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23

Phage-Displayed Recombinant Peptides for Non-Invasive Imaging Assessment of Tumor Responsiveness to Ionizing Radiation and Tyrosine Kinase Inhibitors

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1. Introduction

Recent studies have resulted in a variety of therapeutic options for cancer. However, tumor patients, even a same patient at different disease stages, respond(s) to a treatment protocol with different efficacy. A concept of personalized medicine has been developed to deliver individually tailored treatment upon the unique responsiveness of each patient. Currently, it is still challenging to predict treatment outcomes due to the genetic complexity and heterogeneity of cancers, which underlies the varied responses to treatment. To efficiently design treatment strategies and monitor the outcomes of therapies for individual patient, tumor responsiveness to a specific treatment regimen needs to be assesses in a time- and cost-efficient manner.

Non-invasive imaging technologies have demonstrated great potentials in diagnosis and treatment management by monitoring individual patient's disease condition and progression. Currently, anatomic and functional imaging modalities have been generally applied to detect, stage, and monitor tumors. Compared to the anatomic imaging that measures tumor size, functional or molecular imaging provides more information on tumor metabolism, biomarker expression, cell death or proliferation and thus is more relevant to the imaging assessment of tumor responsiveness to a treatment regimen, especially when the treatment affects tumors through blocking the tumor progression instead of shrinking the tumor size. Discovery of novel probes that specifically binds to tumor-limited targets with sound biological relevance is a limiting factor to develop such functional imaging modality.

Compared to antibody (~150 kD), peptide is in a much smaller size (1-2 kD) that enables an improved tissue penetration, faster clearance from circulation system, and less immunogenic property that are expected for a imaging probe, especially in the repeated assessment of treatment responsiveness in solid tumors. Advances in phage display-related technologies have facilitated the discovery and development of peptide derivatives as imaging probes for a variety of tumors. By using one example of HVGGSSV peptide that has

been discovered and tested for non-invasive imaging assessment of tumor response to ionizing radiation (IR) and receptor tyrosine kinase (RTK) inhibitors in multiple tumor types, this review demonstrates that phage-displayed peptides hold potentials in personalized medicine by facilitating molecular imaging, discovery of diagnostic biomarker or therapeutic target, and tumor-targeted drug delivery.

2. Advancement in radiation therapy of cancer

Cancer is the leading cause of death worldwide, deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030 (World Health Organization). Currently, radiotherapy is one of the most important modalities for the treatment of cancers. Over 60% of cancer patients received radiotherapy as part of their treatments.

2.1 Radiotherapy

Radiotherapy is the medical use of ionizing radiation as part of cancer treatment to control malignant cells. Radiation therapy may be used to treat localized solid tumors, such as cancers of the skin, tongue, larynx, brain, breast, lung, prostate or uterine cervix. It can also be used to treat leukemia and lymphoma. It works by damaging the DNA of cancerous cells through the use of one of two types of high energy radiation, photon or charged particle. This damage is either direct or indirect ionizing the atoms which make up the DNA chain. Indirect damage happens as a result of the ionization of water by high energy radiations, such as X-ray or gamma ray, forming free radicals, notably hydroxyl radicals, which then damage the DNA and form single- or double-stranded DNA breaks. Direct damage to DNA occurs through charged particles such as proton, boron, carbon or neon ions. Due to their relatively large mass, charged particles directly strike DNA and transfer high energy to DNA molecules and usually cause double-stranded DNA breaks. The accumulating damages to cancer cells' DNA cause them to die or proliferate more slowly. To minimize the damage to normal cells, the total dose of radiation therapy is usually fractionated into several smaller doses to allow normal cells time to recover. In clinics, to spare normal tissues from the treatment, shaped radiation beams are aimed from several angles of exposure to intersect at the tumor, providing a much larger absorbed dose in the tumors than in the surrounding tissues. Brachytherapy, in which a radiation source is placed inside or next to the cancer area, is another technique to minimize exposure to healthy tissues during treatment of cancers in the breast, prostate and other organs. It is also common to combine radiotherapy with surgery, chemotherapy, hormone therapy or immunotherapy to maximize treatment efficiency.

