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Review Article

Molecular imaging of insulin-like growth factor 1 receptor in cancer

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Abstract: Insulin-like growth factor 1 receptor (IGF1R) plays an important role in proliferation, apoptosis, angiogenesis, and tumor invasion. Histology and in situ hybridization studies have revealed that IGF1R was significantly up-regulated at the protein and mRNA level in many types of cancer. Since measuring IGF1R expression with immunohistochemistry has many limitations, non-invasive imaging of IGF1R can allow for more accurate patient stratification (e.g. selecting the right patient population for IGF1R-targeted therapy) and more effective monitoring of the therapeutic responses in cancer patients. In this review, we will summarize the current status of imaging IGF1R expression in cancer, which includes single-photon emission computed tomography, positron emission tomography, fluorescence, and γ-camera imaging. The four major classes of ligands that have been developed for non-invasive visualization of IGF1R will be discussed: proteins, antibodies, peptides, and affibodies. To date, molecular imaging of IGF1R expression is understudied and more research effort is needed in the future.

Keywords: Insulin-like growth factor 1 receptor (IGF1R), molecular imaging, peptide nucleic acid (PNA), positron emission tomography (PET), single-photon emission computed tomography (SPECT), cancer

Introduction

The insulin-like growth factors (IGFs), proteins that have high sequence homology to insulin, are part of a complex system often referred to as the "IGF axis" [1, 2]. The IGF axis consists of two IGFs (IGF1 and IGF2), two trans-membrane receptor tyrosine kinases (IGF1R and IGF2R), and a family of six IGF-binding proteins (IGFBP1 to IGFBP6). IGF1, generally secreted by the liver as a result of stimulation by growth hormone (GH), is important in both the regulation of normal physiology and a number of pathological states such as cancer [3]. On the other hand, IGF2 is not regulated by GH and it is believed to be a primary growth factor required for early development, such as embryonic growth. Both IGF1 and IGF2 bind to IGF1R. Once bound, intracellular signaling pathways of cell survival and proliferation is activated (**Figure 1**). IGF2R only binds IGF2 and does not act as a signaling molecule since IGF2R has no intracellular kinase domain to initiate downstream signaling pathways. The six IGFBPs, in particular IGFBP3, exhibit similar binding affinities for IGF1 and IGF2 as that of IGF1R [4]. IGF signaling can be either increased or decreased by the IGFBPs in different contexts. However, the mechanism is understudied and poorly understood.

IGF1R plays important roles in proliferation, apoptosis, angiogenesis, and tumor invasion [3, 5]. It has been reported that its expression level is related to resistance to several targeted therapies [6, 7]. Histology and in situ hybridization have revealed that IGF1R was significantly up-regulated at the protein and mRNA level in many types of cancer, including breast, prostate, colon, pancreatic, lung and thyroid cancer [8-11]. In addition, down-regulation of IGF1R was associated with decreased tumor growth in various xenograft tumor models [12-14]. Because of the importance of IGF1R in cancer development, many therapeutic agents such as antibodies [15-17] and tyrosine kinase inhibitors [18, 19] have been developed to target/inhibit IGF1R and several of these agents are currently in clinical investigation.

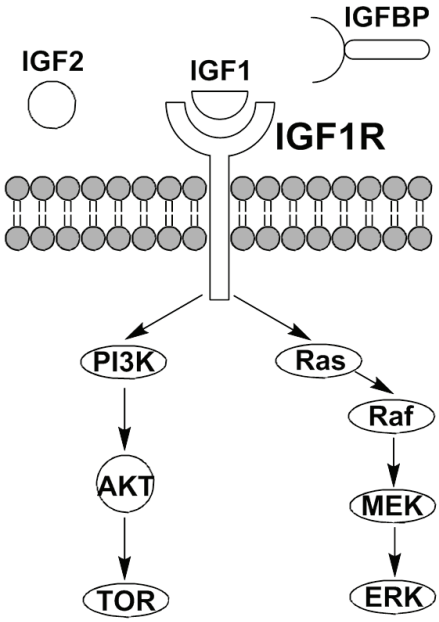

Figure 1. IGF1R activation and downstream signaling.

