Vasoactive intestinal peptide receptor-based imaging and treatment of tumors (Review)

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Abstract. Vasoactive intestinal peptide receptors (VIPRs) are members of the G-protein-coupled receptor superfamily. These receptors are overexpressed in many common malignant tumors and play a major role in the progression and angiogenesis of a number of malignancies. Therefore, VIPRs may be a valuable target for the molecular imaging of tumors and therapeutic interventions. The specific natural ligand or its analogs can be labeled with a radionuclide and used for tumor receptor imaging, which could be used to visualize VIPR-related surface protein expression *in vivo* and to monitor the *in vivo* effects of molecular drugs on tumors. Moreover, the involvement of VIPRs in malignant transformation and angiogenesis renders them potential therapeutic targets for cancer treatment. A variety of VIP antagonists and cytotoxic VIP conjugates have been synthesized and evaluated for VIPR-targeted molecular therapy. The importance of VIPRs in tumor biology and the ability to predict responses to targeted therapy and monitor drug interventions suggest that VIP receptor-based imaging and treatment will be critical for the early diagnosis and management of cancer. Here, we review the current literature regarding VIPRs and their natural ligands and the involvement of VIPRs in tumor growth and angiogenesis, with an emphasis on the present use of VIPRs for the molecular imaging of tumors and therapies targeting VIPRs.

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Abbreviations: VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating polypeptide; PHM, peptide histidine methionine amide; GRF, growth-hormone-releasing-factor; PHI, peptide histidine isoleucine; GPCR, G-protein-coupled receptor; SPECT, single photon emission computed tomography; PET, positron emission tomography; CT, computed tomography; MRI, magnetic resonance imaging; FDG, fluorodeoxyglucose; PRRT, peptide-receptor radionuclide therapy

Key words: VIP, VIP receptor, peptides, imaging, therapy, cancer

Contents

1. Introduction

Malignant tumors have become one of the most lethal diseases in humans, and the incidence, diagnostic studies and therapeutic options for tumor treatment have undergone major changes in recent years. Currently, the standard treatment for a malignancy includes surgical resection followed by the delivery of chemotherapeutic agents. Although surgical resection is the preferred choice for early-stage solid tumors, it is not a suitable treatment for invasive, metastatic or hematologic malignancies. The overall toxicity and the side effects of chemotherapy also limit the dose regimen, thus requiring a relatively narrow therapeutic index, which can lead to insufficient and/or unpredictable responses. Furthermore, during the course of therapy, multidrug resistance can severely limit the effectiveness of chemotherapy and is also associated with tumor recurrence (1-4). Therefore, it is desirable to target therapeutic molecules to primary tumors and their metastases, which represents a major challenge for improving current cancer therapy (5). Another significant challenge is the development of strategies that can guide the use of these targeted molecules and enable the specific delivery of therapeutic agents to malignant tissues (6). Targeted therapies generally take advantage of biological molecules that are uniquely expressed or significantly overexpressed in tumors. Hormone receptors were some of the earliest targets utilized for the targeted treatment of many cancers, such as breast, prostate and thyroid cancers (7,8). Therefore, the molecular imaging of the expression of tumor-related receptors is advantageous because it provides information for receptor-targeted therapy (9).

Molecular imaging has been defined as the visualization, characterization and measurement of biological processes at the molecular and cellular levels (10). Tumor receptor imaging is an important type of molecular imaging and can be used to assess receptor expression for the entire disease burden to facilitate early diagnosis, suggest personalized therapy options, and monitor the *in vivo* effects of a drug on its target. Different receptors are overexpressed in specific tumor types. Several tumor receptors have been utilized for imaging and therapy, including somatostatin receptors, human epidermal growth factor receptor 2 (HER2), integrin receptors, epidermal growth factor receptor (EGFR), and vasoactive intestinal peptide receptors (VIPRs). Vasoactive intestinal peptide receptors (VIPRs) are highly overexpressed in human tumors and metastases. VIPRs also play a major role in the progression of a number of malignancies, thus suggesting that these receptors may serve as molecular targets for cancer diagnosis and treatment. Herein, we describe the biology of VIPRs and VIPR-targeting agents and discuss the potential application of VIPRs for the diagnosis and treatment of cancer.