2.2 Radiosensitizer

Besides the rapid advances in radiotherapy technologies, the increased understanding of cancer biology and signaling networks behind radiotherapy has led to the development of newer chemotherapy agents that help to increase radiation treatment efficiency. Pathways targeted for radiosensitization include DNA damage repair, cell cycle progression, cell survival and death, angiogenesis, or modulation of tumor microenvironment. For example, hypoxia is one general characteristic associated with fast-growing solid tumors. It stimulates tumor malignant progression and induces HIF-1a. A few studies have found that low

oxygen levels in tumors are associated with a poor response to radiotherapy (Overgaard, 2007). Well-oxygenated cells show an approximately 2-3 fold increases in radiosensitivity compared to hypoxic cells (Dasu and Denekamp, 1998). This discovery results in the development of a family of drugs – oxygen radiosensitizers. From initial attempts to increase oxygen delivery to the tumor by using hyperbaric oxygen in radiotherapy (Mayer et al., 2005), to later use oxygen mimetics/Electron-affinity agents, such as nitroimidazoles (Brown, 1975), or transition metal complexes, such as cisplatin (Liu et al., 1997), oxygen radiosensitizers significantly increase the radiotherapy efficiency. Currently, attention has been given to hypoxic cytotoxins, a group of drugs that selectively or preferably destroys cells in a hypoxic environment. These classes of compounds, such as mitomycin (De Ridder et al., 2008; Moore, 1977), are different from classic radiosensitizer in that they can be converted to cytotoxic agents under low oxygenation states, and they provide valuable adjuncts to radiotherapy. Recently, a wide variety of drugs that influence the DNA damage and repair pathways are being evaluated in conjunction with radiation. It includes topoisomerase inhibitors (e.g. camptothecin, topotecan), the hypoxia-activated anthraquinone AQ4N, and alkylating agents such as temozolomide. Proteins involved in tumor malignant progresses are also drawn attention as attractive targets of radiosensitizers, such as HIF-1a (Palayoor et al., 2008), survivin (Miyazaki et al.), Ras (Cengel and McKenna, 2005), epidermal growth factor receptors and related kinases (Sartor, 2004; Williams et al., 2004). Inhibitors for receptor tyrosine kinases such as vascular endothelial growth factor have been extensively studied and applied to improve the therapeutic efficacy of radiotherapy (Vallerga et al., 2004).

3. Assessment of tumor responses to radiotherapy

Different types of cancers possessed different mutations. Even the same type of cancers, they show different growth characteristics at different locations and in different patients. The heterogeneity of cancers underlies the different responses of cancers to the same treatment. Currently, cancer response is measured by imaging assessment of tumor volumes or by repeated biopsy. The whole processes are time consuming and inefficient. The recent advancement in imaging technologies has revolutionized medical diagnosis and prognosis. From the macroscopic anatomical sites down to a functional assessment of processes within tumors, imaging provide us a method to evaluate tumor response to irradiation treatment in a non-invasive, reliable and repeatable way (Lowery et al., 2011). So far, a few biomarkers have been explored for imaging to predict patients' outcomes after radiation treatment.

3.1 Cell metabolism

Cell metabolism is the earliest biomarker being studied after radiation treatment. Positron emission tomography (PET) has been used to evaluate tumor metabolism. ^{18}F-fuorodeoxyglucose (FDG) is the most common PET tracer for metabolism study. FDG, a glucose analog, is taken up by high-glucose-consuming cells, such as cancer cells. But FDG cannot be further metabolized during glycolysis and it becomes trapped and rapidly accumulates within the cell. As a result, the distribution of ^{18}F-FDG is a good reflection of the location of cancer cells. It is routinely used for the staging of cancer and for the monitoring of therapy (Allal et al., 2004).

3.2 Cell proliferation

The development of proliferation probes for PET imaging has enabled the *in vivo* evaluation of cell proliferation (Shields et al., 1998). Among those probes, nucleoside-based imaging probes (3'deoxy-3'-^{18}F-fluorothymidine, FLT) or amino acids based imaging probes are gaining popularity. ^{18}F-FLT is a pyrimidine analog that, after uptake into the cell, is phosphorylated by thymidine kinase 1. The phosphorylated ^{18}F-FLT can not leave the cell and result in the intracellular accumulation of radioactivity. Thymidine kinase 1 is a principal enzyme in the salvage pathway of DNA synthesis and exhibits increased activity during the S phase of the cell cycle. ^{18}F-FLT uptake, therefore, reflects cellular proliferation. Amino acid metabolism is increased in fast proliferating tumor cells. Among the 20 essential amino acids, l-[^{11}C]MET, [^{18}F]fluorotyrosine, l-[^{11}C]leucine, and [18F]fluoro-α-methyl tyrosine have been widely used in the detection of tumors (Laverman et al., 2002). Changes in l-[^{11}C]MET uptake have already been shown to reflect response to radiotherapy treatment in patients suffering from a wide variety of tumors (Team, 2005b).

