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Imaging Tumor Metastases with Molecular Probes

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Abstract: Tumor metastasis is an important prognostic factor regarding long-term survival rate of cancer patients. At present, no imaging modality or technique is ideal for diagnosis of metastases. Molecular imaging has provided a fantastic tool for tumor metastases imaging. Based on the current medical imaging tools such as magnetic resonance imaging (MRI), optical, single-photon emission computed tomography (SPECT), and positron emission tomography (PET), various techniques and functional molecular probes for molecular imaging of tumor metastases have been developed. In this review, we will summarize the current status of nanoprobe based molecular imaging metastases in cancer.

Keywords: Tumor metastasis, Molecular imaging, MRI, Optical Imaging, PET, Nanoparticles.

1. INTRODUCTION

Metastasis is one of the major causes of the death from cancer. To date, preliminary diagnosis of tumor metastases by using conventional imaging tools including computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) or ultrasound is still suffering from low sensitivity or specificity [1]. Originated from the above-mentioned medical imaging tools as well as widely investigated pre-clinical optical imaging instruments, molecular imaging using functionalized molecular probes provide potential alternatives for conventional image diagnostics. Among the large amount molecular probes, nanoparticles have provided promising applications for molecular imaging of metastases due to several unique merits: Firstly, nanoparticles with prolonged blood residence time in comparison to small molecules offer a greater chance in detecting tumor metastases [2, 3]; Secondly, the easily uptake by macrophages in reticuloendothelial system (RES) due to their unique size effect, consequently give rise to the difference between metastases and normal RES tissue [3, 4]; Thirdly, the multiple surface binding sites of nanoparticles provide the functionalization with different molecules for specifically targeting metastases; Last but not least, the intrinsic properties of nanoparticles shine lights on improving the sensitivity and resolution of metastases imaging. Here, we review the *in vivo* applications towards nanoparticle-based probes for imaging of metastases in cancer, highlighting in the nanoprobe construction and imaging approaches for acquiring images. Future perspectives towards clinical applications are also concluded.

Tumor metastatic process consists of dynamic biological processes including: tumor cells invade the stroma of the primary tumor, enter either the lymphatic or blood vessels, are passively transported through the circulation to a secondary site, exit the vessels and extravasate into a tissue for tumorigenesis (Fig. **1**) [5, 6]. Most cancers can form metastatic tumors depending on the specific molecular characteristics of the metastasizing cells [6]. Due to the heterogeneity of the primary tumor, it is extremely challenging to determine the metastatic proclivity [6]. Nevertheless, lymph nodes (LN) are often found to be the first metastatic organ in various cancers, leading to a fact that the prognosis and staging of lymphatic metastases are crucial for the clinical intervention [7]. Overall, the preclinical diagnosis of metastases may guide the therapeutic strategies to identify and prevent tumor dissemination at early stages. Various imaging modalities and molecular probes used for metastases imaging will be discussed in the following sections.

1.1. Molecular Imaging of Tumor Metastases with MRI

MRI provides extraordinarily high temporal and spatial resolution with excellent contrast in soft tissue [8]. Currently, several different MRI techniques are used for characterizing metastases in patients; including the entire body scan for detecting bone metastases as well as some contrast enhanced MRI for lymph node metastases. However, in typical cases, the physicians are using the lymph node size as the criterion for diagnosis of lymph node metastases which is insufficient for identifying metastatic lymph node with normal size. In the 1990's, the iron oxide based ultrasmall superparamagnetic nanoparticles (USPIOs) with 30 to 50 nm in hydrodynamic size were applied for lymphatic metastases detection. The particular size regime of USPIOs and the surface modification with dextran enabled the prolonged circulation time and eventually led to the uptake by lymph nodes in animals [2] and humans [3]. Potential routes for USPIO transportation is that the particles can either pass through transcapillary passage from the venules to the medullary sinuses of lymph nodes or extravasate across the permeable capillaries to interstitium, from which they are transported to lymph nodes by the lymphatic vessels [3]. Then the USPIOs are internalized by the macrophages in either benign or malignant lymph nodes, giving rise to a T_2 enhanced effect on MRI. The abnormal patterns of nanoparticles distribution resulting from the disturbances in lymph flow or in nodal architecture caused by metastases can be detected on T_2-weighted and T_2*-weighted MR images [4]. Although several clinical trials demonstrated the effectiveness for accurately staging lymphatic status [3, 9, 10], the heterogeneous enhancement in normal lymph nodes due to the passively uptake of all lymph nodes strongly impedes the applications of USPIOs in clinical use [11]. Furthermore, the low crystallinity of the iron oxide core of USPIOs derived from the decomposition synthetic method in water system also attribute to the low efficacy on metas-

