Molecular imaging is speeding up drug development

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Abstract

Many imaging techniques have been routinely used in the drug development process to rapidly identify new targets, screen lead compounds, directly monitor the pharmacokinetics and evaluate the effects of the drug in the context of tumor. In this article, authors first brief introduce the basic principles of molecular imaging modalities in living subjects, and then discuss how molecular imaging is speeding up the drug development based on the research work that has been performed in our laboratory in the past decade.

Key words

Drug development; molecular imaging; molecular imaging technique; pharmacokinetics; target identification; therapeutic efficacy; translational research

Introduction

The drug discovery and drug development process (Fig 1) is a relatively lengthy, high-risk and costly endeavour. Research efforts and novel techniques have been conducted and introduced to accelerate the process while minimizing costs. New strategies that aid the early selection of promising candidates to move to pivotal trials, or termination of candidates that are unlikely to be successful, could significantly improve the overall drug development process.

Many imaging techniques have been routinely used in the drug development process to rapidly identify new targets, screen lead compounds, directly monitor the pharmacokinetics and evaluate the effects of the drug in the context of tumor. Molecular imaging attempts to characterize and quantify biological processes at the cellular and subcellular level in intact living subjects. It usually exploits specific molecular probes as well as intrinsic tissue characteristics as the source of image contrast, and provides the potential for understanding of integrative biology,
earlier detection and characterization of disease, and evaluation of treatment. Molecular imaging allows a repetitive, non-invasive, uniform and relatively automated study of the same living subject using identical or alternative biological imaging assays at different time points, thus harnessing the statistical power of longitudinal studies, and reducing the number of animals required and cost.

**Molecular imaging**

Molecular imaging refers to the characterization and measurement of biological processes at the molecular level[3]. Molecular imaging technologies provide a non-invasive or minimally invasive means of visualizing, characterizing, and quantifying anatomical structures and physiological processes at the cellular and subcellular levels with exquisite spatial, temporal, and biochemical resolution *in situ* and *in vivo*. Molecular imaging techniques include positron emission tomography (PET), single-photon emission computed tomography (SPECT), molecular magnetic resonance imaging, magnetic resonance spectroscopy, optical bioluminescence, optical fluorescence, and targeted ultrasound (Fig 2) [4]. Molecular imaging can give whole body readout in an intact system, help to decrease the workload and speed up the drug development process, provide more statistically relevant results because longitudinal studies can be done in the same animals, aid in lesion detection and patient stratification, and help in individualized anticancer treatment monitoring and dose optimization[2].

Molecular imaging can hasten drug development at the target identification and validation stages, in the synthesis and optimization of drug candidates, and in pre-phase I to phase II clinical trials, i.e., at almost any point in the drug development process. It provides the link between *in vitro* studies and those performed *in vivo* and in humans.

**Molecular imaging in drug development**

**Target identification**

By introducing molecular imaging probes into applications in molecular imaging techniques, the expression of indicative molecular markers at different stages of diseases can be determined. The
introduction of new imaging probes, methods and advanced imaging instrumentation is significantly speeding up the processes of drug discovery and development.

The cell adhesion molecule integrin $\alpha_v \beta_3$ is highly expressed on activated and proliferating endothelial cells during tumor angiogenesis, and on some tumor cells, but not on quiescent vessels and normal cells [5], thus it has been suggested that integrin $\alpha_v \beta_3$ is a specific marker of tumor angiogenesis [6, 7]. Integrin $\alpha_v \beta_3$ binds a wide range of extracellular matrix (ECM) molecules with an Arg-Gly-Asp (RGD) tripeptide motif, including fibronectin, fibrinogen, von Willebrand factor, vitronectin, and proteolysed forms of collagen and laminin. The specific recognition of RGD with integrin $\alpha_v \beta_3$ allows the specific determination of the integrin $\alpha_v \beta_3$ expression level in vivo with radiolabeled RGD peptides using PET or SPECT. An $^{18}$F-labeled RGD peptide $^{18}$F-FP-P-PRGD2 was prepared and investigated for integrin $\alpha_v \beta_3$ detection in vivo by microPET [8]. It was demonstrated that $^{18}$F-FP-P-PRGD2 can provide important information on integrin expression on the tumor vasculature. The high integrin binding affinity and specificity, excellent pharmacokinetic properties and metabolic stability make $^{18}$F-FP-P-PRGD2 a promising probe for PET imaging of tumor angiogenesis and for monitoring the efficacy of antiangiogenic treatment [8].