Clearly, tumor expression of IGF1R is necessary for efficacious response to anti-IGF1R therapies [20]. The current clinical assessment of IGF1R expression has been primarily based on immunohistochemistry of tumor tissue sections, which is invasive and has several limitations. For example, it requires multiple procedures to measure IGF1R expression in different lesions, while some tumor tissues may be difficult to obtain. In addition, the expression of IGF1R can be quite heterogeneous within the same tumor, which may also change during the course of anti-cancer therapies. Therefore, a clinically feasible technique to non-invasively image and quantify IGF1R expression is of great importance to cancer patient management.

Molecular imaging, "the visualization, characterization and measurement of biological processes at the molecular and cellular levels in humans and other living systems" [21], has evolved dramatically over the last decade and played an increasingly more important role in cancer diagnosis and patient management. Non-invasive imaging of IGF1R will provide invaluable information in three major aspects: patient stratification where patients with high IGF1R expression can be selected for IGF1R-targeted clinical trials; treatment monitoring where non-invasive imaging of IGF1R expression can indicate the therapeutic response; and facilitating the drug development process through monitoring the therapeutic efficacy of various drugs that target the IGF1R signaling pathway. In this review, we will summarize the current status of imaging IGF1R expression in cancer. To date, four major classes of ligands have been employed for imaging of IGF1R expression: proteins (e.g. IGF1 and its analogs), antibodies, peptides, and affibodies.

Imaging of IGF1R with IGF1-based ligands

IGF1 is consisted of 79 amino acids (molecular weight: 7,649 Da) in a single chain with three intra-molecular disulfide bridges. It binds to both IGF1R and insulin receptor (IR) [1, 22]. Being the naturally occurring ligand for IGF1R and commercially available, IGF1 is an interesting targeting ligand for positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging of IGF1R expression. However, IGFBPs in serum may restrain IGF1 from binding to IGF1R on tumor cells [23], which makes in vivo targeting and imaging of IGF1R with IGF1-based ligands quite challenging. In order to escape from the IGFBPs in the serum, many IGF1 analogs were engineered and investigated. These analogs either have a few of the terminal amino acids of the natural IGF1 sequence truncated, or have an additional amino acid sequence. Such modifications can result in reduced binding to most IGFBPs and make the IGF1 analogs more desirable than IGF1 itself for IGF1R targeting.

IGF1 and its analogs have been labeled with radioisotopes for imaging of IGF1R in xenograft tumor models. In one pioneering study, IGF1 was labeled with ^{125}I and the biodistribution of ^{125}I-labeled IGF1 was compared between two groups of mice: those injected with ^{125}I-labeled IGF1 alone and those co-injected with unlabeled IGF1 [24]. It was found that the binding of radiolabeled IGF1 to IGFBPs was dose dependent in vitro. However, due to the binding of heparin with IGFBPs which decreased their affinity for IGF1, there was more radioactivity associated with the free IGF1 in heparinized plasma. Therefore, it was suggested that tumor targeting could be improved by partial or complete blocking of IGF1-IGFBP complex formation with heparin or heparin-like glycosaminoglycans. In tumor-bearing nude mice, the radioactivity in normal tissues cleared rapidly. When co-injected with

unlabeled IGF1, there appeared to be more ^{125}I-labeled IGF1 in the serum due to partial saturation of IGFBPs in these mice, which led to lower radioactivity accumulation in most normal tissues and higher tumor uptake. Since catabolism of the tracer and loss of ^{125}I occurred rapidly in tumor cells and mice, the use of radiometals may be more advantageous in achieving high tumor-to-non-tumor ratio, which do not readily diffuse through the cell membrane and will remain intracellular in the tumor cells upon internalization. Furthermore, the use of an IGF1 analog with low affinity for IGFBPs may also increase the tumor-to-non-tumor ratio.

A truncated analog of IGF1, termed des(1-3) IGF1, was also labeled with ^{125}I and investigated in tumor-bearing nude mice [25]. Des(1-3)IGF1 is a 67-amino acid analog of human IGF1 with several of the N-terminal residues truncated, which retains the affinity to IGF1R but exhibits little binding to IGFBPs in vitro. Similar as ^{125}I-labeled IGF1, ^{125}I-labeled des(1-3)IGF1 also underwent rapid catabolism and exhibited fast clearance from normal tissues in mice. Co-injection with unlabeled ligand, which decreased the complex formation with IGFBPs, also led to higher tumor uptake of ^{125}I-labeled des(1-3)IGF1. Interestingly, even though the binding of des(1-3)IGF1 to IGFBPs was low in vitro, several IGF1-IGFBP complexes could be detected by high performance liquid chromatography (HPLC) at various times post-injection. This phenomenon was partly attributed to the rapid clearance of des(1-3)IGF1 which made the complexes more visible.