2. Structural and functional features of VIP/PACAP and VIPRs

Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are two members of a structurally related family of peptides that includes mammalian peptide histidine methionine amide (PHM), secretin, glucagon, and growth hormone-releasing factor (GRF) (11). VIP was isolated from the small intestine of a pig and was characterized in the early 1970s by Mutt and Said. VIP exhibits various physiological effects, including increased vasodilatation and reduced arterial blood pressure (12), smooth muscle relaxation (13), stimulation of electrolyte secretion (14), immunosuppression, hormonal secretion, cell proliferation and increased gastric motility (15,16). The human VIP gene is located on chromosome 6 at band q25 (17,18) and encodes the 170-amino acid precursor protein, prepro-VIP, which undergoes post-translational modification to produce the 28 amino acid VIP peptide (12,19), in addition to the 27 aa peptide histidine methionine (PHM) in humans or the peptide histidine isoleucine (PHI) in rodents (20). These two peptides are co-synthesized with VIP as part of the same precursor peptide, which is encoded by an adjacent exon in the human genome, suggesting that the family of peptides has evolved via exon duplication coupled with gene duplication (21). The N-terminus of VIP plays an important role in this protein's biological activity, and several studies have shown that the N-terminus is essential for receptor activation; however, it is not involved in the recognition of the receptor-binding site, which seems to involve amino acids in the C-terminal domain (22,23). The human PACAP gene is located on chromosome 18p11 and encodes a 176-amino acid precursor protein (preproPACAP) that contains PACAP and PACAP-related peptide (PRP) in the C-terminal domain and a 24 amino acid signal protein in the N-terminal domain (23,24).

These two peptides share a similar sequence and exert their functions through the binding of vasoactive intestinal peptide receptors (VIPRs), which are functional receptors for VIP and PACAP. VIPRs are members of the G-protein-coupled receptor (GPCR) family, which is comprised of three classes of receptors: VPAC1, VPAC2 and PAC1. VPAC1 and VPAC2 respond to VIP and PACAP with comparable affinity, whereas PAC1 displays a high affinity for PACAP and a low affinity for VIP (25-27). The VIPRs share a similar structural organization; they contain seven transmembrane domains (TM), a large N-terminal segment that includes the binding site of VIP/PACAP, and an intracellular C-terminal region that is associated with signal transduction (28). Upon ligand binding, these receptors mediate their signal transduction activity via the binding of heterotrimeric G-proteins to the receptor's intracellular loop, which ultimately results in cAMP production (29,30), protein kinase A (PKA) pathway activation (25,26), inositol phosphate (IP) turnover (23), the activation of MAPKs (mitogen-activated protein kinases) (31,32), and the activation of the NF-κB pathway (Fig. 1) (33).

The effects of VIP and PACAP are mediated by VIPRs and have many overlapping functions. These peptides play roles in the gastrointestinal, immune, reproductive, respiratory, cardiovascular and endocrine systems (34,35). For example, VIP is involved in gut motility and acts as a potent vasoregulatory hormone, whereas PACAP is associated with the nervous system and exerts a hypophysiotropic effect on pituitary hormone secretion (11,23). In addition to the crucial roles that VIPRs play in many physiological processes, VIPRs are involved in the progression of a number of malignancies and can stimulate tumor growth and angiogenesis through the transactivation of epidermal growth factor receptor (EGFR) and the expression of vascular endothelial growth factor (VEGF) (36,37).

