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Review

Optical small animal imaging in the drug discovery process

S. Dufort [a,b,c,1], L. Sancey [a,b,1], C. Wenk [a,b], V. Josserand [a,b], J.L. Coll [a,b,*]

[a] CRI-INSERM U823, Cibles diagnostiques ou thérapeutiques et vectorisation de drogues dans les cellules tumorales, Institut Albert Bonniot, BP 170, 38 042 Grenoble cedex 9, France
[b] Université Joseph Fourier, BP 53, 38 041 Grenoble cedex 9, France
[c] UF Cancérologie Biologique et Biothérapie, pôle de biologie et pathologie, CHU de Grenoble, BP 217, 38 043 Grenoble cedex 9, France

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ABSTRACT

Molecular imaging of tumors in preclinical models is of the utmost importance for developing innovative cancer treatments. This field is moving extremely rapidly, with recent advances in optical imaging technologies and sophisticated molecular probes for *in vivo* imaging. The aim of this review is to provide a succinct overview of the imaging modalities available for rodents and with focus on describing optical probes for cancer imaging.

Contents

Abbreviations: CT, X-ray computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography; SPECT, single photon emission computed tomography; ^{18}F, fluorine-18; ^{15}O, oxygen-15; ^{13}N, nitrogen-13; ^{11}C, carbon-11; ^{123}I, iodide-123; ^{125}I, iodide-125; ^{99}mTc, technitium-99m; NIR, near-infrared; BLI, bioluminescence; 2D-FRI, two-dimension fluorescence reflectance imaging; GFP, green fluorescent protein; CCD, charge-coupled device; FMT, fluorescence molecular tomography; FDOT, fluorescence diffuse optical tomography; EGF, epidermal growth factor; EGFR, EGF receptor; HER-2, epidermal growth factor receptor 2; RGD, Arg-Gly-Asp peptide; RAFT, regioselectively addressable functionalized template; cRGD, cyclic RGD; SNR, signal-to-noise ratio; ICG, indocyanine green; MMP, matrix metalloproteinases; AOMK, acyloxymethyl ketone; RTK, receptor tyrosine kinase; EPR, enhanced permeability and retention; ROS, reactive oxygen species; uPA, urokinase plasminogen activator; uMUC-1, underglycolated mucin 1; siRNA, small interfering RNA; PAT, Process Analytical Technologies

* Corresponding author. INSERM U823, Equipe 5, Institut Albert Bonniot, BP 170, 38 042 Grenoble cedex 9, France. Tel.: +33 4 76 54 95 53; fax: +33 4 76 54 94 13.
E-mail address: Jean-Luc.Coll@ujf-grenoble.fr (J.L. Coll).
[1] These authors contributed equally to this work.

1. Introduction

Molecular imaging can be used as a non-invasive means to evaluate pathophysiological processes, such as cancer, in rodents. Indeed, these techniques provide real-time information for early diagnosis, allow longitudinal follow-up of tumor development, and facilitate studies of therapeutic activity and antitumor efficacy of new anti-cancer drugs. Because drug development is an expensive and complicated process with an extremely slim chance of success for any given molecule, molecular imaging can play an important role in drug discovery in the laboratory, during the translation phase from *in vitro* assays to preclinical systems, and eventually in evaluating the biodistribution, pharmacokinetics and biological activity of potentially therapeutics molecules [1].

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These applications necessitate the generation of suitable imaging devices and imaging probes. This review presents the most commonly used methods for small animal imaging. We will focus mainly on optical imaging techniques and probes that are actively being developed for their sensitivity, inherent biological safely, and relative ease of use (see Table 1). In addition, we will present the translation of novel optical devices, methods and techniques into the clinic, which are areas of increased interest.

2. Commonly used small animal imaging technologies

2.1. X-ray computed tomography (CT)

CT is very commonly used on human patients, but until recently its application in rodents has been limited by its spatial resolution. X-rays are absorbed to varying degrees by different biological tissues. Recently, micro-CT devices have been developed to image rodents with a spatial resolution ranging from 10 to 100 µm [2]. Micro-CT is a powerful and cost-effective method for imaging soft-tissue structures, skeletal abnormalities and tumors. Micro-CT systems provide excellent sensitivity for skeletal tissue (Table 1).

2.2. Magnetic resonance imaging (MRI)

MRI is another standard method of clinical imaging, and recent improvements and adaptations have expanded its use to specialized animal facilities. ^{1}H-MRI is based on the fact that when a sample lying within a magnetic field is subjected to a radio-frequency pulse its protons absorb energy and generate a detectable signal during the relaxation phase. The strength of the signal is a function of the number of protons. The relaxation process can be described by two fundamental rate constants: T1 (longitudinal relaxation) and T2 (transverse relaxation) [3]. The sensitivity of this method is low (mM concentrations) but its spatial resolution is extremely good (µm). MRI is very useful for detecting tumors and measuring morphologic parameters. Because there is no damaging radiation, multiple imaging sessions can be performed safely, allowing longitudinal follow-up of tumor growth. Finally, contrast agents influencing either the T1 or T2 relaxation time constants are being developed to allow functional imaging [4–7].