In the abovementioned reports, only biodistribution of the radiolabeled IGF1 (analog) was evaluated but not non-invasive imaging. Over the last decade, radionuclide-based imaging techniques (i.e. SPECT and PET) have been routinely used in clinical oncology [26-29]. Because of the wider availability of γ-cameras and SPECT scanners in the past, IGF1R imaging with protein-based ligands has been achieved with SPECT but not with PET yet. In one study, an IGF1 analog called IGF1(E3R) was labeled with ^{111}In for SPECT imaging of IGF1R in a human breast cancer xenograft model (**Figure 2**) [30]. IGF1(E3R) is an synthetic analog of IGF1 with the Glu^3 residue replaced by Arg^3, which disrupts its binding to IGFBPs but not to IGF1R based on in vitro competition assays. Not only were subcutaneous MCF-7/HER2-18 (HER2

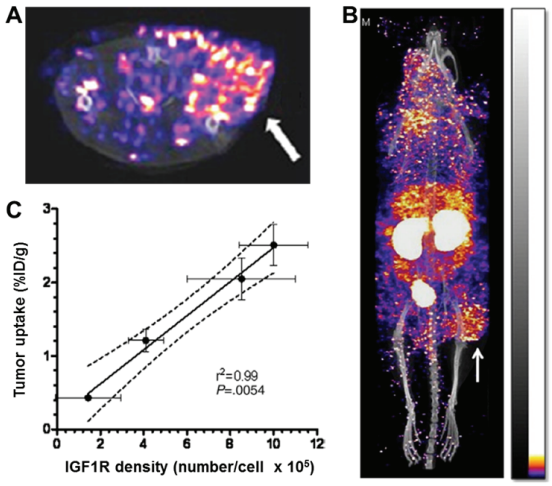

Figure 2. In vivo imaging of IGF1R with ^{111}In-IGF1 (E3R) in a mouse bearing a MCF-7/HER2-18 tumor. A. A transaxial slice of the mouse demonstrating tumor (indicated by an arrow) uptake of radioactivity at 4 h post-injection. B. A whole-body image of the mouse at 4 h post-injection. C. Correlation between IGF1R density measured on human cancer cell lines in vitro and tumor uptake in vivo at 4 h post-injection. The four tumor cells plotted (left to right) were H2N, HR2, MDA-MB-231, and MCF-7/HER2-18. Broken lines represent the 95% confidence interval of the fitting. Adapted from reference [30].

denotes human epidermal growth factor receptor 2) tumors visualized by ^{111}In-IGF1(E3R) microSPECT/CT with good tumor contrast, a strong linear correlation (R^2 = 0.99) also existed between tumor IGF1R expression level and tracer uptake. Furthermore, there was a direct relationship between IGF1R density and the resistance of the tumor cells to trastuzumab (an anti-HER2 monoclonal antibody [mAb] [31]) in vitro, despite overexpression of HER2. Therefore, imaging with such IGF1R-targeted tracers may be useful for identifying HER2-positive breast cancer patients that are resistant to trastuzumab, who should be treated with alternative therapeutic agents.

To the best of our knowledge, no IGF1 analogs have been reported for imaging of IGF1R in cancer other than with SPECT. Measurement of IGF1R expression level in tumors through PET should be investigated in the future, which is more sensitive and quantitative than SPECT and may enable accurate stratification of cancer patients for specific molecular therapies.

Imaging of IGF1R with antibody-based ligands

Several anti-IGF1R mAbs are in pre-clinical development and a few are currently in clinical investigation [15-17, 32]. One major advantage of these mAbs is their high specificity and affinity to IGF1R. Due to the relatively large size of mAbs (150 kDa), there are more sites for bioconjugation chemistry per mAb than each IGF1 analog. In addition, anti-IGF1R antibodies for molecular biology experiments (e.g. Western blotting, histology, and flow cytometry) are widely available from many commercial sources, which can potentially be used for imaging of IGF1R expression in cancer.

