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Review Article

Molecular imaging of integrin $\alpha_v\beta_6$ expression in living subjects

Hao Liu[1,2], Yue Wu[1,2], Fan Wang[1,2], Zhaofei Liu[1,2]

[1]Medical Isotopes Research Center, Peking University, Beijing 100191, China; [2]Department of Radiation Medicine, School of Basic Medical Sciences, Peking University, Beijing 100191, China

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Abstract: Integrins, a family of cell adhesion molecules composed of α and β heterodimeric subunits, are involved in a wide range of cell-extracellular matrix and cell-cell interactions. The study of integrin family members as targets for molecular imaging and therapy has been generally limited with the exception of integrin $\alpha_v\beta_3$. $\alpha_v\beta_6$, a member of the integrin family, is expressed at low or undetectable levels in normal tissues, but is widely upregulated during many pathological and physiological processes, especially cancer and fibrosis, making it a promising target for molecular imaging. Noninvasive and quantitative imaging of integrin $\alpha_v\beta_6$ expression would be very useful for disease diagnosis, treatment monitoring, and prognosis assessment. Although various molecular probes have been developed for positron emission tomography and single-photon emission computed tomography imaging of integrin $\alpha_v\beta_6$ expression in preclinical animal models, further research efforts are required to optimize integrin $\alpha_v\beta_6$-targeting probes for future potential clinical applications in the fields of oncology and beyond.

Keywords: Molecular probe, noninvasive imaging, positron emission tomography (PET), single-photon emission computed tomography (SPECT), cancer, fibrosis, wound healing

Introduction

Molecular imaging attempts to visualize, characterize, and measure biological processes at the molecular and cellular levels in living subjects [1]. There are a variety of modalities that can be used for molecular imaging, such as positron emission tomography (PET), single-photon emission computed tomography (SPECT), molecular magnetic resonance imaging (mMRI), magnetic resonance spectroscopy, optical bioluminescence, optical fluorescence, targeted contrast-enhanced ultrasound, and photoacoustic tomography [2, 3]. Many multimodality systems (e.g., PET/CT and PET/MRI), which combine two or more imaging modalities, are also commercially available or under extensive development. Molecular imaging of key biomarkers in disease progression using specific and sensitive imaging agents (probes) provides unique opportunities not only for the noninvasive determination of biological processes *in vivo*, but also for early detection of lesions, optimization of therapy, and prediction and assessment of treatment response.

Integrins are a family of cell adhesion receptors that directly bind to the extracellular matrix (ECM) and provide the traction necessary for cell motility and invasion [4]. Integrins are heterodimeric transmembrane receptors consisting of an α subunit and a β subunit. There are 18 α and 8 β subunits in humans that are known to comprise at least 24 different subtypes of integrins [5]. Eight out of the 24 integrins recognize native ligands such as fibronectin, vitronectin, and collagen through the Arg-Gly-Asp (RGD) triple peptide motif, whereas other integrins serve as collagen, laminin, and leukocyte-specific receptors (**Figure 1**). Several integrin subtypes are highly over-expressed on many types of cancer cells [6-10]. Integrin members such as integrin $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta_1$ are also crucial mediators of angiogenesis in solid tumor. Integrins expression is important for tumor progression and metastasis by promoting tumor cell migration, invasion, proliferation, and survival. In addition to cancer, integrins are also highly expressed during many normal and abnormal processes, such as fibrosis, wound healing, and inflammation [5].

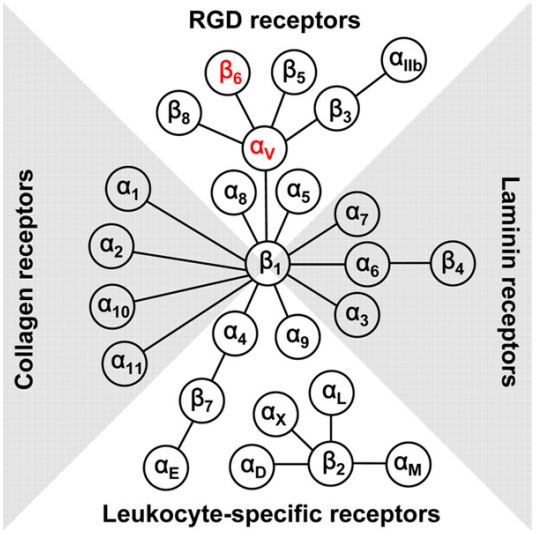

Figure 1. The integrin family in humans: 18 α and 8 β subunits assemble into 24 different functional heterodimers.

In the last decade, noninvasive imaging of integrin $\alpha_v\beta_3$ using RGD peptides has been extensively investigated for diagnosing cancer and other disorders and monitoring treatment response. Many excellent reviews have been published on this topic [11-15]. In contrast to integrin $\alpha_v\beta_3$, there are relatively few reports on the molecular imaging of other integrin subtypes. In recent years, emerging evidence indicates that $\alpha_v\beta_6$, another member of the integrin family, plays pivotal roles in the development of cancer, fibrosis, and other diseases [16, 17]. Noninvasive molecular imaging of integrin $\alpha_v\beta_6$ expression is expected to lead to better disease management. To date, several molecular probes targeting integrin $\alpha_v\beta_6$ have been developed for PET and SPECT imaging of integrin $\alpha_v\beta_6$ expression. In this review, we summarize the currently integrin $\alpha_v\beta_6$-targeting probes and discuss the progress to date in the molecular imaging of $\alpha_v\beta_6$ expression in living subjects to diagnose and monitor cancer and other diseases.