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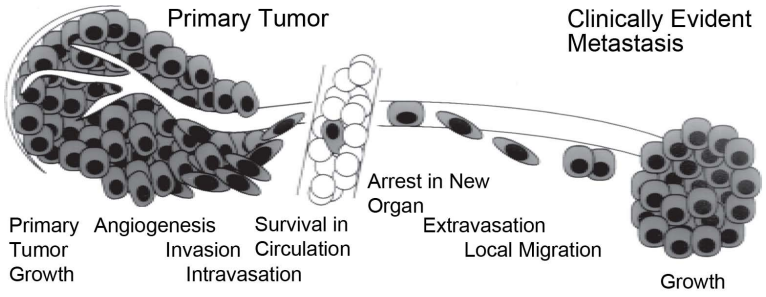

Fig. (1). Illustration of metastatic process. Tumor cells are believed to proceed through sequential steps to form metastases. Figure reproduced with permission from ref. 5, ©1997 Oxford University Press.

tases detection [12]. To further improve the quality of the iron oxide nanoparticles, tremendous efforts have been paid on the synthetic strategies and the thermodecomposition method triggered the applications of new generation of USPIOs for *in vivo* tumor diagnosis [13-26].

Based on the USPIOs acquired from the thermodecomposition method, Nie and co-workers designed and constructed a dual-modality molecular imaging nanoprobe by conjugating a cy5.5-labeled amino-terminal fragment (ATF) to magnetic iron oxide nanoparticles (IO) capped by amphiphilic polymers [27]. ATF enabled the specific targeting of the probe to urokinase plasminogen activator receptor (uPAR), which is highly expressed in pancreatic cancer and tumor stromal cells. *In vivo* MRI and optical imaging by intravenous injection of the uPAR-targeted nanoprobe to an orthotopically transplanted pancreatic tumor model showed selectively targeting of both primary and metastatic cancer lesions.

1.2. Molecular Imaging of Tumor Metastases with PET

^{18}F-fluoro-deoxyglucose (FDG) can be considered as the "work-horse" of PET/CT and PET/MR imaging modalities. FDG provides insight in the pathophysiology of tumors and metastases from the point of view of sugar consumption [28]. Since the brain derives its energy from metabolism of glucose, ^{18}F-FDG is a good candidate for metabolic imaging of the brain. ^{18}F-FDG diffuses into the brain from blood by crossing the BBB and is metabolized in the brain cells wherein FDG is phosphorylated to FDG-6-phosphate mediated by hexakinase. Simply because FDG lacks a hudroxyl group at the 2-position, its first metabolite FDG-6-phosphate is not a substrate for glyncolysis and does not undergo further metabolism [29]. Because of its negative charge FDG-6-phosphate remains trapped in the brain for several hours, thus faciltating imaging of the brain at convenience. ^{18}F-FDG PET imaging is useful in differentiating the metastatic brain tumor [30], which exhibit high glucose metabolism in comparison to the non-metabolizing glucose necrotic brain tissue. Thus ^{18}F-FDG PET shows increased uptake in the metastatic brain tumor, whereas decreased uptake is seen in necrotic brain tissue [31].

On the other hand, amino acid metabolism, expression of various receptors in the cells or on the surface of the cells, angiogenesis, appearance of hypoxic tissues and apoptosis also participate in the pathophysiological processes and may have importance in determining the treatment strategy for patients or in monitoring the tumor metastases. Many molecules involved can be labeled by ^{18}F radionuclide but certain metabolisms require ^{11}C-labelled agents. Various metal complexes containing ^{44}Sc,^{64}Cu, ^{68}Ga,^{86}Y, ^{89}Zr positron emitters can be very useful in molecular imaging of tumor metastases.