R1507, a fully humanized recombinant anti-IGF1R mAb, has been evaluated in multiple clinical trials [17, 33, 34]. It binds to the extracellular domain of IGF1R with high selectivity and affinity, which can lead to the displacement of bound IGF1 and loss of IGF1R on the cell surface. Currently, no clinically efficacious therapeutic agents are available for treating patients with triple-negative breast cancer [35, 36], which does not express estrogen receptor (ER), progesterone receptor (PR), or HER2. Therefore, R1507 was labeled with ^{111}In and ^{89}Zr for SPECT and PET imaging, respectively, in a triple-negative breast cancer model (**Figure 3**) [37]. The mAb was first labeled with ^{125}I to determine the pharmacokinetics of ^{125}I-R1507, which was compared with that of ^{111}In-R1507 in a subcutaneous SUM149 tumor model. Higher tumor uptake of ^{111}In-R1507 than that of ^{125}I-R1507 was observed at all time points examined, since R1507 is an internalizing mAb and radiometal is readily trapped intracellularly than radiohalogen.

Through a dose-escalation study of ^{111}In-R1507, the optimal antibody dose was determined to be of 3 μg or less per mouse. The tumors were clearly visualized with both SPECT and PET imaging (**Figure 3**), with prominent tumor uptake of both ^{111}In-R1507 and ^{89}Zr-R1507 [37]. One issue that remains to be addressed in future studies and clinical translation of radiolabeled R1507 is that the mAb only binds to human IGF1R, but does not cross-react with murine IGF1R. In human patients, IGF1R is widely expressed in normal tissues such as muscle and bone [38], which may lead to significant background signal of radiolabeled R1507 and decrease the tumor-to-non-tumor ratio to some extent. Feasibility study of imaging IGF1R in cancer patients with radiolabeled mAbs is eagerly anticipated.

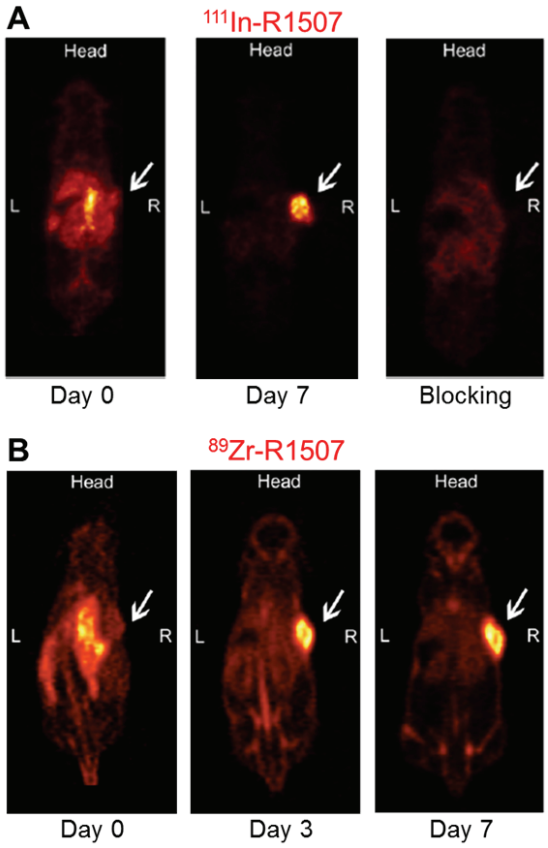

Figure 3. In vivo imaging of IGF1R with radiolabeled R1507 in mice bearing subcutaneous SUM149 tumors. A. SPECT images of a mouse injected with ^{111}In-R1507 at 0 and 7 days post-injection, and a mouse co-injected with 1 mg of unlabeled R1507 (i.e. blocking). B. PET images of a mouse administered with ^{89}Zr-R1507 at 0, 3, and 7 days post-injection. Tumors are indicated by arrows. Adapted from reference [37].