3. VIP receptors as potential targets for imaging and therapy

VIPRs have been identified in several normal tissues and in various types of tumors. In normal human tissues, VPAC1 receptors are abundant in the brain, T lymphocytes, and most peripheral tissues including the liver, lungs and intestines (38,39). VPAC2 receptors are mainly expressed in the hippocampus, the brainstem, the spinal cord, and most smooth muscle tissues (39,40). PAC1 receptors are predominantly found in the adrenal medulla and on the surface of neuroendocrine neoplasms (41,42). However, VIPRs are highly overexpressed in the majority of human tumors and are particularly common in frequently occurring cancers. Reubi *et al* (43) reported that VPAC1 receptors are overexpressed in most frequently occurring malignant epithelial neoplasms, such as cancers of the colon, breast, lungs and prostate, in a pattern similar to that found in normal tissues. In contrast to the ubiquitous expression of VPAC1 receptors in most human tumors, tumors that express VPAC2 receptors are rare, and this phenomenon is only observed in some leiomyomas and gastrointestinal stromal tumors (43,44). A high incidence of PAC1 receptor expression was found in neuroendocrine neoplasms located in the brain and the endocrine and neuroendocrine systems and included gliomas, neuroblastomas, and pituitary adenomas, as well as endometrial cancers (43). The VIPRs are significantly overexpressed in most human tumors, and the differences in the cell surface receptor profiles between cancer cells and their normal counterparts can be utilized as a molecular signature for targeted imaging. Thus, the VIPRs may be potential targets for molecular imaging.