3.3 Tumor vasculature and hypoxia

Although being characterized as vasculature-rich structures, tumors often develop regions of hypoxia due to the leaky and disorganized tumor blood vessels. Low oxygen environment will promote tumor angiogenesis, metastasis and render tumors resistant to radiation treatment (Tatum et al., 2006). Therefore, the tumor vasculature structure and oxygen level are valuable biomarkers for prognosis after treatment. ^{18}F-fluoromisonidazole is the most widely used PET tracer for detecting tumor hypoxia. After uptake in cell, it is reduced and binds selectively to macromolecules under hypoxic conditions (Team, 2005a). One recent study indicates that ^{18}F-fluoromisonidazole uptake is correlated with radiation treatment outcome in Head and neck cancer (Thorwarth et al., 2005). As to the tumor vasculature, several studies have been proposed using two different techniques - quantified power Doppler sonography or Dynamic contrast-enhanced MRI (DCE-MRI). And both showed promising results (Hormigo et al., 2007; Kim et al., 2006; Mangla et al., 2010).

3.4 Apoptosis

Since its recognition as one of the major forms of cell death after radiation, apoptosis is being increasingly studied as a biomarker of cellular radiosensitivity and a prognosis marker for radiotherapy outcome. During the apoptosis process, phosphatidylserine (PS) flips from the inner leaflet of the cell membrane to the exterior of the cells. Annexin V, a cellular protein of the Annexin family, binds to the exposed PS. To date, Annexin V has been fluorinated for PET and radioiodinated for SPECT. Annexin V labeled with 99mTc has demonstrated significant uptake in patients suffering form myocardial infarction (Narula et al., 2001). Studies assessing quantitative ^{99m}Tc-Annexin V uptake in human tumors and their relationship to radiotherapy outcome are underway.

4. Phage-displayed peptides as novel imaging probes for assessing tumor response to treatment

Recently, advances in phage display-related technologies facilitate the use of small peptide derivatives as probe molecules for recognition and targeting tumors. Phage display enables discovery and optimization of affinity probes for the known tumor-specific biomarkers.

Furthermore, this technology makes it possible to *de novo* discover novel imaging probes, and eventually identify novel diagnostic markers or therapeutic targets of cancer. In vivo screening against heterogeneous tumor targets have generated a diverse group of peptides for cancer-targeted delivery of imaging or therapeutic agents.

4.1 Principle of phage display

A phage is a type of viruses that infect bacteria. Typically, phages consist of a protein capsid enclosing genetic materials. Due to its simple structure, phages have been developed into a powerful tool in biological studies. Phage display was originally invented by George P. Smith in 1985 when he demonstrated the display of exogenous peptides on the surface of filamentous phage by fusing the DNA of the peptide on to the capsid gene of filamentous phages (Smith, 1985) (Fig. 1). This technology was further developed and improved to display large proteins such as enzymes and antibodies (Fernandez-Gacio et al., 2003; Han et al., 2004). The connection between genotype and phenotype enables large libraries of peptides or proteins to be screened in a relative fast and economic way. The most common phages used in phage display are M13 filamentous phage and T7 phage (Krumpe et al., 2006; Smith and Petrenko, 1997). The functional moiety on the phage surface can be short peptides, recombinant proteins, engineered antibody fragments or scaffold proteins. Screening can be conducted on the purified organic or inorganic materials, cells, or tissues.

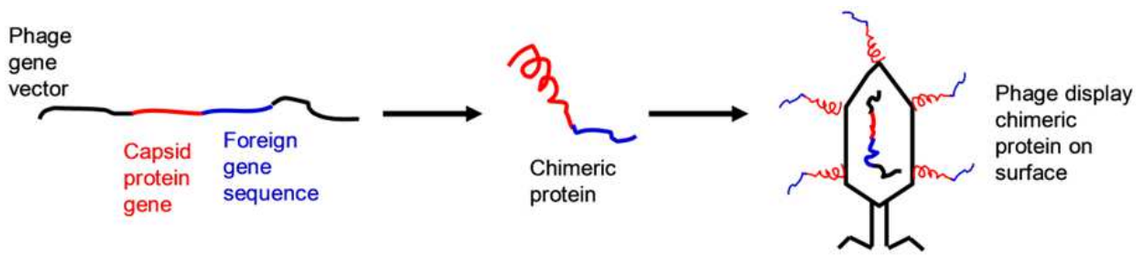

Fig. 1. Schematic illustration of phage display. Foreign gene sequences encoding short peptides, recombinant proteins or large antibody fragments can be fused with capsid protein genes with recombinant DNA technologies. As a result, the recombinant phages express the foreign peptides or proteins on the phage surface for affinity-based selection. The affinity-selected phages can be replicated in bacterial host for further rounds of selection or DNA-sequencing to identify the affinity peptides or proteins expressed on the phage surface.