Integrin $\alpha_v\beta_6$ expression and signaling

Integrin $\alpha_v\beta_6$ is a subtype of the integrin family that is expressed exclusively on epithelial cells. The β_6 subunit only combines with α_v to generate a single heterodimer [16]. Integrin $\alpha_v\beta_6$ is usually expressed at low or undetectable levels in normal adult tissues but can be highly upregulated during pathological and physiological processes such as wound healing, fibrosis, inflammation, and cancer [4]. Thus, integrin $\alpha_v\beta_6$ has become a promising target for disease diagnosis and therapy. Integrin $\alpha_v\beta_6$ has been found to be highly upregulated in various kinds of cancers (**Table 1**) and is usually a predictor of poor patient outcome [18]. Integrin $\alpha_v\beta_6$ can also increase cancer metastasis by promoting cancer cell invasion and migration [19-21].

Integrin $\alpha_v\beta_6$ can bind extracellularly to the ECM and intracellularly to the cytoskeleton. Integrins transduce signals from the outside into the cell to modulate invasion, inhibit apoptosis, regulate matrix metalloproteinase (MMP) expression, and activate transforming growth factor-beta (TGF-β) [22-24]. Increasing evidence indicates that there is extensive crosstalk between integrins and TGF-β signaling in many physiological and pathological phenomena. The activation of TGF-β by integrin $\alpha_v\beta_6$ has been reported in several studies [25-27]. TGF-β is usually secreted as a large latent complex (LLC). LLCs are formed by disulfide covalent binding of latent TGF-β binding proteins to the small latent complexes, which consist of TGF-β noncovalently linked to the latency associated protein (LAP). Integrin $\alpha_v\beta_6$ binds to the LAP and then to the actin cytoskeleton. This induces a conformational change in the LLC, leading to TGF-β release to its receptors and subsequent pathway activation [28]. TGF-β activation can also upregulate MMPs through the extracellular signal-regulated kinase/mitogen-activated protein kinase pathway [29, 30].

In addition to cancer, *de novo* or increased expression of integrin $\alpha_v\beta_6$ also occurs during wound healing. During the re-epithelialization of wounds, the expression of integrin $\alpha_v\beta_6$ is highly upregulated at the wound edge as a consequence of an $\alpha_v\beta_5$ to $\alpha_v\beta_6$ switch [31-33]. The absence of integrin $\alpha_v\beta_6$ has been shown to delay epidermal and corneal wound healing [34, 35]. During wound healing, elevated $\alpha_v\beta_6$ expression is usually accompanied by a concomitant increase in TGF-β, which is important in wound healing for the regulation of re-epithelialization, suppression of inflammation, deposition of ECM, and formation of scar tissue [36]. Several studies have indicated that the role of integrin $\alpha_v\beta_6$ in wound healing is associated with TGF-β pathway activation [37-39].

The expression of integrin $\alpha_v\beta_6$ is also dramatically increased during fibrogenesis. Integrin

Table 1. Integrin $\alpha_v\beta_6$ expression in various cancers

Cancer Type	Population Size	Integrin $\alpha_v\beta_6$ expression (%)	Ref.
Endometrial	126	42	[81]
Basal cell	15 nodular	7	[82]
	13 morphoeic	77	
Oral squamous cell	30	90	[31]
	17	100	[83]
	5	80	[84]
	40	100	[85]
	11	100	[86]
Head and neck squamous cell	38	94.7	[87]
Esophagus	56	68	[47]
Liver	63	71.4	[88]
Colon	358	34	[88]
	488	37	[19]
Gastric	38	71	[89]
	300	36.7	[90]
Pancreas	34	100	[91]
Breast	90	18	[92]
Skin	49	84	[47]
Lung	51	50	[93]
	311	54	[18]
Ovary	45	100	[94]
Cervical squamous	85	59	[95]
	46	92	[47]
Thyroid	62 papillary	79.03	[96]
	28 follicular	78.57	

$\alpha_v\beta_6$-mediated TGF-β activation has been demonstrated to play an important role in animal models of fibrotic kidney, liver, and lung disease [27]. Various groups have reported that β_6 knockout mice were partly or completely protected from fibrosis in different organs, and that fibrosis can be inhibited by treatment with TGF-β antagonists and $\alpha_v\beta_6$ antibodies [37, 40-45].

