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Tumor Molecular Imaging with Nanoparticles

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ABSTRACT

Molecular imaging (MI) can provide not only structural images using traditional imaging techniques but also functional and molecular information using many newly emerging imaging techniques. Over the past decade, the utilization of nanotechnology in MI has exhibited many significant advantages and provided new opportunities for the imaging of living subjects. It is expected that multimodality nanoparticles (NPs) can lead to precise assessment of tumor biology and the tumor microenvironment. This review addresses topics related to engineered NPs and summarizes the recent applications of these nanoconstructs in cancer optical imaging, ultrasound, photoacoustic imaging, magnetic resonance imaging (MRI), and radionuclide imaging. Key challenges involved in the translation of NPs to the clinic are discussed.

1. Introduction

Cancer is now the leading cause of death in the world [1]. China constitutes approximately 20% of the world's population, and comprises 29.42% of new cancer cases and 27% of cancer deaths worldwide. In 2012, the cancer incidence rate in China was 1.74%, and the cancer mortality rate was 1.22%. In China, cancers of the stomach and liver have traditionally occurred with the highest frequency; however, the incidence of lung cancer has increased as the leading cause of cancer in recent years [2]. Despite the fact that huge advances in diagnostic technologies have led to an explosion of knowledge in cancer research, only a minute number of cancer patients are diagnosed at early stages due to the poor selectivity and sensitivity of conventional diagnostic techniques.

Traditional imaging technologies reflect mostly anatomical changes that differentiate pathological from normal tissue rather than measuring the biological processes responsible for disease. Molecular imaging (MI) is a rapidly emerging biomedical research method that enables visual representation, characterization, and quantification of biological processes at the cellular and/or molecular level within living organisms [3–5]. Several MI modalities are currently available, including fluorescence and bioluminescence imaging, targeted ultrasound (US), molecular magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), single-photon-emission computed tomography (SPECT), and positron emission tomography (PET). Probes or beacons, which can accumulate at the site of interest and allow for imaging, are required for MI. However, the limitations of some MI techniques include poor spatial resolution, low sensitivity, or poor signal penetration through tissues. For example, optical imaging is restricted in its depth-penetration capability, leading to poorer resolution, whereas MRI can image deep tissues but is much less sensitive (Table 1).

Nanoparticles (NPs) are emerging as a new class of MI agent to overcome these major hurdles in detecting human disease. NPs have great potential for accurate cancer diagnosis via passive accumulation and/or active-targeting approaches [6]. Combining

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Table 1
Molecular imaging modalities.

Modality	Form of energy used	Spatial resolution (mm)	Advantages	Imaging cost	Clinical translation
Fluorescence imaging	Visible to infrared light	< 1 (fluorescence reflectance imaging, FRI); 1 (fluorescence molecular tomography, FMT)	High sensitivity; multiplexed imaging	Low (FRI); medium-high (FMT)	Yes
Bioluminescence imaging	Visible to infrared light	3–5	High sensitivity; high-throughput	Low	No
Ultrasound (US)	High frequency sound waves	0.04–0.1 (small-animal US); 0.1–1 (clinical US)	High sensitivity; portable	Low-medium	Yes
Positron emission tomography (PET)	Annihilation photos	1–2 (micro PET); 6–10 (clinical PET)	High sensitivity; quantitative; tracer amount of probe	High	Yes
Single-photon-emission computed tomography (SPECT)	Gamma rays	0.5–2 (micro SPECT); 7–15 (clinical SPECT)	High sensitivity; quantitative; tracer amount of probe	Medium-high	Yes
Magnetic resonance imaging (MRI)	Radio frequency waves	0.01–0.1 (small-animal MRI); 0.5–1.5 (clinical MRI)	High sensitivity; quantitative; tracer amount of probe	High	Yes

their large payload-carrying capacity, high signal intensity, and stability, NPs can deliver high concentrations of imaging agents to the target region. NPs are typically smaller than cells [7], and are comparable in size to molecules and proteins [8].