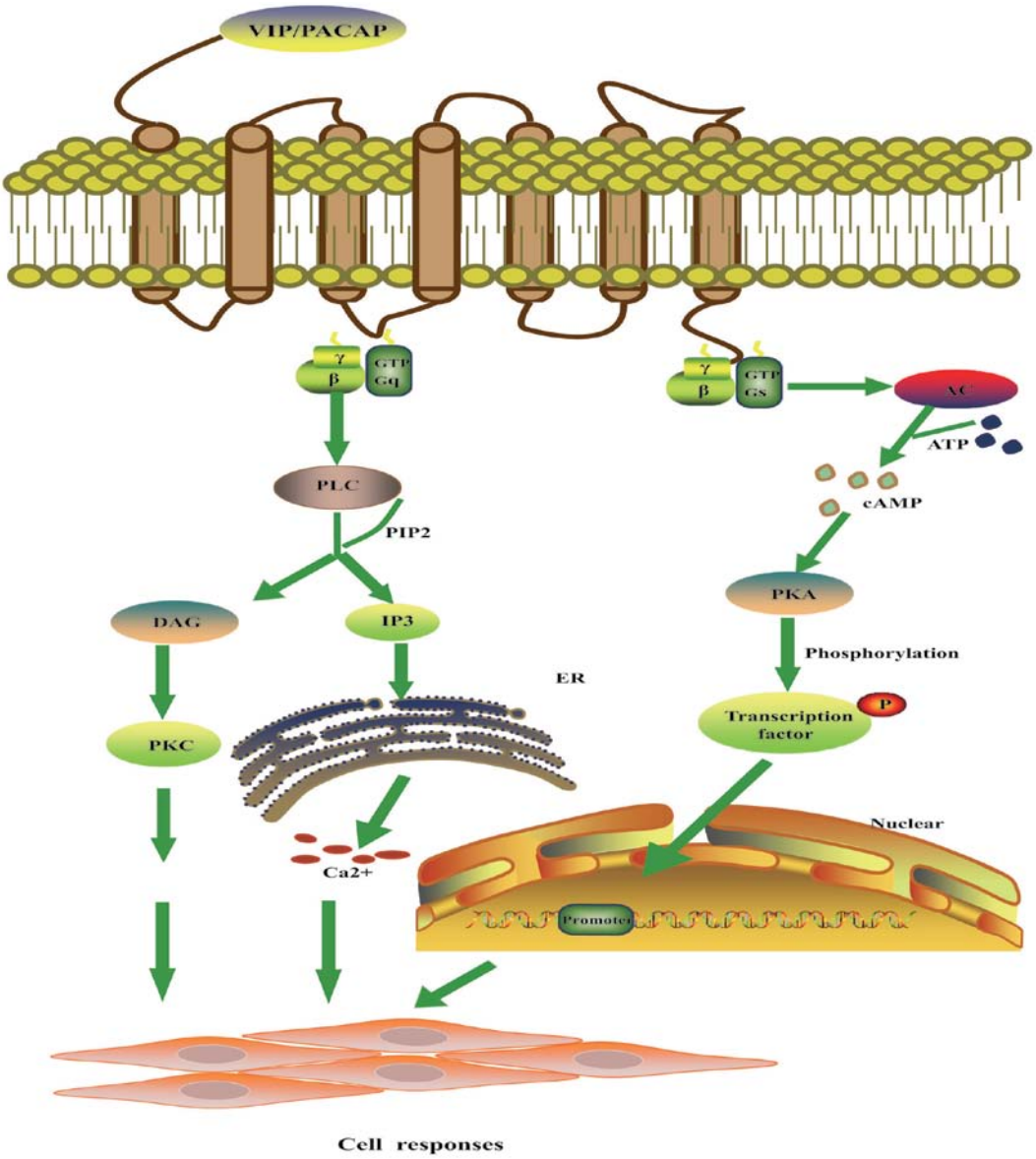

Figure 1. Molecular mechanism of VIP signal transduction. The main signals of VIP are mediated through VIP/PACAP receptors via coupling to adenylate cyclase (AC)-stimulation G protein and PI signal transduction pathways. AC-cAMP pathway: VIP and receptor binding triggers the GDP-GTP exchange of the Gs protein and activate AC. AC catalyzes the synthesis of cAMP from ATP, and cAMP elicits its cellular responses by the phosphorylation of a number of cellular proteins or transcription factors via cAMP-dependent protein kinase (PKA). PI pathway: VIP and receptor binding is coupled to Gq protein and activates phospholipase C (PLC), which cleaves PIP2 into DAG and IP3. DAG activates PKC which in turn phosphorylates a number of cellular proteins and exerts biological functions. IP_3 causes the release of Ca^{2+} from endoplasmic reticulum (ER) which leads to exocytosis and various other cellular responses. cAMP, cyclic adenosine 5'-monophosphate; GTP, guanosine 5'-triphosphate; DAG, diacylglycerol; IP3, inositol-1,4,5-triphosphate; PIP2, phosphatidylinositol-4,5-bisphosphate.

It is well documented that excessive VIPR signaling, which arises from receptor overexpression and autocrine stimulation by VIP/PACAP or other factors, is a hallmark of a wide variety of tumors. Previous research has shown that the serum levels of VIP in several tumors are significantly increased, which may serve as an indicator of the presence of certain tumors and could become a part of their diagnosis (45). Since most tumors overexpress VIPRs, VIP/PACAP can bind to these VIPRs and activate them, leading to an interaction with a stimulatory guanine nucleotide binding protein (Gs) and ultimately resulting in the stimulation of adenylate cyclase. Research has shown that the addition of VIP to lung cancer cells elevated the cAMP levels and led to the activation of a series of transcription factors that promoted the expression of nuclear oncogenes and growth factors (46,47). In addition, VIP activation increased the secretion of VEGF and facilitated tumor angiogenesis via the cAMP/PKA and PI3K signaling pathways (36). The involvement of VIPRs in malignant transformation and angiogenesis renders them potentially valuable targets for cancer therapy.

4. Targeting VIPRs for molecular imaging

Tumor receptor imaging plays an important role in molecular imaging, the characterization of receptor expression, tumor biology, the identification of molecular targets, and the application of targeted cancer therapy (48). The advantages of tumor receptor imaging include its noninvasive nature,