Integrin $\alpha_v\beta_6$-targeting ligands

Because the expression pattern of integrin $\alpha_v\beta_6$ is restricted to tumors and other pathological tissues, it has become a promising diagnostic and therapeutic target. Several integrin $\alpha_v\beta_6$-specific antibodies have significantly blocked $\alpha_v\beta_6$ binding to the LAP and subsequent TGF-β activation [46]. Studies have also suggested that antibody-mediated blockade of integrin $\alpha_v\beta_6$ inhibits tumor progression in *in vivo* animal models [47, 48].

Several $\alpha_v\beta_6$-targeting peptides have also been developed, such as A20FMDV2 (NAVPNLRGDLQVLAQKVART). The sequence of A20FMDV2, a 20-mer peptide, was derived from the G-H loop of an envelope protein of the foot-and-mouth disease virus [49]. Although A20FMDV2 contains the RGD sequence, it has demonstrated a high specificity for $\alpha_v\beta_6$ but not for other RGD-recognizing integrins [50]. Furthermore, the RGDLXXL (X indicates a nonspecific amino acid) sequence was found to be a key motif for $\alpha_v\beta_6$ binding, whereas other integrins such as $\alpha_v\beta_3$ and $\alpha_v\beta_5$ only minimally interacted with A20FMDV2 [51]. Using a phage display approach, Oyama et al. isolated a 20-mer peptide with the sequence RGDLATLRQLAQEDGVVGVR, named H2009.1, from a panning peptide library of the lung adenocarcinoma cell line H2009 [52]. H2009.1 was shown to deliver chemotherapeutic agents specifically to tumor cells *in vitro* by targeting integrin $\alpha_v\beta_6$ [18, 53, 54] Further studies demonstrated that the tetrameric version of this peptide has a higher affinity for its cellular targets than the corresponding monomers [55]. Furthermore H2009.1 tetrameric peptide-functionalized liposomes [56] and multifunctional micelles encapsulated with superparamagnetic iron-oxide nanoparticles and doxorubicin [57] demonstrated significantly increased tumor cell targeting in MRI and drug-delivery applications. Similar to H2009.1, another $\alpha_v\beta_6$-targeting peptide, namely HBP-1 (H_2N-SPRGDLAVLGHKYCONH$_2$), was also identified via phage display screening against a cell line. HBP-1 showed preferential binding of $\alpha_v\beta_6$ over $\alpha_v\beta_3$. ^{125}I/^{131}I-labeled HBP-1 showed relatively stable and high affinity for $\alpha_v\beta_6$ [58]. As linear peptides may suffer from *in vivo* instability and rapid *in vivo* clearance, several engineered cysteine knots were recently designed and developed that exhibit high affinity for $\alpha_v\beta_6$ but not for the related subtypes $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta_1$ [59].

Because of their specific integrin $\alpha_v\beta_6$-targeting properties, several above mentioned ligands

Table 2. Selected list of the molecular imaging probes targeted to integrin $\alpha_v\beta_6$

Imaging modality	Probe	Targeting sequence	Disease model	Ref.
PET	[^{18}F]FBA-A20FMDV2	NAVPNLRGDLQVLAQKVART	Melanoma	[50]
	[^{18}F]FBA-PEG$_{28}$-A20FMDV2		Pancreatic cancer	[62]
	[^{18}F]FBA-(PEG$_{28}$)$_2$-A20FMDV2			
	^{64}Cu-PEG$_{28}$-A20FMDV2 (using different chelators)		Melanoma	[67, 68]
	[^{18}F]FBA-C$_6$-ADIBON$_3$-PEG$_7$-A20FMDV2		Melanoma	[64]
	^{64}Cu-DOTA-R$_0$1	GCILNMRTDLGTLLFRCRRDSDCPGACICRGNGYCG	Pancreatic and epidermoid cancer	[59]
	^{64}Cu-DOTA-S$_0$2	GCRSLARTDLDHLRGRCSSDSDCLAECICLENGFCG		
	^{18}F-fluorobenzoate-R$_0$1	GCILNMRTDLGTLLFRCRRDSDCPGACICRGNGYCG	Pancreatic cancer	[70]
	^{18}F-fluorobenzoate-S$_0$2	GCRSLARTDLDHLRGRCSSDSDCLAECICLENGFCG		
	^{64}Cu-AcD10	RGDLATLRQL	Non-small cell lung cancer	[69]
SPECT	^{125}I/^{131}I-HBP-1	SPRGDLAVLGHKY	Head and neck squamous cell carcinoma	[58]
	^{111}In-DTPA-A20FMDV2	NAVPNLRGDLQV LAQKVART	Breast cancer and melanoma	[71]
			Pulmonary fibrosis	[78]
	^{123}I-IFMDV2		Pancreatic cancer	[72]
	^{111}In-DTPA-Streptavidin-biotinylated-D25p	EPRGDLRTLAAREKENFNETLARL QEKGI	Melanoma	[73]
	^{99m}Tc-SAAC-S$_0$2	GCRSLARTDLDHLRGRCSSDSDCLAECICLENGFCG	Lung carcinoma	[74]
	^{99m}Tc-HYNIC-HK	RGDLATLRQLAQEDGVVGVRK	Pancreatic cancer	[75]

have been labeled with PET or SPECT radionuclides for *in vivo* imaging in animal models of cancer and other diseases. Representative probes are listed in **Table 2**.