4.2 Applications of phage display

4.2.1 General applications

The application of phage display technology include determination of binding partners of organic (proteins, polysaccharides, or DNAs) (Gommans et al., 2005) or inorganic materials (Hattori et al., 2010; Whaley et al., 2000). The technique is also used to study enzyme evolution *in vitro* for engineering biocatalysts (Pedersen et al., 1998). Phage display has been widely applied in drug discovery. It can be used for finding new ligands, such as enzyme inhibitors, receptor agonists and antagonists, to target proteins (Hariri et al., 2008; Pasqualini et al., 1995; Perea et al., 2004; Ruoslahti, 1996; Uchino et al., 2005). Invention of antibody phage display revolutionized the drug discovery (Han et al., 2004). Millions of different single chain antibodies on phages are used for isolating highly specific therapeutic antibody leads. One of

the most successful examples was adalimumab (Abbott Laboratories), the first fully human antibody targeted to TNF alpha (Spector and Lorenzo, 1975).

4.2.2 *In vivo* phage display and its application in clinical oncology

Because isolating or producing recombinant membrane proteins for use as target molecules in phage library screening is often facing insurmountable obstacles, innovative selection strategies such as panning against whole cells or tissues were devised (Jaboin et al., 2009; Molek et al., 2011; Pasqualini and Ruoslahti, 1996). Due to cells inside the body may express different surface markers and possess different characteristics from cell lines in culture, *in vivo* phage bio-panning was developed to identify more physiologically relevant biomarkers (Fig. 2) (Pasqualini and Ruoslahti, 1996). Since its invention, *in vivo* phage display has been used extensively to screen for novel targets for tumor therapy. Majority of those studies focused on analyzing the structure and molecular diversity of tumor vasculature and selecting tumor stage- and type-specific markers on tumor blood vessels (Arap et al., 2002; Rajotte and Ruoslahti, 1999; Sugahara et al., 2010; Valadon et al., 2006). Recently, the use of this technique was expanded to the field of discovering new biomarkers for evaluation of cancer treatment efficacy. (Han et al., 2008; Passarella et al., 2009).