the ability to assess receptor expression, and the ability to evaluate the *in vivo* effects of the drug on the tumor. Several types of imaging agents may be used for the imaging of receptors that are overexpressed in tumors, including antibodies or their fragments, natural peptide ligands or their analogs, and non-peptide small molecules. Although the introduction of antibodies as specific agents to target malignant tumors dates back almost 35 years, these proteins are insufficient for tumor receptor imaging due to their high molecular weight, slow targeting of the tumor, slow clearance from the blood and normal tissues, and relatively low tumor to non-tumor ratios when used in tumor imaging and therapy (49,50). In general, natural ligands and small ligand analogs for targeted imaging are the most common imaging agents in use because small peptide ligands are small and readily diffusible and have rapid kinetics (51). Another important molecular imaging tool is the use of a tracer that accumulates at the sites of targeted receptors. Most molecular imaging tracers include a reporter moiety that emits a signal that can be detected by a special external device and a linker joining the reporter moiety to the targeted ligands. Some of the most commonly used tracers are radionuclide-labeled probes and optical imaging probes. However, the tissue penetration of optical signals is limited to a few millimeters and thus limits the clinical application of optical imaging probes. Therefore, radionuclide-based tracers dominate in clinical molecular imaging techniques due to their high sensitivity and increased tissue penetration. Currently, there are two major radionuclide-based imaging methods used in the pre-clinical or clinical settings: single photon emission computed tomography (SPECT) and positron emission tomography (PET). For the reasons stated above, we focus on radionuclide-labeled peptide ligands and small molecule analogs used for SPECT and PET imaging in this review.

5. ^{123}I-labeled VIP for receptor imaging

Virgolini *et al* (52) first reported that ^{123}I-labeled VIP could be successfully used *in vivo* to scan patients with gastrointestinal adenocarcinomas, carcinoids and insulinomas. After the intravenous injection of the ^{123}I-labeled VIP, ~45% of the radioactivity was found in the lungs within 30 min, and the activity decreased rapidly. Visualization of the primary tumors and metastases was possible within an hour of injection and was still apparent at 24-h post-injection. Most importantly, radiolabeled VIP-receptor scanning is more advantageous than CT scanning because receptor imaging is based on the receptor expression pattern rather than the macroscopic morphology (52). Subsequently, the same group performed a series of clinical studies in which ^{123}I-labeled VIP was used to image various tumors, such as colorectal cancers (53), pancreatic cancers (54), VIPomas (55), and endocrine tumors (56). These imaging results exhibited a high sensitivity, which was an advantage over CT scanning, particularly in patients with tumors of a small size. Raderer *et al* (57) compared the *in vitro* and *in vivo* binding of ^{123}I-VIP and an ^{111}In-labeled monoclonal antibody specific to the TAG-72 protein in patients with intestinal adenocarcinomas in a single-blinded prospectively randomized trial. The results indicate that VIP receptor scanning is more sensitive than immunoscintigraphy for the localization of intestinal adenocarcinomas and metastatic spread (57). Although ^{123}I-VIP has promising potential to localize even small tumors expressing VIPRs and is useful for the early diagnosis and treatment of tumors, naturally occurring peptides like VIP exhibit limitations for *in vivo* tumor imaging because they have a short biological half-life and are rapidly degraded in the liver and kidneys. Due to this metabolic instability, native VIP peptides require further chemical modifications to improve their bioavailability for receptor binding prior to their use in tumor receptor imaging. In addition, the radionuclide ^{123}I is generated by an expensive cyclotron instrument, which increases the cost of treatment. Furthermore, radiolabeled VIP requires further isolation and purification, which greatly limits its clinical applications. Therefore, novel stable analogs of VIP and applicable radionuclides are required for VIPR imaging.

6. ^{99m}Tc-labeled VIP analogs for receptor imaging

In contrast to ^{123}I, ^{99m}Tc has excellent physical characteristics for scintigraphic imaging, is very inexpensive, and is produced by a ^{99}Mo generator system (Table I) (58). In an initial attempt to employ ^{99m}Tc labeling, the N terminus (His1) of VIP was conjugated to one of two well-known bi-functional chelating agents (BFCAs): CPTA [4-(1,4,8,11-tetraazacyclotetradec-1-yl) methyl] benzoic acid or MAG_3[N-[N[N-(benzylthio) acetyl]-glycyl] glycyl] glycine]. These compounds have two major drawbacks: radiochemical impurity and a loss of biological function. It was found that the histidine residue in the number one position of VIP played an important role in the biological activity of VIP (59).