In vivo imaging of integrin $\alpha_v\beta_6$ expression in cancer

Integrin $\alpha_v\beta_6$ is upregulated in numerous cancer types, such as colon, lung, cervical, ovarian, and pancreatic cancers [16], but is expressed at low or undetectable levels in healthy organs. Moreover, high expression of integrin $\alpha_v\beta_6$ in carcinomas is a prognostic factor for poor patient survival [19]. Thus, molecular imaging agents that target integrin $\alpha_v\beta_6$ would be a highly useful noninvasive method for cancer detection and monitoring cancer progression. PET and SPECT are the most commonly used modalities for the molecular imaging of integrin $\alpha_v\beta_6$.

PET

PET imaging offers high sensitivity (10^{-11}~10^{-12} M [60]) and has been extensively used for the noninvasive clinical management of cancer over the last decade. Traditional PET radionuclides include ^{18}F ($t_{1/2}$: 109.7 min), ^{11}C ($t_{1/2}$: 20.3 min), ^{13}N ($t_{1/2}$: 9.97 min), and ^{15}O ($t_{1/2}$: 2 min). In recent years, several PET radionuclides such as ^{68}Ga ($t_{1/2}$: 68 min), ^{64}Cu ($t_{1/2}$: 12.7 h), ^{86}Y ($t_{1/2}$: 14.7), and ^{89}Zr ($t_{1/2}$: 78.41 h) have been under extensive investigation in both preclinical and clinical studies.

Several integrin $\alpha_v\beta_6$-targeting ligands have been radiolabeled with PET radionuclides for *in vivo* imaging. The Sutcliffe group has radiolabeled A20FMDV2 with ^{18}F [50, 61-66] and ^{64}Cu [67, 68] for *in vivo* cancer imaging. By radiolabeling the N-terminus of A20FMDV2 with 4-[^{18}F] fluorobenzoic acid ([^{18}F]FBA), they demonstrated that A20FMDV2 binding is highly specific for integrin $\alpha_v\beta_6$ in *in vitro* cell binding assays [50]. Furthermore, [^{18}F]FBA-A20FMDV2 can be used to selectively image $\alpha_v\beta_6$-positive tumors *in vivo* in mice bearing both DX3puro and DX3puroβ6 human melanoma xenografts (**Figure 2A**) [50]. A20FMDV2 was also labeled with 3 different prosthetic groups, [^{18}F]FBA, [^{18}F]FPA and [^{18}F] FC5. Small-animal PET imaging and biodistribution studies revealed that radiolabeling of the prosthetic groups had a noticeable effect on A20FMDV2 pharmacokinetics, especially tumor uptake and metabolic fate [61]. Although ^{18}F-labeled A20FMDV2 exhibited specific integrin $\alpha_v\beta_6$ targeting *in vivo*, low tumor uptake and retention and metabolic instability of the probe limit its general application. To increase tumor uptake and improve *in vivo* pharmacokinetics of ^{18}F-A20FMDV2, two new radiotracers with polyethylene glycol (PEG) spacers were synthesized. The two modified radiotracers exhibited

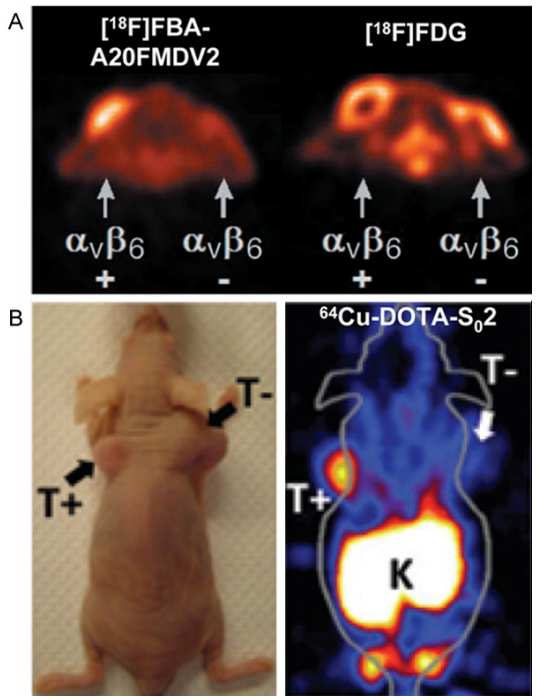

Figure 2. Small-animal PET imaging of integrin $\alpha_v\beta_6$ expression in cancer. A: Representative transaxial PET images of [^{18}F]FBA-A20FMDV2 and [^{18}F]FDG in the same mouse with the positive ($\alpha_v\beta_6$-expressing DX3puroβ_6) tumors located near the left shoulder and the negative (control DX3puro) tumors near the right shoulder. Adapted with permission from the American Association for Cancer Research: ref. [50]. B: The photograph and PET image of a mouse bearing both BxPC-3 $\alpha_v\beta_6$-positive (T+) and $\alpha_v\beta_6$-negative 293 (T-) xenografts injected with ^{64}Cu-DOTA-$S_0$2. Adapted with permission from the American Association for Cancer Research: ref. [59].

significantly improved tumor retention without affecting the high specificity for integrin $\alpha_v\beta_6$ [62]. Recently, a new radiolabeling method, copper-free strain-promoted click chemistry, was used to generate the tracer [^{18}F]FBA-C_6-ADIBON$_3$-PEG$_7$-A20FMDV2. Unfortunately, the new tracer altered A20FMDV2 pharmacokinetics, resulting in its unexpected uptake in the gall bladder and gastrointestinal tract [64].

