is also employed for dynamic and functional imaging. MRI makes investigation of various aspects of tumor cell biology possible and allows assessing the response to a specific therapy. The ability to track the path of therapeutic cells post transplantation is critical to assess effectiveness of such therapies.

Owing to its inherent high spatial resolution, MRI serves as an ideal imaging modality to understand the fate of cellular therapies *in vivo*[16] . To detect the transplanted cells by MRI with high sensitivity, it is necessary to label them with magnetic nanoparticles (MNPs) prior to their implantation. This technique induces contrast that allows for MRI imaging without altering the functional properties and also

allows for detection of few hundred cells. Thus, when paired with other imaging techniques, like PET or BLI, MRI can provide insight into important grafting parameters such as optimal cell "dose", that can be in the clinical implementation of a novel cell therapies.[17] To determine some of these parameters in a reliable manner, for instance graft volume or cell proliferation, very precise titration controls are needed to determine the detection limit of the MRI equipment.

Radionuclide imaging including PET and single-photon emission computed tomography (SPECT), is based on registering ionizing radiation.[18-20] This imaging modality has a greater sensitivity and the best spatial resolution across all imaging modalities that are currently being employed[21-22]. In the clinics, it has been widely used to assess several pathophysiological functions in humans. Radioisotopes that have a longer decay half-life are selected for labeling and tracking cells.[23] PET imaging quantitatively detects the high-energy γ-rays emitted from isotopes or labeled molecular probes. Advantages of PET imaging are: high sensitivity, it is non-invasive, it allows real time tracking *in vivo*, and it is not dependent of the depth of the emitted signal.

A PET tracer is designed in a way that it promotes reflection of specific biochemical processes being studied in the body. The PET camera captures emitted photons by positron annihilation events radioisotopes, such as F-18. They can be linked to a molecule of interest like 2-deoxy-2- [18F] fluoro-D-glucose (FDG) that aids in tracking glucose metabolism in the body. Jin *et al.* have investigated the potential of in vivo radionuclide imaging of CSCs using Iodine-125-labeled ANC9C5, an anti-human CD133 antibody, in colon carcinoma xenografts. Although a favorable biodistribution profile was not obtained, intratumoral distribution of 125I-labeled ANC9C5 depicted on autoradiography was overlapped with CD133 immunohistochemistry expression in many areas.[24]

Gaedicke *et al.* performed PET imaging to detect an epitope of the second extracellular loop of CD133. Specifically, two cell lines overexpressing CD133 were xenografted in mice and imaged using ^{64}Cu-NOTA-AC133. The imaging yielded accurate and high-resolution images of brain tumor lesions 2-3 mm in size, with significant tumor-to-background contrast. This study disclosed the differences in invasive behavior between orthotopically growing U251 (noninvasive) and NCH421k (invasive) gliomas. More interestingly, PET signal intensity accurately reflected the microscopic pattern of tumor AC133+ expression. This was observed in sharply delineated PET images of U251 tumor that has compact and spherical microscopic appearance. On the other hand, the chaotic and infiltrative growth pattern in NCH421k tumors was represented by more diffuse PET signal.

The tumor cell homing of MSC has been investigated by employing reporter gene labeling.[25] MSC were engineered to express HSV-TK, which serves as an imaging tracer for PET studies. Using the fluorine-18-labeled acycloguanosine derivative substrate (9-(4-[^{18}F] Fluoro-3-hydroxymethylbutyl) – guanine) tracer [^{18}F] FHBG, an increased tracer uptake in glioblastoma tumors following FHBG administration and imaging was observed.[25] Further, these findings were validated employing a multimodal PET/MRI system and a 2-photon laser scanning microscopy.

Thus, radionuclide imaging has proven to be extremely efficient in tracking cells *in vivo* and is often used in conjunction with modalities to constitute a multimodal imaging approach.

Future of imaging based approaches to determine fate of therapies

Rapid advances in imaging technologies over the past decade certainly promise a bright future in this field. Tremendous potential exists to further advance imaging capabilities in basic science, translational and clinical practice. These novel tools will most definitely allow for newer types of measurements. The techniques that specifically are capable of quantitative and comprehensive assessment of the cellular and/or subcellular events *in vivo* will be very valuable to validate efficacy of promising therapeutics. Today a number of opportunities certainly exist in the world of imaging such as methods to improve clinical detection of earlier

forms of cancers to <1 mm^3, strategies to develop single cell imaging techniques to image beyond the current depth capabilities and development of techniques to achieve resolution at the cellular level. These are a few of the very many challenges that could be tackled to make medical discoveries more promising and to accelerate translation of promising therapies from bench to bedside.

To conclude, the world of imaging has critically contributed to various aspects of basic science, translational sciences, and clinical medicine. We are confident that the future holds promise and novel developments will emerge allowing us to push the boundaries in terms of how efficiently the fate of novel therapeutics can be evaluated at a much deeper level.

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