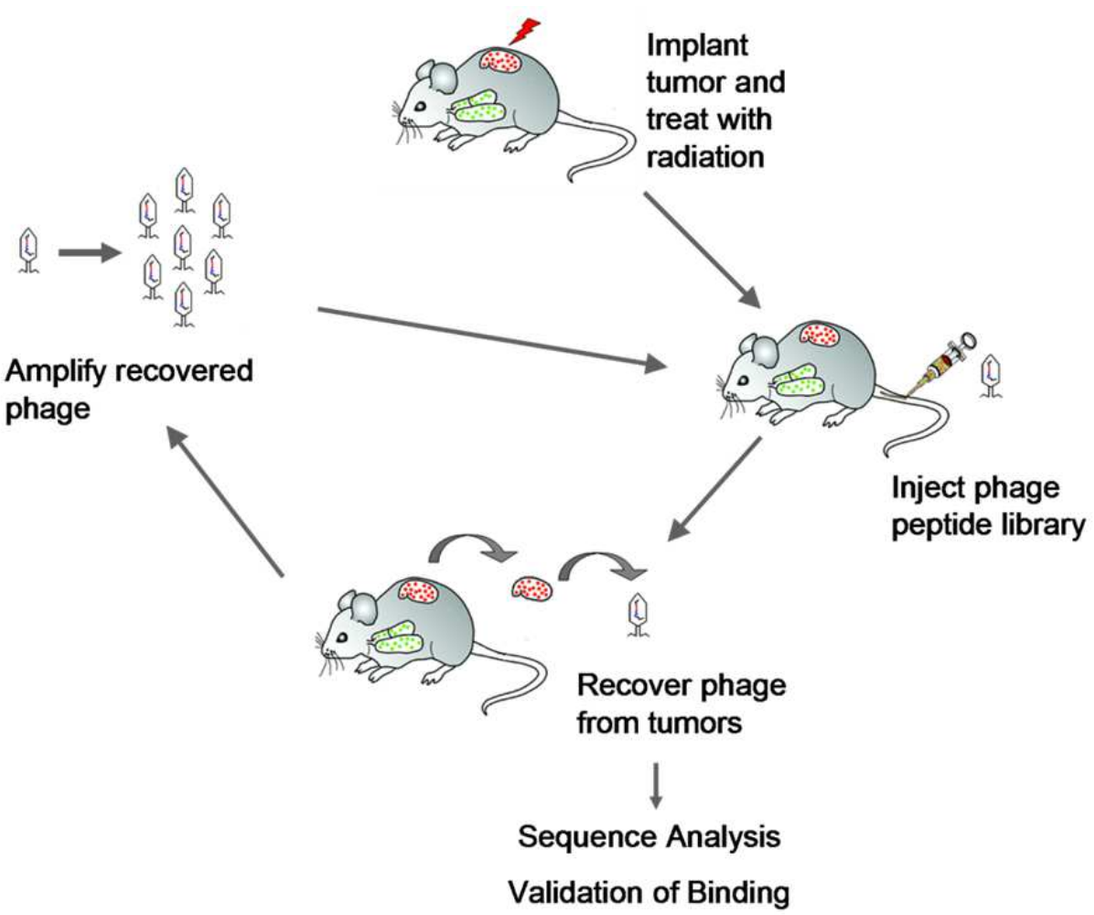

Fig. 2. *In vivo* phage display for screening peptides specifically target to radiation- or drug-treated tumors.

4.3 Peptides as probes for tumor targeted imaging

4.3.1 Advantages of peptide vs. antibody for tumor targeting

Antibodies, especially monoclonal antibodies, have been successfully utilized as cancer-targeting therapeutics and diagnostics due to their high target specificity and affinity. However, due to antibody large size (150 kDa) and limited tissue permeability, non-specific uptake into the reticuloendothelial system, and immunogenicity, most antibody-based therapeutics are of limited efficacy (Lin et al., 2005; Stern and Herrmann, 2005). In contrast to antibodies, peptides are much smaller molecules (1-2 kDa). Peptides have favorable biodistribution profiles compared to antibody, characterized by high uptake in the tumor tissue and rapid clearance from the blood. In addition, peptides have increased capillary permeability, allowing more efficient penetration into tumor tissues. Also peptides are easy to make and safe to use, they will not elicit an immune response (Ladner et al., 2004). With all these advantages, peptides have been increasingly considered as a good tumor targeted imaging probe (Aloj and Morelli, 2004; Okarvi, 2004; Reubi and Maecke, 2008).

4.3.2 Peptide as imaging probe

To date, a large number of peptides derived from natural proteins have already been successfully identified and characterized for tumor targeting and tumor imaging, such as integrin (RGD), somatostatin, gastrin-releasing peptide, cholecystokinin, glucagon-like peptides-1 and neuropeptide-Y (Cai et al., 2008; Hallahan et al., 2003; Korner et al., 2007; Miao and Quinn, 2007; Reubi, 2003; Reubi, 2007). A list of a few tumor homing peptides isolated using phage display technique is shown in Table 1.

Tumor Types	**Tumor- targeting peptides**
Prostate carcinoma	IAGLATPGWSHWLAL (Newton et al., 2006) ANTPCGPYTHDCPVKR (Deutscher et al., 2009) R/KXXR/K (Sugahara et al., 2009)
Colon carcinoma	CPIEDRPMC (Kelly et al., 2004)
Breast carcinoma	EGEVGLG (Passarella et al., 2009)
Hepatocellular carcinoma	AWYPLPP (Jia et al., 2007) AGKGTAALETTP (Du et al., 2010)
Pancreatic carcinoma	KTLLPTP (Kelly et al., 2008)
Head and Neck Cancer	SPRGDLAVLGHKY (Nothelfer et al., 2009)
Osteosarcoma	ASGALSPSRLDT (Sun et al., 2010)
Fibrosarcoma	SATTHYRLQAAN (Hadjipanayis et al., 2010)
Esophageal Cancer	YSXNXW and PXNXXN (Zhivotosky and Orrenius, 2001)
Bladder Cancer	CSNRDARRC (Ginestier et al., 2007)

Table 1. Phage display-derived tumor-targeting peptides

For use as *in vivo* imaging probes, peptides can be directly or indirectly labeled with a wide range of imaging moieties according to the imaging modality. For instance, near-infrared (NIR) fluorescent dyes or quantum dots have been labeled for optical imaging (Fig. 3), several radionuclides have been employed for positron emission tomography (PET) or single-photon emission computed tomography (SPECT), and paramagnetic agents have

been used for magnetic resonance imaging (MRI) (Frangioni, 2003; Reubi and Maecke, 2008). Peptides can also be conjugated to other tumor targeted polymers or nanoparticles and dramatically increase their tumor targeted selectivity and efficiency (Hariri et al., 2010; Lowery et al., 2010; Passarella et al., 2009).