Because of the relatively rapid radioactive decay of ^{18}F, A20FMDV2 was also labeled with ^{64}Cu, which has a nearly seven times longer half-life compared with ^{18}F. Unfortunately, the ^{64}Cu-labeled tracers showed unexpectedly high and persistent levels of radioactivity in the kidneys and liver, making them less suitable than the ^{18}F-labeled tracers [67]. Four different chelators were further investigated for ^{64}Cu radiolabeling of A20FMDV2; however, the data suggested no clear "best" chelator and no obvious effects of the individual chelators [68].

In addition to A20FMDV2, a 10-mer peptide sequence RGDLATLRQL selected from the $\alpha_v\beta_6$-specific H2009.1 peptide was also labeled with ^{64}Cu and investigated for its utility in *in vivo* PET imaging [69]. Two approaches were used to achieve peptide dimerization: dimerization of the peptide followed by conjugation to the chelator and direct presentation of two copies of the peptide on the chelator scaffold. The divalent probes ^{64}Cu-D10 and ^{64}Cu-$(M10)_2$ showed approximately three-fold higher tumor uptake compared with the monovalent probe, indicating the role of multivalency in signal amplification. To abrogate the nonspecific uptake of the divalent probes in the kidneys, the N-terminus of the peptide was acetylated, and the resulting bivalent probe ^{64}Cu-AcD10 exhibited significantly decreased kidney accumulation while maintaining tumor uptake [69].

As liner peptides generally have poor *in vivo* stability, Kimura et al. [59] engineered several highly stable cysteine knot peptides with high-affinity integrin $\alpha_v\beta_6$ binding, and labeled them with either ^{64}Cu [59] or ^{18}F [70]. Among the ^{64}Cu-labeled knot peptides, ^{64}Cu-NOTA-$S_0$2 showed the best *in vivo* imaging properties including excellent tumor/background ratio, rapid renal clearance, and high serum stability. Small-animal PET imaging demonstrated that ^{64}Cu-NOTA-$S_0$2 targeting of integrin $\alpha_v\beta_6$ can specifically detect pancreatic tumor xenografts (**Figure 2B**) [59]. In another study by the same group [70], two cystine knot peptides were radiolabeled with ^{18}F, which was considered to have better matched kinetics with the rapid clearance of the peptides. The results of their study demonstrated the translational promise of the radiotracer ^{18}F-fluorobenzoate-$R_0$1 for molecular imaging of integrin $\alpha_v\beta_6$ overexpression in pancreatic and other cancers.

SPECT

SPECT is the universally available nuclear medicine imaging technique because of the wider availability of gamma cameras and SPECT scanners. Common radionuclides used for SPECT imaging are ^{99m}Tc ($t_{1/2}$: 6.02 h), ^{111}In ($t_{1/2}$: 2.83 d), ^{123}I ($t_{1/2}$: 13.2 h), and ^{131}I ($t_{1/2}$: 8.04 d) [2]. The radionuclides used for SPECT usually have a longer half-life than those used for PET. More than 70% of the radiotracers used in clin-