To improve the radiolabeling efficiency, Thakur *et al* (60) further modified VIP at the carboxy terminus of Asn^{28}. The research group chose to use 4-aminobutyric acid (Aba) as the spacer and extended the molecule to include Gly-Gly-(D)-Aba-Gly, which resulted in an N_4 configuration that could be used for the chelation of ^{99m}Tc (Fig. 2). This modified analog was referred to as TP3654 (VIP labeled with ^{99m}Tc). The biological activity of ^{99m}Tc-TP3654 was equivalent to that of the native VIP peptide, and the 24-h tumor uptake of ^{99m}Tc-TP3654 was higher than that of ^{123}I-VIP. These results implied that a simple and efficient hybrid peptide technique had been developed for labeling peptides with ^{99m}Tc. Subsequently, Thakur *et al* (58,60,61) evaluated the pharmacokinetics and imaging characteristics of ^{99m}Tc-TP3654 in normal volunteers and patients with a history of cancer. No adverse reactions or changes were noted in the imaging process. Furthermore, ~70% of the injected radioactivity was eliminated in the urine, and 20% was cleared through the liver. The tumor uptake of ^{99m}Tc-TP3654 was significantly higher than that of ^{111}In-octreotide, which is concordant with the higher VIP receptor density observed in most tumors when compared to the density of somatostatin receptors. This VIP analog promises to be a nontoxic and reliable agent for imaging human tumors that overexpress VIPRs.

Kothari *et al* (62) synthesized three VIP analogs functionalized at the N terminus with histidine [VP05 (28 residue), VD4 (20 residue), VD5 (19 residue)] by solid phase synthesis and radiolabeled the VIP analogs with ^{99m}Tc via a novel tricarbonyl synthon. All of the radiolabeled complexes were stable at room temperature and were produced with high yields.

Table I. Radionuclides for labeling of molecular imaging and therapeutic probes.

Nuclide	Half-life (h)	Production	Energy (keV)	Application
^{99m}Tc	6.01	Generator	γ 140	SPECT imaging
^{123}I	13.27	Cyclotron	γ 159	SPECT imaging
^{18}F	1.8	Cyclotron	β^+ 634 γ 511	PET imaging
^{64}Cu	12.7	Cyclotron	β^+ 655 β^- 573	PET imaging
^{111}In	67	Cyclotron	γ 171 Auger e- 30	SPECT imaging
^{68}Ga	1.1	Generator	β^+ 1899 γ 511	PET imaging
^{90}Y	65	Generator	β^- 2964	Therapy
^{131}I	192	Cyclotron	β^+ 607 γ 364	Therapy
^{177}Lu	161	Cyclotron	β^- 498 γ 208	Therapy