**Compound optimization**

Once a target is chosen and identified, the following stage is typically high-throughput screening of large libraries of chemicals for their ability to modulate the target. In contrast to the high-throughput screening that is usually restricted to cell-based assay, molecular imaging can also allow rapid compound screening using noninvasive animal-based in vivo assays. The two new cyclic RGD dimers, $\text{E[PEG}_4\text{-c(RGDfK)]}_2$ ($\text{P}_4\text{-RGD}_2$, $\text{PEG}_4=15$-amino-4,7,10,13-tetraoxa-pentadecanoic acid) and $\text{E[Gly}_3\text{-c(RGDfK)]}_2$ ($\text{G}_3\text{-RGD}_2$, $\text{G}_3=$Gly-Gly-Gly) were designed and synthesized for integrin targeting. $\text{P}_4\text{-RGD}_2$ and $\text{G}_3\text{-RGD}_2$ was radiolabeled with $^{68}$Ga, and then investigated by microPET imaging [9]. The new RGD dimers with the Gly$_3$ and PEG$_4$ linkers showed higher integrin $\alpha_v \beta_3$ binding affinity than
nolinker RGD dimer (RGD2). Both $^{68}$Ga-NOTA-P$_2$-RGD2 and $^{68}$Ga-NOTA-G$_3$-RGD2 exhibited significantly higher tumor uptake and tumor-to-normal tissue ratios than $^{68}$Ga-NOTA-RGD2. The results demonstrated that P$_2$-RGD2 and G$_3$-RGD2 are more promising compounds for integrin targeting, and can be used for integrin targeted cancer detection as well as possible delivery of cancer therapeutics.

**Measurement of pharmacokinetics**

Studies on drug absorption, distribution, metabolism and excretion play important roles in any drug development process. Poor pharmacokinetics is usually one of the major causes of drug inefficacy. By labeled with radioisotopes, the drug precursors can then be used for drug validation in living animals for the assessment of pharmacokinetics and pharmacodynamics using molecular imaging.

Abegrin™, also known as MEDI-522 or Vitaxin™, is a humanized monoclonal antibody against human integrin $\alpha_v\beta_3$, which is currently in clinical trials for the treatment of stage IV metastatic melanoma and androgen-independent prostate cancer [10, 11]. In a recent clinical study, Abegrin™ was well-tolerated with no evidence of immunogenicity in patients with advanced solid tumors, which guaranteed its further clinical investigation [12]. We labeled Abegrin™ with $^{111}$In, and investigated the *in vivo* behaviors by noninvasive gamma imaging [13]. Imaging results showed that the tumor uptake of $^{111}$In-DOTA-Abegrin™ in integrin $\alpha_v\beta_3$-positive U87MG tumors was much higher than that in integrin $\alpha_v\beta_3$-negative HT-29 tumors, indicating the specific targeting of Abegrin™ *in vivo* [13]. Predominant liver accumulation of $^{111}$In-DOTA-Abegrin™ was also found due to the liver clearance of Abegrin™.

**Monitoring of therapeutic efficacy**

Molecular imaging can be used as a patient or disease biomarker to stratify and identify responders and enrich proof-of-concept studies, enabling shorter and potentially more successful clinical trials. Such stratification is a key goal of efforts to improve the efficiency of drug development and, ultimately, deliver the right therapy to the right patient at the right dose. In addition to staging and diagnosis of disease, molecular imaging is playing an increasing role in monitoring treatment and recurrence of cancer during chemotherapy.

Cyclophosphamide (CTX) is a cell cycle-dependent DNA and protein alkylating agent that has a broad spectrum of activity against variety of neoplasms, and is widely used in the clinical management of human malignancies [14]. Cyclophosphamide is inactive until it undergoes hepatic transformation to form 4-hydroxycyclophosphamide, which then breaks down to form the ultimate alkylating agent, phosphoramid mustard. In clinical settings, treatment of cancers by high dose of CTX is often accompanied by host cytotoxic effects including cardiac and renal toxicity [15]. Therefore, a method to assess its efficacy early during treatment is needed to improve patient care by identifying the only responding patients to continue the CTX treatment. The patients, who have no response and therefore may not benefit from the therapy, would be able to avoid unnecessary toxic side effects and switch to different, more effective therapeutic approaches in a timely manner. The *in vivo* monitoring the therapeutic efficacy of CTX in the HCC-LM3-fLuc human hepatocellular carcinoma nude mouse model by the dual-modality PET and bioluminescence imaging (BLI) was carried out [16]. CTX induced a 25.25% and 35.91% tumor growth inhibition rate on day 9 and 12 post-treatment, respectively, as determined by BLI. $^{18}$F-FDG imaging revealed a significant uptake reduction in the tumors of CTX-treated group compared with that in the saline control.
group (5.30±1.97 vs 3.00±2.11 %ID·g⁻¹) on day 16 after CTX treatment. It was concluded that dual-modality molecular imaging using BLI and small-animal PET can play important roles in the process of the chemotherapy, and will provide noninvasive and reliable monitoring of the therapeutic response.[16]

¹⁸F-FDG and ¹⁸F-FLT based microPET imaging was used to determine the radioimmunotherapy efficacy of ⁹⁰Y-Abegrin™ in animal models[17]. ¹⁸F-FDG and ¹⁸F-FLT were used to quantify tumor cell metabolic activity and DNA synthesis, respectively. ¹⁸F-FDG imaging revealed a reduction of cell proliferation and metabolic activity, whereas ¹⁸F-FLT reflected decreased DNA synthesis in the ⁹⁰Y-Abegrin™ group[17]. By using the noninvasive imaging techniques, the therapeutic efficacy of ⁹⁰Y-Abegrin™ can be longitudinally monitored.