Historically, many of the developed NPs are considered to accumulate in tumors based on the enhanced permeability and retention (EPR) effect, which in nanomedicine research has been considered a universal feature of solid malignant tumors that can serve as the basis for passive tumor-targeting by therapeutic and diagnostic NPs [9–17]. The EPR effect was first reported as a concept of the delivery of macromolecular drugs to tumors [18]. However, the heterogeneity of the EPR effect provoked a debate about the real value of this effect. Many experimental scientists, pharmacologists, and nanotechnology engineers hold to the premise that solid tumors consist of uniform tissue; that is, that tumors are homogeneous [19]. However, as this is not the case, new strategies such as active targeted NPs have been pursued. Targeted NPs can perform with both high sensitivity and specificity to achieve high accumulation at the tumor site [20–23]. Active targeting requires the therapeutic agent to be guided to the target by conjugating the therapeutic agent or carrier system to a tissue or cell-specific ligand. In addition, using targeted NPs with a variety of moieties to reach multiple binding sites can provide higher binding efficiencies for targeting specific tumor sites.

A number of articles review the current applications of nanotechnologies for MI [24]. This paper presents a brief overview of recent developments in nanotechnology for MI, and will also address the drawbacks and future challenges of current nanoplatforms for the clinical translation of imaging.

2. Nanoplatforms

A wealth of NP-based systems exploits nanoplatforms for many imaging modalities. These NPs come in a wide variety of compositions, sizes, shapes, and structures from a variety of materials [25,26]. These different materials and shapes range from spheres, rods, and cubes to resembling snowflakes, flowers, thorns, hemispheres, worms, discoids, and chains—a wide variety that enables many imaging modalities (e.g., optical imaging, MRI, US, and/or nuclear imaging) for combining disease diagnosis and therapy into so-called "theranostic" (therapy plus diagnostic) applications. As mentioned earlier, NPs have been known to target tumors via passive- and/or active-targeting pathways. Due to abnormally leaky vasculature and the lack of an effective lymphatic drainage system in tumor tissues, NP platforms can passively accumulate in tumor tissues. These unique phenomena are jointly referred to as the EPR effect. Moreover, NPs can recognize, bind to, and internalize into tumor cells via receptor-mediated endocytosis when modified with tumor-targeting moieties such as antibodies, nucleic acids, proteins, or other ligands. In this section, we will describe exemplary theranostic nanoplatforms along with their applications regarding cancer.

2.1. Optical nanoparticles

Optical imaging offers high sensitivity, cost-effectiveness, non-ionizing radiation, and great potential for small-animal studies. However, the penetration depth of light prohibits deep-tissue imaging and is the major disadvantage for *in vivo* imaging in humans; thus, optical imaging is typically not quantitative for living-subject imaging studies. An abundance of probes, including synthetic fluorophores, semiconductor fluorescent crystals, and probes based on lanthanide rare-earth ions, have been developed for small-animal imaging [27–29].

Quantum dots (QDs) are the most widely studied NPs for preclinical optical imaging applications. Compared with organic fluorophores, QDs possess many superior properties for biological imaging, such as a strong resistance to photobleaching and chemical degradation, high quantum yields, continuous absorption spectra from ultraviolet (UV) to near-infrared (NIR), and large effective Stokes shifts [30–33]. Arginine-glycine-aspartic acid (RGD)-QDs could selectively bind to luminal endothelium in mouse tumor neovasculature [34]. However, because of their small size, QDs demonstrated poor retention inside the tumor, easily washing back out into the bloodstream [33] (Fig. 1). Nevertheless, the major drawbacks for the clinical utility of QDs are their potential toxicity and lack of deep-tissue imaging and quantification ability [35]. With the help of small-animal PET, radiolabeled QDs were found to have rapid uptake into the reticuloendothelial system (RES), liver, and spleen [36,37]. In order to reduce the uptake of QDs into the RES, human serum albumin (HSA) was explored as a coating for QD800-mercaptopropionic acid (MPA) NPs. The resulting QD800-MPA-HSA NPs show reduced localization in mononuclear phagocytic system-related organs over QD800-MPA, plausibly due to the low uptake of QD800-MPA-HSA in macrophage cells. QD800-MPA-HSA may have great potential for *in vivo* fluorescence imaging [38]. Affibody-modified QDs also showed high specificity for targeting human epidermal growth factor receptor 2 (HER2)-expressing cells and tumors [39].

Dyes that can be incorporated or encapsulated in polymeric NPs include indocyanine green (ICG) [40], NIR region fluorescent cyanine 7 (Cy7) [41], and dialkylcarbocyanine fluorophores [42], which have been approved by the United States Food and Drug Administration (FDA). A nanoplatform strategy is a promising method to enable optical imaging to overcome the drawbacks of free and small dyes [43,44]. Various nanocarriers have been investigated for the delivery of NIR dye to tumor sites, including liposomes, silica, polymersomes, and targeted rare-earth nano-