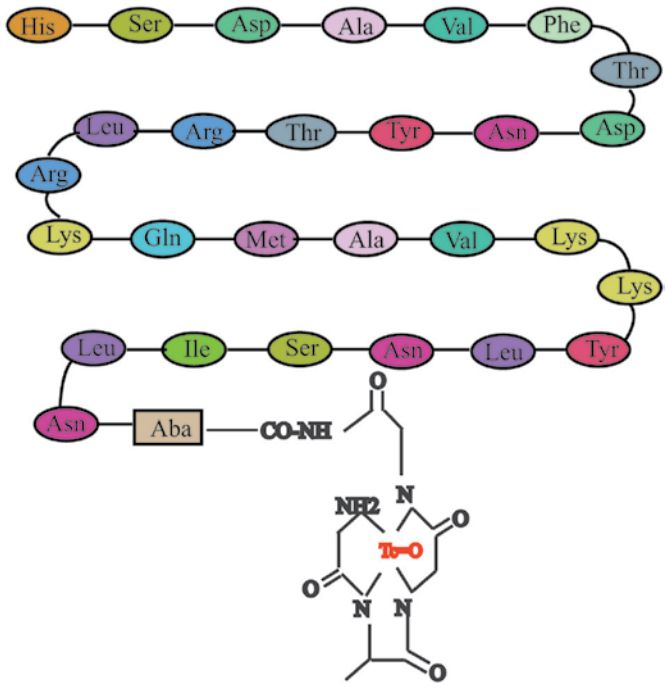

Figure 2. Amino acid sequence and proposed structure of ^{99m}Tc-TP3654. Gly-Gly-(D)-Aba-Gly is used as a chelating moiety that provides an N_4 configuration for chelation with ^{99m}Tc which has been shown to bind ^{99m}Tc strongly and efficiently. In addition, the 4-aminobutyric acid (Aba) is used as the spacer to extend the molecule including Gly-Gly-(D)-Aba-Gly. VIP-Aba-Gly-Gly-(D)-Ala-Gly had the observed molecular weight of 3654.48 and is referred to as TP3654.

Biodistribution studies revealed good tumor uptake kinetics; however, the soft tissue uptake was also high due to the lipophilic nature of the peptide. Thus, more research is required to modify the reported analogs and create novel radiolabeled analogs to enhance their ability to target VIP receptors.

7. PET molecular imaging of the VIP receptor

Although PET is not as widely available as SPECT, PET is advantageous for tumor diagnosis because it exhibits higher resolution and sensitivity than computed tomography (CT), magnetic resonance imaging (MRI) and SPECT. Therefore, labeling VIP with positron emission radionuclides is important for PET molecular imaging of tumors expressing VIP receptors. Moody *et al* (63) used ^{18}F to radiolabel VIP analogs (at Arg^{15}/Arg^{21}) and obtained a labeled complex (^{18}F-RR) VIP that bound to breast cancer cells with high specificity and affinity and acted as an agonist. The tumor imaging results showed that 4 h after injection, the density of (^{18}F-RR) VIP was 4-fold greater in the tumor than in the normal tissue with the highest uptake (intestinal) and was ~15-fold greater than in the normal breast, indicating that (^{18}F-RR) VIP could localize to breast tumors *in vivo*. However, a comparison of (^{18}F-RR) VIP and ^{18}F-FDG showed that FDG exhibited a 2- to 3-fold greater tumor accumulation and target-to-non-target ratio relative to (^{18}F-RR) VIP (64). To further improve the stability and biodistribution characteristics of ^{18}F-labeled VIP, Cheng *et al* (65,66) synthesized ($R^{8,15,21}$, L^{17}) VIP by replacing Asp8, Lys15, and Lys21 with Arg and Met17 with Leu in the amino acid sequence of VIP. The radiolabeled ^{18}F-($R^{8,15,21}$, L^{17}) VIP was obtained with high radiochemical purity, high specific radioactivity and good stability *in vitro*. Biodistribution data showed higher tumor-to-muscle and tumor-to-blood ratios, indicating its potential application as a PET imaging agent for tumors overexpressing VIPRs.

The radionuclide ^{64}Cu has a longer half-life and a wealth of known chemistry and provides nearly quantitative yields so that the radiolabeled compound can be prepared without further purification (Table I). ^{64}Cu-1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA) VIP was the first reported ^{64}Cu-VIP analog, but no further preclinical or clinical data have been reported (67). Subsequently, Thakur *et al* (68) synthesized the VIP analog TP3982 to harbor a carboxy-terminal lysine residue separated from asparagine by 4-aminobutyric acid (Aba) as a spacer. The biological activity of ^{64}Cu-TP3982 was not compromised. It also exhibited *in vivo* stability and a 74-fold increase in tumor uptake relative to ^{99m}Tc-TP3654, which had previously been successfully used to image human tumors. Thus, ^{64}Cu-TP3982 is a desirable PET imaging agent for the imaging of cancers overexpressing VIPRs. Zhang *et al* (69,70) used the same method to synthesize the VIP analog TP3939 and label it with ^{64}Cu. The imaging data obtained for ^{64}Cu-TP3939 in experimental and spontaneous prostate cancer indicated that with its uncompromised biological activity, ^{64}Cu-TP3939 could detect xenografts and cases of occult prostate cancer that were not detectable with ^{18}F-FDG or CT. Thus, ^{64}Cu-TP3939 is worthy of further investigation for the PET imaging of human

Table II. Some analogs of VIP and their therapeutic effects.