Clinical translational

Molecular imaging techniques can be used for translational clinical research and characterization of pharmacokinetics, tumor targeting efficacy, toxicity, and dose optimization of drugs, thereby accelerating the clinical translation.

⁹⁰mTc-3PRGD2 was reported as a new radiotracer for tumor imaging in athymic nude mice bearing U87MG glioma and MDA-MB-435 breast cancer xenografts[18]. ⁹⁰mTc-3PRGD2 exhibited increased tumor uptake and improved the in vivo kinetics. Importantly, the labeling procedure of ⁹⁰mTc-3PRGD2 is simple, efficient, and reproducible, which allows a kit formulation and the easy availability for routine clinical use. Following the continuous interest in the translation of ⁹⁰mTc-3PRGD2 to clinical SPECT imaging of integrin αvβ3, we recently investigated the blood clearance kinetics, biodistribution characteristics, and radiation dosimetry estimate of the kit-formulated ⁹⁰mTc-3PRGD2 in healthy non-human primates[19]. ⁹⁰mTc-3PRGD2 could be easily obtained from freeze-dried kits with high radiochemical purity (95%) and high specific activity (~5 Ci·μmol⁻¹). ⁹⁰mTc-3PRGD2 had a rapid blood clearance with less than 1% of the initial radioactivity remaining in the blood circulation at 60 min postinjection. No adverse reactions were observed up to 4 weeks after the repeated dosing. The whole-body images exhibited high kidney uptake of ⁹⁰mTc-3PRGD2 and high radioactivity accumulation in the bladder, demonstrating the rapid renal clearance of this tracer. The highest radiation doses of ⁹⁰mTc-3PRGD2 were found in the kidneys (13.2±1.08 μGy/MBq) and the bladder wall (33.1±1.91 μGy/MBq), which is much less than the clinical routinely used radiotracer ¹⁸F-FDG[19].

New molecular imaging techniques in drug development

Bioluminescence tomography (BLT) is a newly emerging optical molecular imaging modality that can quantitatively and accurately analyze a bioluminescent source distribution in animal models. BLT reveals molecular and cellular signatures critically important for numerous biomedical studies and applications, and also for drug discovery and development. Most recently, we developed a dual modality BLT prototype system with micro-computed tomography (microCT) registration approach. The quantitative reconstruction algorithm was improved based on adaptive hp finite element method (hp-FEM). Source reconstruction was performed in a mouse model with implanted luminescence source and also in nude mice bearing firefly luciferase transfected PC-3 tumor cells. Our results demonstrated that the reconstruction based on heterogeneous mouse model is more accurate for the localization and quantification of a bioluminescent source than that based on the homogeneous mouse model,
and BLT allows super-early in vivo tumor detection based on tomographic reconstruction of heterogeneous mouse model signal [20]. Although optical imaging based modality is currently not well investigated in humans directly, its application in animal models can provide great power in drug screening and monitoring treatment efficacy, and thereby accelerating the clinical translation of drugs.

Because no single imaging modality can provide information on all aspects of structure and function, an obvious approach is to interrogate a subject using multiple imaging modalities. There has been considerable interest over the past decade in examining the possibility of building multimodality imaging systems (e.g. PET/CT, SPECT/CT, etc.) in which two or more imaging modalities are integrated to a greater or lesser extent into a single imaging unit. Dual- or multi-modality molecular imaging would provide complementary information on the disease progression, and also on the effects of drugs on disease management. Currently, dual- or multi-functional molecular imaging probes that are labeled with more than one molecular imaging moieties and used for simultaneously molecular imaging with different modalities are also extensively investigated. The development of these multi-functional imaging probes allows the strengths of each modality to be combined, thereby improving diagnostic accuracy and providing greater insight into underlying disease processes.

Conclusions

Molecular imaging has a very special role to play in drug development ranging from target identification through the study of drug metabolism to possible clinical translational. Molecular imaging has the greatest influence during the initial stages of drug development by aiding the early selection of lead candidates that are likely to be successful ultimately before pivotal trials, and de-prioritizing or terminating the development of others.

Development of molecular imaging probes is essential for the application of multimodality molecular imaging techniques to drug development process. To foster the continued discovery and development of molecular imaging probes, cooperative efforts are needed from biologists to identify and validate molecular imaging targets, chemists to synthesize and characterize the imaging probes, and medical physicists to develop high-sensitivity/high-resolution imaging devices/ hybrid instruments. With the advances in the development of molecular imaging probes, optimized imaging modalities and regarding operating comfort and data acquisition/analysis, as well as clinically relevant animal models, molecular imaging is believed to play a more important role in accelerating and improving drug development in the near future.

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