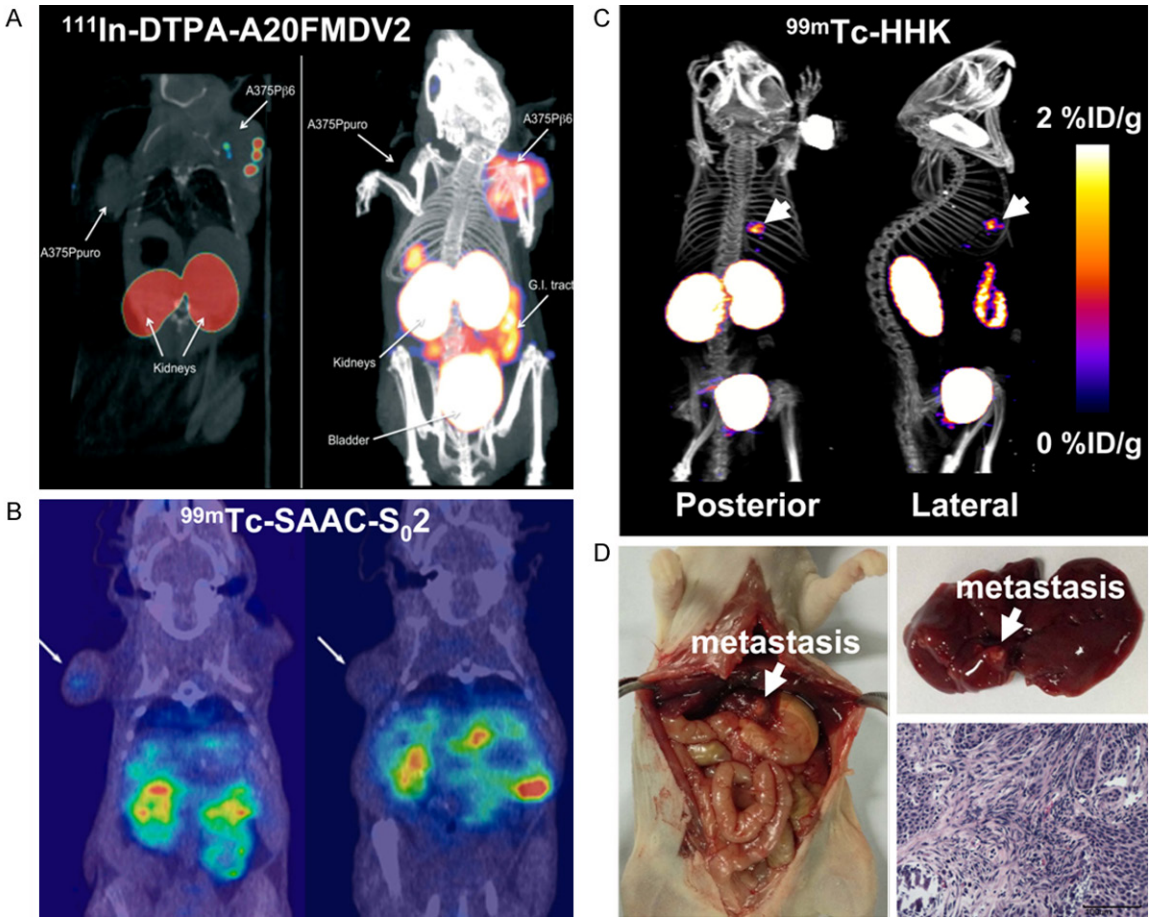

Figure 3. Small-animal SPECT imaging of integrin $\alpha_v\beta_6$ expression in cancer. (A) Small-animal SPECT (left) and SPECT/CT (right) images of mice bearing $\alpha_v\beta_6$-negative A375Ppuro (left shoulder) and $\alpha_v\beta_6$-positive A375Pβ_6 (right shoulder) tumors with ^{111}In-DTPA-A20FMDV2. Adapted with permission from ref. [71]. Copyright (2010) Pathological Society of Great Britain and Ireland. (B) Coronal SPECT/CT images of a mouse bearing $\alpha_v\beta_6$-positive HCC4006 xenograft (left) and a mouse bearing $\alpha_v\beta_6$-negative H838 xenograft (right) after injection of ^{99m}Tc-SAAC-S$_0$2. Adapted with permission from ref. [74]. Copyright (2014) American Chemical Society. (C) Whole-body posterior and right lateral SPECT/CT images of nude mice with liver metastasis of $\alpha_v\beta_6$-positive BxPC-3 tumors after ^{99m}Tc-HHK injection. (D) Mouse from (C) was sacrificed, and the liver metastasis was verified. This research was originally published in ref. [75]. Copyright (2014) the Society of Nuclear Medicine and Molecular Imaging, Inc.

ics are ^{99m}Tc compounds mainly because of their optimal nuclear properties, availability and low cost.

Compared with PET-based radiotracers, SPECT radiotracers for integrin $\alpha_v\beta_6$-targeting are relatively rare. A20FMDV2 was radiolabeled with ^{111}In-diethylenetriamine pentaacetic acid (DTPA), and the resulting probe ^{111}In-DTPA-A20FMDV2 was tested in SPECT imaging studies. ^{111}In-DTPA-A20FMDV2 was found to efficiently image $\alpha_v\beta_6$-positive cancers with high resolution (**Figure 3A**) [71]. However, as ^{111}In-DTPA-A20FMDV2 is degraded in serum, this probe may need to be modified to increase its stability. In a recent study, Man et al. [72] identified a single chain fragment variable, named D25p, using a 3-dimensional geometry-based library designed from ligand-receptor binding. ^{111}In-labeled D25p exhibited similar targeting capabilities to that of A20FMDV2 [73].

Zhu et al. conjugated the cysteine knot peptide S$_0$2 with a single amino acid chelate and labeled it with ^{99m}Tc [74]. The resulting probe was evaluated in two different tumor models and demonstrated several positive features such as high stability, quick tumor targeting, and rapid renal clearance (**Figure 3B**). However, because of the high lipophilicity of the ^{99m}Tc-(CO)$_3$ core, the probe showed high blood and liver uptake and retention [74]. Recently, we modified the