^{111}In-R1507 SPECT has also been utilized to predict the response to anti-IGF1R therapy in human bone sarcoma xenografts [39]. Three IGF1R-expressing human bone sarcoma xenografts (OS-1, EW-5, and EW-8) that exhibit high, modest, or no response to R1507 therapy, as well as an IGF1R-negative tumor model (OS-33), were generated in nude mice. Prominent and specific accumulation of ^{111}In-R1507 in OS-1 and EW-5 xenografts was observed. Interestingly, tracer uptake in IGF1R-positive, but unresponsive, EW-8 xenograft was similar to that of

the IGF1R-negative OS-33 tumor. These findings suggested positive correlation between target accessibility measured by non-invasive imaging and degree of response to the IGF1R inhibitor R1507, which indicated that ^{111}In-R1507 SPECT may be used as an independent method to screen for target accessibility thereby predicting the therapeutic response.

The different target accessibility of mAbs may be caused by the various tumor penetrating capacity of the same mAb in different tumors. In the abovementioned study [39], histological examination showed that the OS-1 tumors were well perfused without any necrotic areas. On the other hand, EW-5 and EW-8 tumors exhibited little extracellular matrix with multiple necrotic lesions surrounded by hypoxic borders. Clearly, both target expression level and target accessibility is needed for positive therapeutic response, especially in antibody-based therapies. Since tumors may develop resistance to anti-IGF1R mAbs and exhibit independence of the IGF1R pathway for growth (e.g. loss of IGF1R expression during treatment), non-invasive imaging of IGF1R can be employed for not only initial patient screening, but also potentially monitoring the resistance level during anti-IGF1R therapy in cancer patients.

Quantum dots (QDs) are inorganic fluorescent semiconductor nanoparticles with many attractive optical properties for imaging applications, such as strong signal intensity, continuous absorption spectra spanning the range from UV to near-infrared (700-900 nm), narrow emission spectra, etc. [40-42]. A humanized anti-IGF1R mAb AVE-1642 was conjugated to QD705 (emission maximum: 705 nm) or Alexa680, and the ability of the resulting probes to detect receptor expression and down-regulation of IGF1R was compared in vivo [43]. Both conjugates showed uptake in an orthotopic human breast cancer MCF-7 model. However, tumor accumulation of Alexa680-labeled AVE-1642 was demonstrated to be mostly attributable to active targeting, whereas tumor uptake of the QD conjugate was mainly due to the enhanced permeability and retention (EPR) effect. For many nanomaterial-based tumor targeting and imaging, poor extravasation of the probe into the tumor tissue is the major drawback [44, 45]. Due to the relatively large size of QD705-labeled AVE-1642 and the fact that IGF1R is expressed mainly on the tumor cells instead of on the tumor vasculature, the majority of QD705-labeled AVE-1642 could not efficiently extravasate to reach the target on tumor cells.

One major obstacle for translation of optical probes to clinical applications is the poor tissue penetration of light [46-49]. Over the last decade, guiding surgery with molecularly targeted fluorescent agents has attracted enormous interest. Recently, a proof-of-principle study investigating the potential benefit of intraoperative tumor-specific fluorescence imaging in staging and debulking surgery for ovarian cancer using a systemically administered targeted fluorescent agent (folate-FITC, which emits in the visible range) was reported [50]. The use of a near-infrared fluorescent agent (e.g. a labeled anti-IGF1R mAb), which has much better signal penetration in an imaging window with significantly less autofluorescence than in the visible range, may be more desirable for surgery guidance with the development of suitable intraoperative imaging systems.

Imaging of IGF1R with peptide-based ligands

Peptides have been widely investigated for molecular imaging of various types of cancer and tremendous progress has been made over the last decade [51-53]. A classic example is radiolabeled Arg-Gly-Asp (RGD) peptides and their derivatives for imaging tumor expression of integrin $\alpha_v\beta_3$, a cell adhesion molecule which plays important roles during tumor angiogenesis and metastasis [54-56]. Precise mapping of the IGF1-binding site on IGF1R was recently reported using single point mutations of IGF1R [57]. It was found that the IGF1 C-domain (residues 29-37; T-G-Y-G-S-S-R-R) interacts with the cysteine-rich domain of IGF1R [58]. By molecular modeling of IGF1 [58], several small IGF1-like peptides were designed to bind IGF1R. These synthetic peptides were modeled on the domains with the least similarity between IGF1 and insulin. To date, direct labeling of IGF1-like peptides to image IGF1R expression has not been reported yet. However, taking advantage of IGF1R-mediated endocytosis, IGF1-like peptides have been used as the targeting ligand to deliver peptide nucleic acids (PNAs) for imaging overexpressed oncogene messenger RNA (mRNA) in preclinical tumor models.