^{18}F-fluoro-2-deoxy-D-glucose-positron emission tomography/computed tomography (PET/CT) would not be appropriate to diagnose metastases of the lateral lymph node. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of predicting metastases were not highly, respectively, upon PET/CT. ^{18}F-fluoro-2-deoxy-D-glucose-PET/CT diagnosis alone was not suitable for detecting lateral lymph node metastases [32].

Kitajima K's study was to compare ^{11}C-choline PET/CT with pelvic multiparametric MR imaging for detection of recurrent prostate carcinoma in patients with suspected recurrence after radical prostatectomy. The patient-based sensitivity, specificity, and accuracy of multiparametric MR imaging for diagnosing local recurrence were 88.5% (54/61), 84.6% (22/26), and 87.4% (76/87) whereas those of PET/CT for detecting body LN or bone metastases were 92.3% (72/78), 100% (18/18), and 93.8% (90/96), respectively. Multiparametric MR imaging with endorectal coil is superior for the detection of local recurrence, PET/CT is superior for pelvic LN metastases, and both were equally excellent for pelvic bone metastases. ^{11}C -choline PET/CT and pelvic multiparametric MR imaging are complementary for restaging prostatectomy patients with suspected recurrent disease [33].

Gastroenteropancreatic neuroendocrine tumors (GEPNETs) are indolent neoplasms presenting unpredictable and unusual biologic behavior that causes many clinical challenges. Tumor size, existence of metastases, and histopathologic classification remain incapable in terms of treatment decision and prognosis estimation. The sensitivity of ^{68}Ga-DOTATATE PET/CT in GEPNETs in detecting liver metastases, lymph nodes, bone metastases, and primary lesion was 95%, 95%, 90%, and 93%. ^{68}Ga-DOTATATE PET/CT is helpful in the individual therapeutic approach of GEPNETs and can overcome the shortcomings of histopathologic grading especially in GEPNETs [34].

Recently, it was reported that radiogallium-labeled 1,4,7,10-tetraazacyclodod- ecane-1,4,7,10-tetraacetic acid (DOTA)-conjugated (Asp)n peptide [Ga-DOTA-(Asp)n] has great potential for bone metastases imaging. In the current study, a compound containing an aspartic acid peptide linker (D11) as a carrier to bone metastases, an RGD peptide [c(RGDfK) peptide] as a carrier to the primary cancer, and Ga-DOTA as a stable radiometal complex for imaging in one molecule, Ga-DOTA-D11-c(RGDfK), was designed, prepared, and evaluated to detect both the primary cancer and bone metastases simultaneously using ^{68}Ga, which is easy to handle. *In vitro*, ^{68}Ga-DOTA-D11-c(RGDfK) had a high affinity for hydroxyapatite and $\alpha_v\beta_3$ integrin. *In vivo*, ^{68}Ga-DOTA-D11-c(RGDfK) exhibited high uptake in bone and tumor. The accumulation of ^{68}Ga-DOTA-D11-c(RGDfK) in tumor decreased when it was co-injected with c(RGDfK) peptide. ^{68}Ga-DOTA-D11 - c(RGDfK) has great potential as a PET tracer for the diagnosis of both the primary cancer and bone metastases simultaneously [35].

Based on the high and consistent expression of prostate-specific membrane antigen (PSMA) in metastatic prostate cancer (PC), the DOTAGA-conjugate PSMA inhibitor labeling with ^{68}Ga for imaging and therapy (PSMA I&T) was the PSMA inhibitor for imaging of positron emission tomography (PET) in patients with metastatic and castration resistant disease. Tumor targeting and tracer of ^{68}Ga-DOTAGA-conjugate PSMA I&T kinetics *in vivo* were fast, with

the highest uptake in tumor xenografts and kidneys (both PSMA specific). First human ^{68}Ga-PSMA I&T PET imaging allowed high contrast detection of bone lesions, lymph node and liver metastases [36]. Complete remission of metastatic neuroendocrine paragastric carcinoma after ^{68}Ga-DOTATATE PET/CT and ^{177}Lu-DOTATATE peptide receptor radionuclide diagnose and therapy. Usually, peptide receptor radionuclide (PRRT) therapy is considered a palliative treatment. Few patients demonstrate a very favorable response allowing resection of the primary tumor after downstaging metastatic disease burden [37].