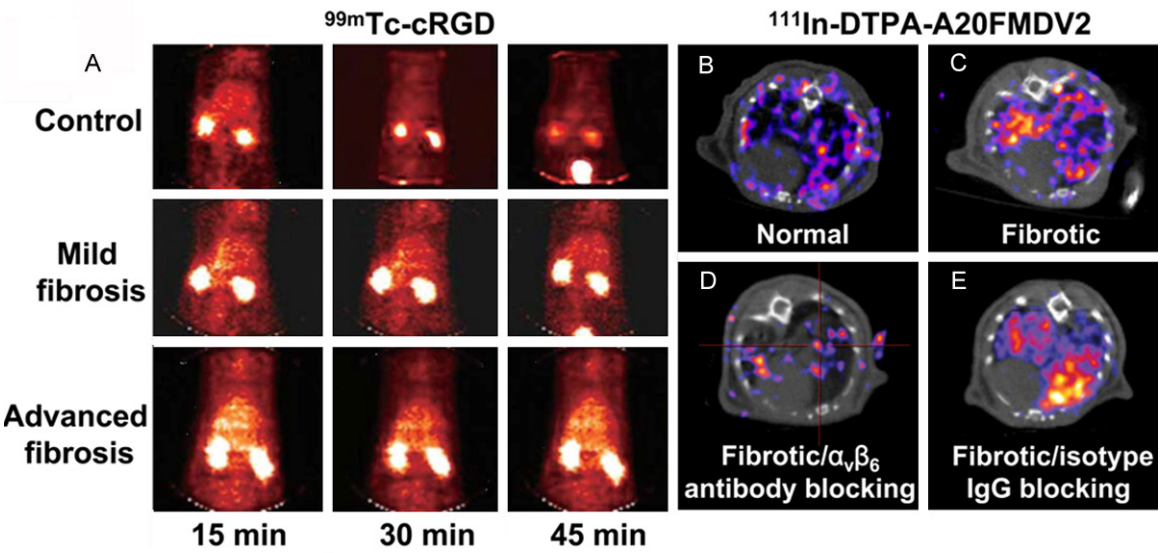

Figure 4. SPECT imaging of fibrosis. (A) SPECT images of rat with or without liver fibrosis after injection of an integrin $\alpha_v\beta_3$-targeting probe ^{99m}Tc-cRGD. Adapted with permission from ref. [76]. Copyright (2011) American Association for the Study of Liver Diseases. (B, C) Transaxial small-animal SPECT/CT images of ^{111}In-DTPA-A20FMDV2 in normal (B) and fibrotic (C) lungs of the mice. (D, E) Transaxial small-animal SPECT/CT images of ^{111}In-DTPA-A20FMDV2 in fibrotic lungs of the mice receiving integrin $\alpha_v\beta_6$-blocking antibody (D) or IgG isotype control (E). This research was originally published in ref. [78]. Copyright (2013) the Society of Nuclear Medicine and Molecular Imaging, Inc.

H2009.1 peptide by adding a lysine residue to the C-terminus [75]. The new peptide was conjugated with 6-hydrazinonicotinyl and then labeled with ^{99m}Tc to generate the integrin $\alpha_v\beta_6$-targeting probe ^{99m}Tc-HHK. Compared with ^{18}F-FDG, ^{99m}Tc-HHK can sensitively distinguish $\alpha_v\beta_6$-expressing tumors from $\alpha_v\beta_6$-negative tumors. In addition, SPECT imaging with ^{99m}Tc-HHK was found to be an effective approach for the noninvasive detection of liver metastatic lesions of integrin $\alpha_v\beta_6$-positive tumors (**Figure 3C**, **3D**) [75]. However, a major drawback of ^{99m}Tc-HHK is its relatively low tumor uptake, which may be partly due to its *in vivo* metabolic instability [75].

In vivo imaging of integrin $\alpha_v\beta_6$ expression in other disorders

Most integrin $\alpha_v\beta_6$ probes were designed for imaging $\alpha_v\beta_6$ expression in cancer. Besides cancer imaging, integrin $\alpha_v\beta_6$ probes can also be used to detect and monitor other pathological processes. Because $\alpha_v\beta_6$ is also upregulated in fibrogenesis, it has become a promising imaging target for lung and liver fibrosis.

The integrin family member $\alpha_v\beta_3$ has previously been used for the imaging of liver fibrosis. Li et al. [76] demonstrated that the expression of integrin $\alpha_v\beta_3$ was markedly upregulated on activated hepatic stellate cells in fibrotic livers and correlated with the stage of fibrosis. They successfully used a ^{99m}Tc-labeled RGD peptide, ^{99m}Tc-cRGD, to image $\alpha_v\beta_3$ expression in fibrotic livers. The probe showed potential to noninvasively distinguish different stages of liver fibrosis (**Figure 4A**) [76]. Because integrin $\alpha_v\beta_6$-mediated TGF-β activation is essential to the pathogenesis of idiopathic pulmonary fibrosis (IPF), integrin $\alpha_v\beta_6$ represents a promising therapeutic target for the treatment of this disease [77]. Recently, it has been reported that integrin $\alpha_v\beta_6$ may be a useful target for imaging IPF. The ability to use SPECT imaging of ^{111}In-labeled A20FMDV2 to monitor and predict the development of lung fibrosis was investigated in a bleomycin model of IPF (**Figure 4B**). This probe showed the potential to be used to detect the expression of $\alpha_v\beta_6$, which may be a promising biomarker to facilitate the stratification of IPF treatment [78].