PNA is a synthetic polymer similar to DNA or RNA, in which the deoxyribose/ribose phosphate backbone is replaced by a peptide backbone composed of repeating N-(2-aminoethyl)-

glycine units [59]. The binding of PNA/DNA strands is stronger than that of DNA/DNA strands, since the backbone of PNA does not contain charged phosphate groups hence there is no electrostatic repulsion between strands. PNA displays higher T_m (i.e. "melting" temperature) values for duplexes formed with single-stranded DNA or RNA, which is also more resistant to both proteases and nucleases [60, 61]. In addition, the backbone of PNA typically does not exhibit significant non-specific binding to intracellular nucleic acid-binding proteins, which usually bind negatively charged oligonucleotide analogs. The antisense effect of PNA has been demonstrated in vitro by microinjection into individual cells [62]. However, unmodified PNA cannot efficiently cross cell membranes to reach its intracellular target [63], which can be improved by covalent linkage to a cell penetrating peptide.

The schematic design of a PNA-based dual-specific imaging agent is shown in **Figure 4A**. It was proposed that by targeting two species (i.e. mRNA and IGF1R) at the same time, these agents may allow for non-invasive imaging and quantification of both targets and provide more information per PET/SPECT scan. The peptide ligand can bind to IGF1R that is expressed on tumor cells and lead to internalization, after which the PNA will hybridize with the overexpressed oncogene mRNA. Cyclin D1 (CCND1) is a member of the highly conserved cyclin family. Overexpression of CCND1 mRNA has been reported to distinguish invasive and in situ breast carcinomas from non-malignant lesions [64]. Based on these findings, a CCND1-specific PNA hybridization sequence (CTGGTGTTCCAT), separated by a C-terminal spacer to a cyclized IGF1-like peptide (D-Cys-Ser-Lys-Cys), was labeled with ^{64}Cu (for PET) or ^{99m}Tc (for SPECT) [65, 66]. It was demonstrated that the resulting chimeras were able to non-invasively detect CCND1 mRNA in IGF1R-overexpressing human MCF-7 breast cancer xenografts in nude mice. Furthermore, the chimeras were also able to lower the tumor CCND1 protein level after intratumoral injection. PET imaging revealed prominent tumor accumulation of the ^{64}Cu-labeled chimeras after intravenous injection, which exhibited faster and higher tumor uptake than the ^{99m}Tc-labeled chimeras (**Figure 4B**).

Using the same cyclized IGF1-like peptide as the targeting ligand, two other PNAs targeting different oncogene mRNAs (i.e. MYC and KRAS) have been employed for the imaging of breast and pancreatic cancer xenografts [67-70]. Further efforts to improve the targeting and internalization efficiency of the IGF1-like peptides are needed to enhance the cellular/tumor uptake. In order to increase the circulation half-life and tumor uptake of the imaging probe, an extended polydiamidopropanoyl (PDAP) dendrimer with increasing numbers of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelators was conjugated to a KRAS2 PNA-peptide chimera via an N-terminal aminoethoxyethoxyacetate (AEEA) linker, resulting in a $(DOTA\text{-}AEEA)_n\text{-}PDAP^m\text{-}AEEA_2\text{-}KRAS2$ PNA-AEEA-D(Cys-Ser-Lys-Cys) nanoparticle [71]. After labeling the nanoparticle with ^{111}In for in vivo investigation, it was found that PDAP dendrimer with up to 16 DOTA chelators attached to the PNA-IGF1-like peptide chimeras enhanced accumulation of the probe and improved the retention time in the tumor.