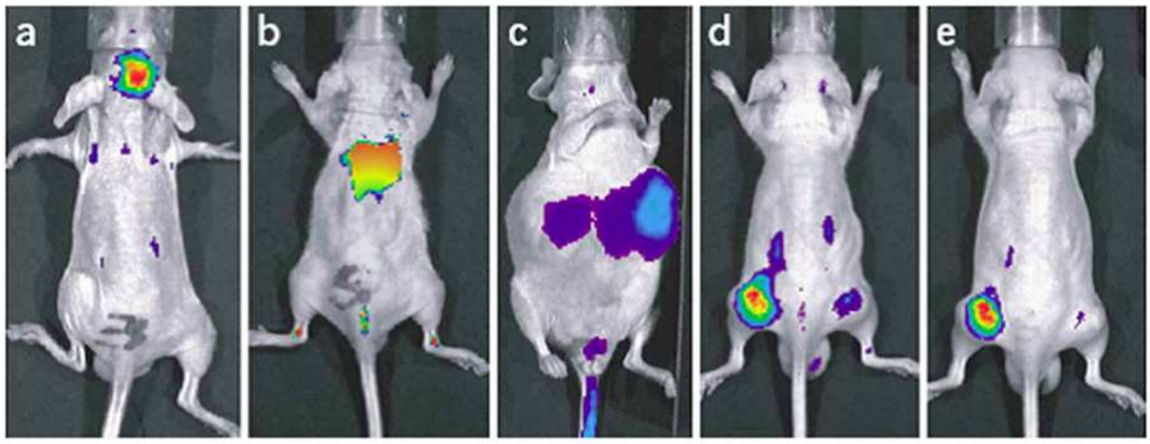

Fig. 3. HVGGSSV peptide labeled with near-infrared (NIR) fluorescent dyes specifically located to radiation-treated tumors. a) brain tumor (D54 human glioblastoma cell), b) lung tumor (H460 cell), c) colon cancer liver metastasis (HT22 cell), d) prostate cancer subcutaneous model (PC-3 cell), and e) breast cancer subcutaneous model (MDA-MB-231 cell). (Adapted from Han et al., 2008).

5. HVGGSSV peptide as one imaging probes to detect tumor response to radiation and tyrosine kinase inhibitor (TKI) *in vivo*

5.1 Discovery of HVGGSSV peptide

In our recent studies, we employed *in vivo* phage display technique and intended to identify peptides that will specifically home to radiation or drug treatment responsive tumors (Han et al., 2008; Passarella et al., 2009). During the studies, we first treated tumors in mice with radiation and tyrosine kinase inhibitors. Then a peptide phage library was injected from the tail vein of tumor bearing mice for tumor binding screening. After several rounds of *in vivo* screening and enrichment of phages isolated from the treated tumors (Fig. 2), one phage clone, encoding HVGGSSV peptides, was identified preferentially target to treatment responsive tumors. The binding preference of those phages were confirmed by fluorescence labeled phage or peptide imaging (Han et al., 2008; Passarella et al., 2009).

5.2 HVGGSSV peptide as imaging/targeting probe for radiation responsive tumors

To explore HVGGSSV peptide's clinical application in noninvasive imaging of tumor response to treatment, fluorescent labeled HVGGSSV peptide were used to target human tumors in several mouse models. Optical imaging studies indicated that the signal intensities of peptide binding within tumors correlate to the overall efficacy of treatment regimens on tumor growth control in multiple tumor models that had been treated with a variety of RTK inhibitors with or without combination of radiation (Han et al., 2008). SPECT/CT provides high spatial resolution and sensitivity in functional imaging. We