Nevertheless, owing to the limited resolution with only 4-5 mm of the FDG-PET, the in total sensitivity of this technique is rather low to moderate on metastases imaging [38]. For example, 14.5% of metastatic lymph nodes in gastric cancer have a largest diameter with less than 3 mm, which can be missed by the FDG-PET imaging [1]. Other drawbacks involve the low FDG uptake and the uptake by adjacent primary tumor which causes ambiguous signal for differentiating the metastases [39].

1.3. Molecular Imaging of Tumor Metastases with Optical Imaging

As a pre-clinical imaging tool, optical imaging holds great potential in molecular imaging for its easy operation, short acquisition time as well as relatively lower cost [40]. However, the *in vivo* applications of optical imaging strongly relies on the development of optical probes, which generating the luminescent or fluorescent signals for acquiring optical images. Bioluminescent or fluorescent imaging using genetically transfected luciferase- or green fluorescent protein (GFP) cell lines are frequently mentioned in the molecular imaging as one type of optical imaging of tumor, which is a non-irrelevant concept for molecular imaging of tumor detection. An exogenous optical probe for targeting tumor lesions is the essential requirement because the naturally formed tumors are not genetically transfected with gene reporters.

As the matter of fact, the optical imaging for the detection of conventional fluorescence probes are suffering from very limited depth of tissue penetration for both excitation and emission lights in

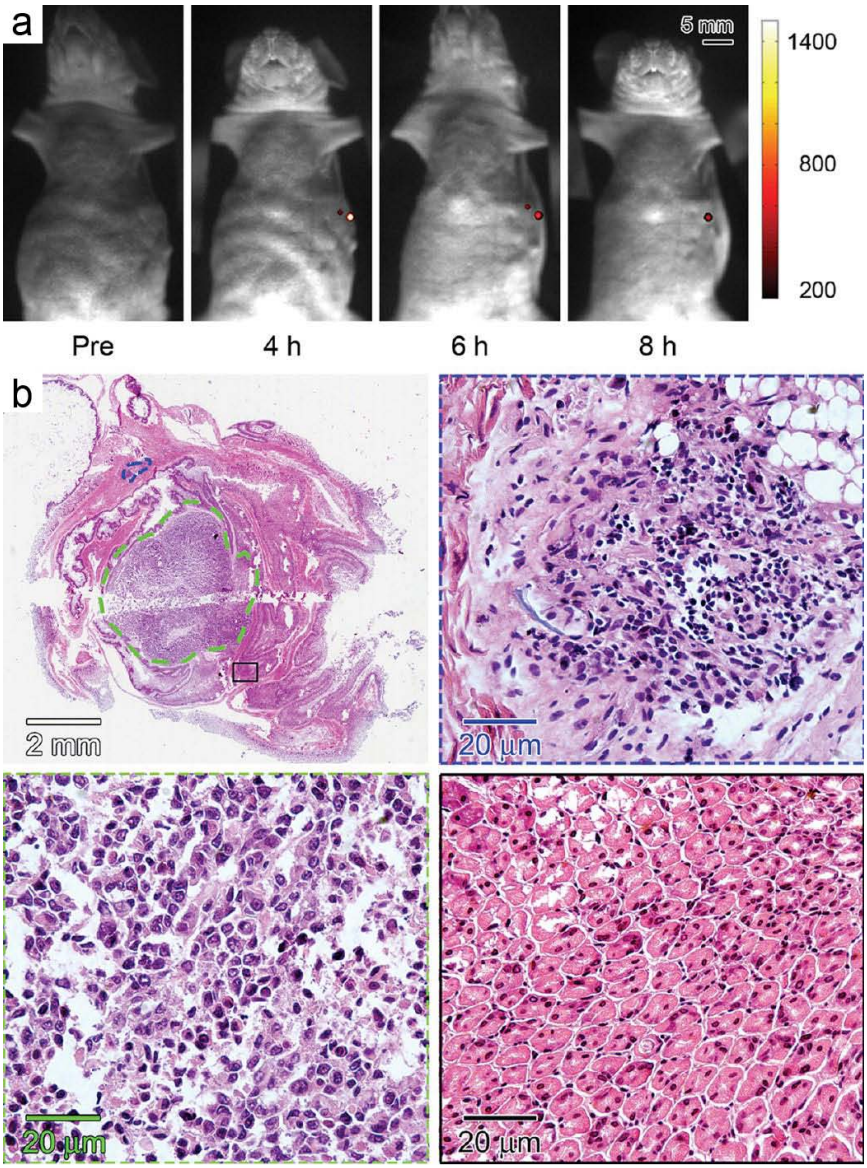

Fig. (2). *In vivo* optical images with intravenous administration of NP-MGb_2 nanoprobes on an orthotopically transplanted gastric cancer model. **a**. Bright-field images superimposed with color-coded upconversion luminescence images showing two distinguishable signals at stomach region. **b**. The primary tumor and lymphatic metastasis were evident from the histological studies. Figure reproduced with permission from ref. 51, © 2015 American Chemical Society.