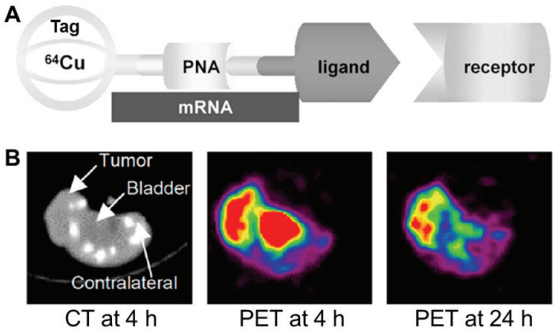

Figure 4. Imaging of IGF1R and mRNA with PNA-peptide chimeras. A. Schematic design of a PNA-peptide chimera targeting IGF1R and mRNA. B. Transaxial PET/CT images of a mouse bearing MCF-7 human breast cancer xenograft at 4 and 24 h post-injection of a ^{64}Cu-labeled chimera targeting CCND1 mRNA and IGF1R. Adapted from reference [65].

These PNA-based studies represent an intriguing strategy for imaging of cancer-related mRNA and indirect visualization of IGF1R expression. The major advantages of using IGF1-like peptides to target IGF1R are: 1) The small size of the resulting probe can have higher tumor penetrating capacity than the much larger antibody-based probes; 2) The short circulation half-life of the probes can lead to faster binding to the target and whole body clearance, which gives less background signal and potentially better tumor contrast; 3) The labeling condition is

more tolerant for peptides than antibodies/proteins, which have to be labeled under mild conditions. However, with such a short peptide sequence (four amino acid residues with two cysteines to form a disulfide bridge), the binding affinity/specificity of the resulting probes for IGF1R may be compromised. Direct labeling and imaging of these IGF1-like peptides for evaluation of IGF1R expression, and side-by-side comparison with the PNA-peptide chimera-based imaging, will be interesting and should be investigated in future studies.

Imaging of IGF1R with affibody-based ligands

Affibody molecules are a class of small (6.5 kDa) phage display-selected scaffold-based robust proteins, based on one of the independently folding IgG-binding domains of staphylococcal protein A [72, 73]. Unlike antibodies, affibody molecules are composed of α-helices and lack disulfide bridges. Combinatorial randomization of 13 amino acid residues in two helices that comprise the original Fc-binding surface of the Z-domain results in a library from which affibody molecules can be selected for specific binding to a target of interest [74]. Using this strategy, affibody molecules with specific binding to a variety of targets have been selected, with affinities in the picomolar to micromolar range. Combining small size with high affinity and specificity, affibody molecules are promising targeting ligands for both molecular imaging and many other biomedical applications [75, 76]. The small size and simple structure makes affibody production feasible by chemical synthesis. In addition, the robustness and refolding properties of affibodies can tolerate harsh labeling conditions that can denature most proteins.

An IGF1R-binding affibody molecule $Z_{IGF1R:4551}$ was site-specifically labeled with ^{111}In, via a cysteine residue at the C-terminus, for imaging of IGF1R in prostate cancer xenografts [77]. ^{111}In-labeling was carried out at 90°C for 30 min, which did not alter the IGF1R binding properties of $Z_{IGF1R:4551}$. In vitro evaluation of ^{111}In-DOTA-$Z_{IGF1R:4551}$ in prostate cancer DU-145 cells demonstrated that internalization of the tracer was inefficient, at < 40% after incubation for 4 h. Biodistribution studies in normal mice showed that tracer clearance was predominantly through the renal pathway, and tracer uptake in IGF1R-expressing tissues was saturable while

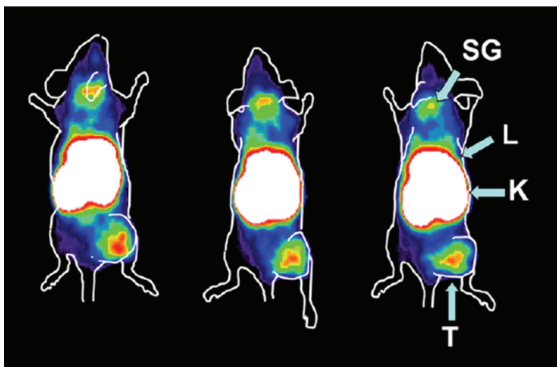

Figure 5. In vivo imaging of IGF1R expression in DU-145 prostate cancer xenografts in mice with ^{111}In-DOTA-$Z_{IGF1R:4551}$. Images were acquired at 8 h post-injection with a planar γ-camera. T: Tumor, K: Kidney, L: Liver, SG: Salivary Gland. Adapted from reference [77].