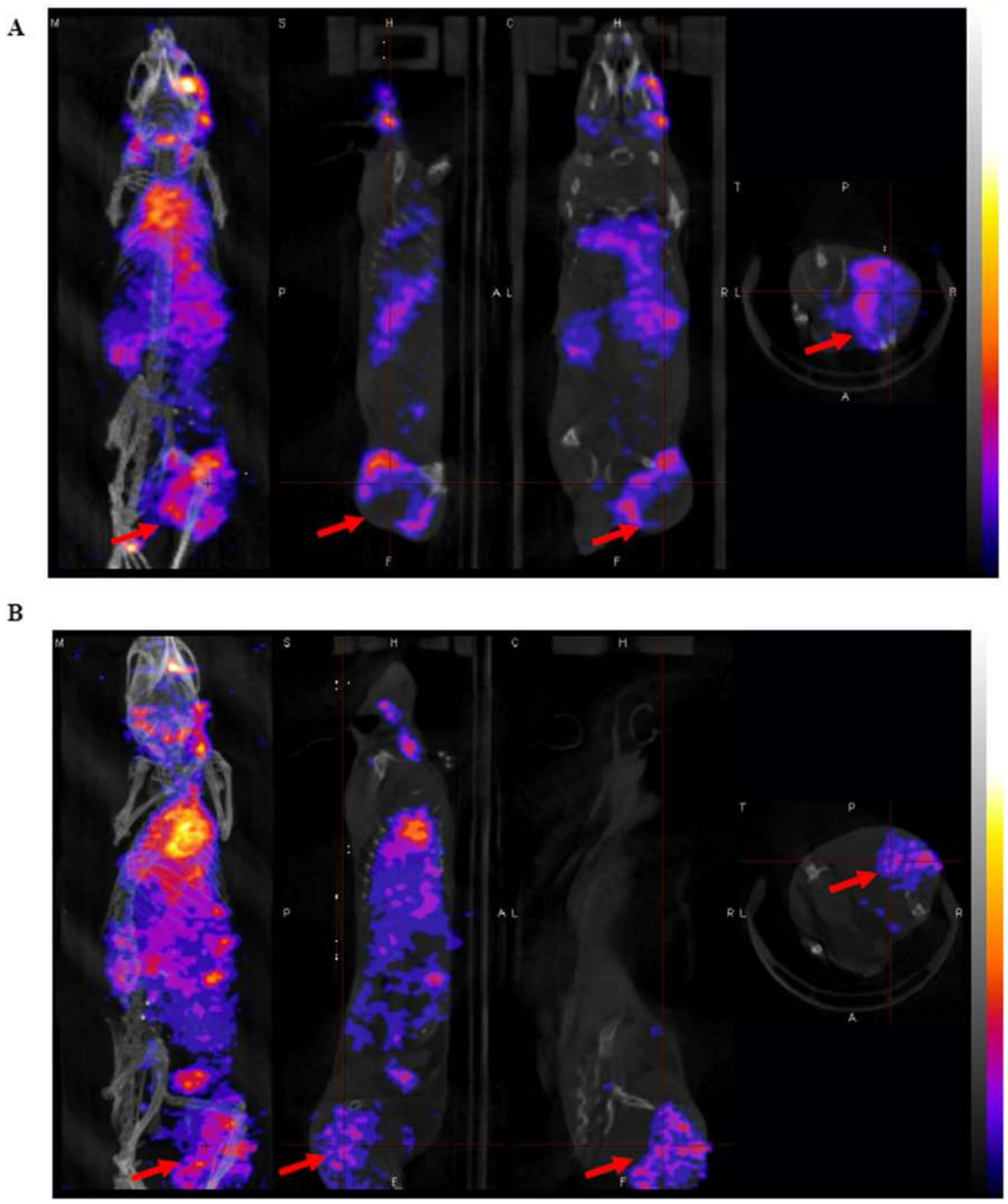

Fig. 4. SPECT/CT imaging of the HVGGSSV peptide within LLC tumors after treatment. The biotinylated HVGGSSV peptide was complexed with iodine-125-labeled streptavidin. The implanted tumor was treated once with radiation (5 Gy) alone (**A**), or combination of Sunitinib (40 mg/Kg) and radiation (5 Gy) (**B**) before intravenous administration of the imaging probe. Shown are 3D virtual rendering (3D-VR) images (far left) and hybrid SPECT/CT fusion images in the coronal, sagittal, and transaxial planes (the second to the fouth from the left, respectively) acquired 4 hours after the imaging probe administration. The LLC tumors was pointed with arrows, high resolution images enable spatially localizing the radiation-responding cells within the peripheral and central regions of the tumors.

employed this imaging modality to detect tumor response to radiation by using the HVGGSSV peptide. The mice were treated with radiation alone or combination of radiation and one TKI - Sunitinib (40 mg/Kg,). After the treatment, the HVGGSSV peptide complexed with ^{125}I-labeled streptavidin was selectively targeted to the tumors treated with radiation or radiation combined with Sunitinib. High resolution SPECT/CT images (Fig. 4) also showed that majority of the imaging probes were located in the peripheral area of the tumors that were treated with radiation alone. However, treatment with radiation and Sunitinib extended the imaging probe binding to both the peripheral and central parts of the subcutaneous tumors. This data might reflect the radiosensitization effect of Sunitinib.