the visible range in addition to the high background signal caused by autofluorescence from normal tissues. Therefore, optical probes with emission and/or excitation windows in the near-infrared windows (NIR, 650-1400 nm), which is minimally absorbed by hemoglobin, muscle, and fat tissues, and minimizes autofluorescence of tissues [41-43]. Most importantly, NIR-fluorophore allows deeper tissue penetration of photons, which offers great opportunities in metastasis imaging [44].

Some conventional NIR fluorophores, such as indocyanine green (ICG), have shown the potential for detection of metastasis in animal models [44]. Under the use of ICG-labeled anti-EGFR antibody, peritoneal micrometastases with 1-2 mm size in diameter could be detected. However, the sensitivity of the optical imaging were largely limited at a shallow tissue with a relatively low resolution. Owing to the superior optical properties such as the combination of higher quantum yield and stability, nanoparticles such as quantum dots have shown promising potential in detecting lymphatic metastases [42]. It has been demonstrated that sentinel lymph nodes with 1 cm deep were able to be detected in real time, favoring a future application for visualizing guidance through the surgery.

Benefiting from the unique anti-Stokes mechanism, upconversion nanoparticles (UCNPs) have driven much attention recently for offering greater penetration of the tissue by the NIR excitation and eliminating the interference of endogenous fluorescence [45-48]. We recently have reported the construction of UCNPs-based nanoprobes by conjugating PEGylated $NaGdF_4$:Yb,Er nanoparticles with anti-EGFR antibody. A tumor size with ~2 mm in intraperitoneal LS180 tumor xenografts was able to be detected through the upconversion imaging. Moreover, MR imaging with the enhancement on T_1-weighted images can also be acquired from the intrinsic Gd ions in the nanoparticles [49, 50]. Recently, we have further applied the core-shell structured $NaGdF_4$:Yb,Er@$NaGdF_4$ nanoparticles with similar surface structure for detecting lymphatic metastases in gastric cancer. The gastric cancer specific probe $NaGdF_4$:Yb,Er@ $NaGdF_4$-MGb_2 (NP-MGb_2) was constructed through the "click" reaction between the maleimide residue on the surface of the nanoparticle and MGb_2 antibody. Cell staining results demonstrated that the probe could specifically recognize the SGC7901 cells, which is a typical gastric cancer cell line. To further testify the effectiveness of the probe, an *in vivo* imaging method for achieving highly sensitive and specific detection of gastric cancer was developed by applying the above-mentioned targeting probe on an orthotropic model. The upconverting imaging results revealed that the probe could be useful for not only tiny tumor lesion diagnosis but also for lymphatic metastasis detections (Fig. **2**), indicating potential clinical applications in the early gastric cancer diagnosis and lymph node status evaluation [51].

PERSPECTIVE (CONCLUSION)

The motivation for imaging metastasis is to construct reliable technique for getting the preclinical guidance for therapeutic decisions. Due to the heterogeneity of tumor and metastases, it still remains great challenge for detecting metastasis by using conventional imaging tools. Molecular imaging based on functional probes offers great opportunities in metastasis detection and it has been demonstrated that some probes can successfully detect tiny tumor metastasis at a size around 1 mm. However, further achievements need to be made in optimizing the physical and chemical properties of nanoprobes. Reliable surface modification strategy is also essential for providing longer blood circulation time to ensure the accumulation of nanoprobes at the metastatic tumor lesions. Moreover, the use of more efficient and sensitive reporters towards the clinical and practical challenges is also an ongoing area for improving the efficacy of molecular imaging probes. Last but not least, reliable and representable animal models have to be also taken into consideration for evaluation of the acquired nanoprobes.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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