the amount of radioactivity in the liver was independent of the affibody dose. In tumor-bearing mice, ^{111}In-DOTA-$Z_{IGF1R:4551}$ had modest tumor uptake and tumor-to-blood ratio of about 3 at 8 h post-injection (**Figure 5**). This study represents the first example of using affibody molecules for in vivo imaging of IGF1R expression, whereas most previous reports on affibody-based imaging was focused on the HER-kinase axis [75, 78]. Since ^{111}In-DOTA-$Z_{IGF1R:4551}$ binds to both human and murine IGF1R, the xenograft model used here was considered to be adequate for evaluation of IGF1R targeting in vivo and be able to mimic the potential clinical situation, since imaging results can be significantly influenced by the expression of IGF1R in normal tissues. The relatively high tracer uptake in the kidneys and the liver will need to be reduced before future clinical translation of these imaging agents.

Conclusion and future perspectives

An array of targeting ligands has been investigated for imaging of IGF1R expression in cancer, ranging from small peptide-based ligands (~1 kDa) to full-size antibodies (~150 kDa). Considering the important roles that IGF1R plays during tumor development [3, 79], molecular imaging of IGF1R has been understudied. To date, non-invasive imaging of IGF1R has been achieved only with radionuclide-based imaging techniques (i.e. SPECT and PET) and fluorescence imaging. Visualization of IGF1R

expression has potential clinical applications in many aspects: cancer diagnosis where lesions with high IGF1R expression can be detected; patient stratification where patients with high IGF1R expression can be selected for IGF1R-targeted therapies/clinical trials; treatment monitoring where non-invasive imaging of IGF1R expression can indicate the therapeutic response; facilitating new anti-cancer drug development through monitoring the therapeutic efficacy of various drugs that target the IGF1R signaling pathway; among others. Quantitative correlation of IGF1R expression level with tracer uptake would be very compelling for future treatment monitoring applications, where the biological changes during therapeutic intervention can be non-invasively and quantitatively assessed in each individual patient. Much further effort should be directed towards the development of translatable IGF1R-targeted imaging agents.

Because of their excellent sensitivity and tissue penetration, radionuclide-based imaging techniques possess much higher clinical potential than non-radionuclide-based techniques. To date, most of the IGF1R-targeted imaging agents are radionuclide-based. The naturally-occurring IGF1, its analogs, and PNA-peptide chimeras have been labeled with ^{125}I, ^{111}In, ^{99m}Tc, and ^{64}Cu for biodistribution and SPECT/PET imaging, an IGF1R-targeted mAb R1507 has been labeled with ^{111}In and ^{89}Zr for SPECT and PET imaging respectively, and an IGF1R-binding affibody has been labeled with ^{111}In for SPECT imaging. Other molecular imaging modalities, such as magnetic resonance imaging and ultrasound, have not been employed for IGF1R imaging yet which may also play a role in future imaging of IGF1R in cancer.

The major hurdles/limitations of the reported studies on imaging of IGF1R can be divided into the following categories. First, the existence of IGFBPs in the serum can bind to IGF1, which makes IGF1R targeting with natural IGF1 or IGF1 analogs quite challenging. Second, preclinical imaging with antibody-based ligands was performed under optimal conditions, where IGF1R expression in normal human tissues may not be present in mouse models. In cancer patients, normal tissue expression of IGF1R can result in enhanced background signal and reduced tumor uptake. Third, direct labeling of IGF1-like peptides which do not bind to IGFBPs or IR has not been studied yet. Fourth, the development of more IGF1R-targeting ligands and further optimization of their pharmacokinetics should be pursued in future investigation. Lastly, multimodality imaging of IGF1R expression, where the same probe can be simultaneously detected by two or more imaging modalities, should be developed in the future. Among all of the molecular imaging modalities, no single modality is perfect and each technique has its advantages and disadvantages. A combination of multiple imaging techniques can offer synergistic advantages which no single modality alone can offer. For example, IGF1R-binding PET/optical agents can be used for not only lesion detection and patient stratification (with PET), but also for surgical guidance of tumor removal (with optical imaging). Clinical translation and investigation of optimal IGF1R-targeted imaging probes in cancer patients are eagerly awaited.

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