The tumor targeting potential of HVGGSSV peptide has been further explored in several drug delivery studies. In these studies, HVGGSSV peptide has been conjugated to different nanoparticles, such as liposome, FePt, and nanoparticle albumin bound (nab) (Hariri et al., 2011; Hariri et al., 2010; Lowery et al., 2011), and selectively targeted those nanoparticles to irradiated tumors. One study also showed >5-fold increase in paclitaxel levels within irradiated tumors in HVGGSSV-nab-paclitaxel-treated groups and significantly increase tumor growth delay as compared with controls (Hariri et al., 2010).

5.3 The biological basis of the HVGGSSV peptide imaging

5.3.1 Peptide receptor identification

To understand the physiology underlines peptides binding, we need to identify the molecular targets of peptides. However, peptides are usually unstable. Their surface charges and structures will change dramatically in different environment. And peptides usually interact with their targets with low binding affinity due to their small sizes. Therefore, traditional affinity purification methods are of little use because of high background of non-specific binding. To date, there are very few identified receptors for peptides in contrast to the great number of discovered cancer targeting peptides (Sugahara et al., 2009). New strategies are needed for identifying peptide's receptors.

5.3.2 TIP-1 as a molecular target of HVGGSSV peptide

In our recent studies of one peptide (HVGGSSV), we utilized a phage cDNA library screening to search for peptide's receptors. Because several rounds of phage display screening can significantly enrich the low-affinity or low-abundance proteins, we successfully identified a PDZ protein - TIP-1 as the target of HVGGSSV peptide (Wang et al., 2010). Through the PDZ domain, TIP-1 binds to the classic C-terminal PDZ motif within the HVGGSSV peptide. One TIP-1-specific antibody that inhibited the in vitro interaction between TIP-1 and the HVGGSSV peptide attenuated the peptide's accumulation within irradiated tumors. Imaging with TIP-1-specific antibody recapitulated the pattern of peptide imaging in tumor-bearing mice. Mutation in the classic PDZ binding motif of the HVGGSSV peptide destroyed the specific binding within irradiated tumors. These results also demonstrated the potentials of screening phage-displayed cDNA library in discovery of molecular targets of the peptides with a simple structure and low affinity.

5.3.3 The biological relevance of TIP-1 relocation onto tumor cell surface to the radiation response of tumor cells

With a TIP-1 specific antibody, it was further identified that radiation induced translocation of the basically intracellular TIP-1 protein onto the cell surface in a dose-dependent manner.

The treatment-induced TIP-1 expression on the cell surface is detectable in the first few hours after the treatment and before the onset of treatment associated apoptosis or cell death. Majority of the cells expressing TIP-1 on the cell surface are the live albeit such cells are less potent in proliferation and more susceptible to subsequent radiation treatment (Wang et al., 2010). The increased susceptibility to the subsequent irradiation might explain why the peptide binding is predictive in assessing tumor overall responsiveness in the early stage of treatment course. The treatment-inducible TIP-1 translocation before the onset of cell apoptosis or death further suggests potentials of the HVGGSSV peptide in non-invasive imaging assessment of tumor response to radiation and tyrosine kinase inhibitors.

6. Perspectives

The development of imaging technologies revolutionizes medial diagnosis and clinical management. Functional molecular imaging becomes one critical part of personalized medicine. Peptide, with its small sizes and versatile structures, is increasingly recognized as a promising imaging probe to predict the outcomes of radiotherapy and other medical treatments. Although some disadvantages associated with peptides, such as its degradation inside human body and its low affinity with its targets, with chemical modification to improve its stability and association with nanoparticles to increase its binding affinity, peptides will play a major role in the future molecular imaging.

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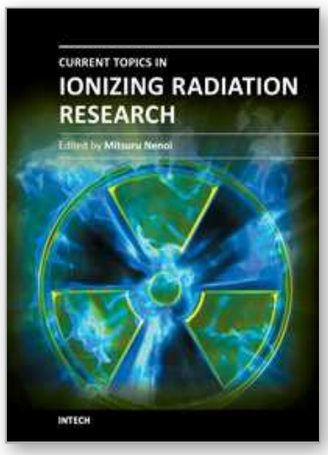

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Since the discovery of X rays by Roentgen in 1895, the ionizing radiation has been extensively utilized in a variety of medical and industrial applications. However people have shortly recognized its harmful aspects through inadvertent uses. Subsequently people experienced nuclear power plant accidents in Chernobyl and Fukushima, which taught us that the risk of ionizing radiation is closely and seriously involved in the modern society. In this circumstance, it becomes increasingly important that more scientists, engineers and students get familiar with ionizing radiation research regardless of the research field they are working. Based on this idea, the book "Current Topics in Ionizing Radiation Research" was designed to overview the recent achievements in ionizing radiation research including biological effects, medical uses and principles of radiation measurement.

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