Conclusion and future perspectives

In consideration of the important role integrin $\alpha_v\beta_6$ plays in many pathological and physiological processes, especially in cancer and fibrosis, noninvasive imaging of integrin $\alpha_v\beta_6$ expression would provide a great deal of information to

benefit disease detection, new drug development and validation, and patients management (e.g., treatment monitoring, dose optimization, and patient stratification). Quantitative correlation of probe uptake with $\alpha_v\beta_6$ expression level and disease progression would be an attractive noninvasive method to monitor clinical response to treatment. Noninvasive quantitative imaging of $\alpha_v\beta_6$ expression may also play a role in personalized medicine by allowing treatment efficacy to be optimized for each individual patient.

Currently, most $\alpha_v\beta_6$-targeted probes are used for radionuclide-based imaging modalities PET and SPECT, which have excellent tissue penetration depth and imaging sensitivity. Most developed probes have been peptide based because of the advantages of peptides as targeting agents, such as small molecular weight, minimal immunogenicity, rapid clearance from normal tissues, and easy synthesis, modification, and storage. However, most reported peptide-based integrin $\alpha_v\beta_6$-targeting probes exhibit limited receptor binding affinity, poor tumor uptake and retention, and metabolic instability. Although some success has been achieved in improving tumor uptake and stability *in vivo*, unresolved problems still remain, such as the unexpectedly high uptake of modified probes in some normal organs. Optimization strategies [13, 15], such as multimerization to increase receptor binding affinity, peptide cyclization to improve stability, and amino acid modifications to improve degradation resistance, may be needed in future studies. After peptide optimization, the next step is to further develop clinically translatable $\alpha_v\beta_6$-targeted probes.

All the currently investigated integrin $\alpha_v\beta_6$-targeting probes contain the RGDXXL or DLXXL targeting peptide sequence (**Table 2**). Previous studies have reported that the interaction of the A20FMDV2 peptide (NAVPNLRGDLQVLAQKVART) with integrin $\alpha_v\beta_6$ requires the RGD motif and the adjacent DLXXL motif, which forms the C-terminal helix, whereas the residues N-terminal to the RGD motif are not required for binding [49]. Further studies of the structural basis of natural ligand (e.g., fibronectin and TGF-β-LAP) recognition by integrin $\alpha_v\beta_6$ are needed to elucidate the essential elements required for high specificity and affinity peptide-$\alpha_v\beta_6$ interactions, thereby allowing for the rational design of targeting peptides for *in vivo* molecular imaging applications. In addition to peptides, antibody and engineered small molecule proteins could also be developed as they have high binding affinity and long tumor retention. Future research efforts are expected to be directed at the development of such molecular imaging probes for integrin $\alpha_v\beta_6$ targeting.

Recent advances in nanotechnology have provided numerous strategies for designing nanoplatform-based imaging and theranostic agents that efficiently deliver imaging signals or drugs to tumor sites by overcoming many biological barriers [79]. Nanoparticles have large surface areas to which a variety of targeting ligands and imaging moieties could be incorporated for multivalent and signal amplified molecular imaging. Nanoparticle-based targeting probes can also significantly increase tumor uptake and retention through passive targeting based on the enhanced permeability and retention effect. Nanoparticle-based theranostic agents designed to target integrin $\alpha_v\beta_6$ may broaden the applications of integrin $\alpha_v\beta_6$-targeted molecular imaging. However, caution should be taken for *in vivo* applications of nanoparticle-based therapeutic agents when functionalized by ligands with relatively low affinity such as peptides. For example, in a recent study, Gray et al. [80] studied a liposomal drug platform functionalized with tetramatic H2009.1 peptide for integrin $\alpha_v\beta_6$-targeted drug delivery for cancer therapy. However, the *in vivo* targeting of H2009.1 tetrameric peptide liposomal doxorubicin was similar to that of the control peptide and liposomes alone. Therefore, ligand targeting does not guarantee the enhanced efficacy of a liposomal drug, highlighting the complexity of *in vivo* targeting [80].

Current applications of integrin $\alpha_v\beta_6$-targeting probes are limited to PET and SPECT. The use of integrin $\alpha_v\beta_6$-targeting probes for multimodality imaging should also be explored. The combined advantages of different imaging modalities would provide more detailed and accurate information of pathological and physiological processes. This information is essential to better understand the mechanisms of integrin $\alpha_v\beta_6$ in biological processes. Advances in biology will help to further identify the key role of integrin $\alpha_v\beta_6$ in normal as well as abnormal biological processes, which may further

broaden the application of molecular $\alpha_v\beta_6$ imaging in living subjects beyond the field of oncology.

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Address correspondence to: Dr. Zhaofei Liu, Medical Isotopes Research Center, Peking University, 38 Xueyuan Road, Beijing 100191, China. Tel: +86-10-82802871; Fax: +86-10-82802871; E-mail: liuzf@bjmu.edu.cn

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