2.3. PET and SPECT

Positron emission tomography (PET) requires the use of radioactive isotopes that emit positrons, such as ^{18}F, ^{15}O, ^{13}N, and ^{11}C [8], while SPECT (single photon emission computed tomography) uses tracers that emit gamma ray or high-energy X-ray photons, such as ^{123}I, ^{125}I, and ^{99}mTc. Positrons move a short distance through tissues, losing energy as they collide with other molecules, and eventually combine with electrons ("annihilation"), producing two high-energy gamma rays or photons traveling outward and in opposite directions. In SPECT, a single photon per event is directly emitted, and this photon interacts with electrons and nuclei of nearby atoms within the tissue. Unlike positrons, these energetic photons do not "slow down" but are attenuated. Because there is only one photon per event, electronic collimation is not possible, and a physical collimator must be added. Sensitivities are on the order of 10^{-14}–10^{-15} M for PET and 10^{-14} M for SPECT, and spatial resolution can reach 1.3 mm for PET and is sub-millimetric for SPECT [9]. PET and SPECT give information about physiological functions at the molecular level and are thus well suited to monitoring many vital processes, such as glucose metabolism, blood flow and perfusion, receptor-ligand binding rates, and oxygen utilization.

Table 1
Characteristics of the different imaging devices.

Technique	Resolution	Depth	Time	Quantitative	Clinical use
CT	20 µm	No limit	Minutes	Yes	Yes
MRI	10 µm	No limit	Minutes to hours	Yes	Yes
PET	1–2 mm	No limit	Minutes to hours	Yes	Yes
SPECT	1 mm	No limit	Minutes to hours	Yes	Yes
FRI	µm to mm	<1 cm	Seconds	No	Yes
FMT	1 mm	<5 cm	Minutes to hours	Yes	Soon
BLI	1 mm	<1.5 cm	Minutes	No	No

2.4. Optical imaging

Optical imaging is based on the detection of light passing through the tissues. Several major obstacles must be resolved for optical imaging, including surface reflectance, absorption, scattering (deviation of the photon from its original path) and autofluorescence. Absorption and autofluorescence are important factors in fluorescence imaging in the visible wavelengths (400–650 nm) but are less problematic in the near-infrared (NIR) (650–900 nm). Above 900 nm, however, water absorption is an issue because it prevents deep penetration of the light. Absorption is affected by the thickness and optical properties of the tissues. The body heterogeneity will compromise the linearity of measurements. *In vivo*, scattering of far-red photons affects the spatial resolution, which is dependent on the depth of observation. Spatial resolution is mainly affected by an animal's skin, which reflects light, but intravital microscopy greatly improves the resolution [1].

Light can be produced in live animals by two main types of reaction: bioluminescence (BLI) and fluorescence (for reviews see [10,11]). BLI is based on the self-emission of green to yellow light due to the oxidation of luciferin in the presence of luciferase enzymes (Firefly, Renilla, Aequoria…). BLI has minimal background signal and an excellent signal-to-noise ratio (SNR). Acquisition times range from seconds to minutes.

Two-dimensional fluorescence reflectance imaging (FRI) uses fluorescent probes to produce detectable signals (Fig. 1). In FRI, the sample is submitted to an NIR light (excitation) that can be absorbed by the fluorescent probe, which then emits light (fluoresces) at a longer wavelength as it returns from an excitated electronic state to its ground level. The probe could be a protein (e.g., green fluorescent protein (GFP) or DsRed) present in genetically modified animals or cells or an exogenous fluorophore. Because these fluorescent proteins are not excitable in the far-red or NIR spectrum, but only in the 450–650 nm window, they are not well adapted to whole-body small animal imaging, and their detection is limited by depth (typically 1–2 mm). In contrast, some other fluorophores (such as cyanines, quantum dots, and lanthanides) can be exited in the far-red window, allowing deeper detection (typically 1–2 cm). Sensitivity is very good (10^{-12} M), spatial resolution varies from 100 µm *in vitro* to 2 mm *in vivo* and temporal resolution is in the range of milliseconds to seconds. However, 2D-FRI is highly surface-weighted, and quantification is at best semi-quantitative.

Recent development of 2D-FRI may allow its application in the clinic for improving cancer surgery. The Fluobeam® system (Fluoptics, Grenoble, France) uses a laser and small charge-coupled device (CCD) camera within a portable 2D-FRI system. It can function under the normal light of an operating room [12]. Another intraoperative device, the FLARE system (fluorescence-assisted resection and exploration), has already been used in preclinical trials [13–15].

Because of problems with absorption and autofluorescence, deep and absorbing tissues like lung, spleen or liver cannot be investigated non-invasively using 2D-FRI. Imaging these tissues requires the use of fluorescence molecular tomography (FMT) [16]. FMT provides 3D volumetric imaging, true quantification independent of depth, tissue optical properties and heterogeneity, and augmentation of the contrast by reducing the autofluorescence. Detecting deep events