Analogs	Selectivity	Affinity (IC_{50}:nM)	Application
VIPhyb	PAC antagonist	300	Inhibition of colon and breast cancer
(SN)VIPhyb	VPAC1 antagonist	30	Inhibition of non-small lung cancer
[K^{15}, R^{16}, L^{27}]-VIP(1-7)/GRF (8-27)	VPAC1 antagonist	10	Inhibition various cancers
VIP-LALA-E	VPAC1 agonist	100	Inhibition of breast cancer
Ro 25-1553	VPAC2 agonist	1	Therapeutic for bronchial athma
BAY 55-9837	VPAC2 agonist	60	Stimulation of insulin secretion
stearyl-[Nle^{17}]-VIP	Nonselective agonist	5	Inhibition of breast cancer
[$R^{15, 20, 21}$, L^{17}]-VIP-GRR	Nonselective agonist	1.4	Treatment of asthma and COPD
[$R^{15, 20, 21}$, L^{17}]-VIP(1-23)	Nonselective agonist	48	Relaxation of muscle

tumors and their metastases or recurrent lesions and for determining the efficacy of tumor therapy.

8. Targeting VIP receptor for cancer molecular therapy

VIP antagonist-based cancer therapy. Since VIP is thought to have growth promoting properties through VIP receptor activation (71,72), the treatment of human tumors with a VIP antagonist may lead to the inhibition of tumor growth (Table II). The VIP-receptor antagonist VIPhyb was synthesized as a hybrid peptide of neurotensin and VIP consisting of an N-terminal Lys-Pro-Arg-Arg-Pro-Tyr (designed to increase membrane permeability), followed by the C-terminal 22 amino acids of VIP (73). This broad spectrum VIP receptor antagonist inhibited non-small cell lung cancer (73), breast cancer (74), and colon cancer (75) growth *in vitro* and *in vivo*. In addition, a further modification of VIPhyb was the addition of a stearyl group at the N-terminus and the exchange of the methionine at position 17 with norleucine. These modifications resulted in an antagonist with improved affinity (SN)VIPhyb (76). (SN)VIPhyb bound the VPAC1 receptor with an ~10-fold greater affinity than VIPhyb and acted as a cytostatic agent in non-small cell lung cancer (77) and pancreatic cancer (Table II) (78). Moreover, (SN)VIPhyb enhanced the anti-proliferative effects of chemotherapeutic agents on cancer cell lines (79).

To avoid the side effects of the broad-spectrum VIP receptor antagonists, new analogs selectively inhibiting each VIP receptor subtype may significantly prevent undesirable side effects, such as the inhibition of VPAC2 activation, which has been associated with glucose-dependent insulin secretion and bronchodilation (80). Recently, a VPAC1 antagonist conjugated to a high-molecular-weight PEG was synthesized, and the pharmacokinetic characteristics were improved without compromising functional activities. The results showed that the selective inhibition of the VPAC1 receptor is sufficient to prevent tumor proliferation, which suggests that a VPAC1-selective antagonist may be a safe and effective tool for treating tumors (unpublished).

Cytotoxic peptide conjugate-based cancer therapy. Conventional chemotherapy is limited by multidrug resistance of tumor cells, toxicity to normal cells and a lack of tumor specificity. The more specific delivery of chemotherapeutic drugs to cancer cells can produce a higher drug concentration in tumors and reduce the toxicity to normal cells (81). Therefore, 'targeted therapy' was introduced with the aim of enhancing the specificity of chemotherapy and reducing the non-specific toxicity to normal cells (82). Since high-affinity VIP receptors are overexpressed on many tumors, they can be targeted using cytotoxic VIP conjugates.

A new cytotoxic analog of VIP-ellipticine was prepared that consisted of tetra- and pentapeptide ellipticine (E) attached to the C-terminus of VIP. The VIP-E conjugates functioned as VPAC1 receptor agonists, bound to VPAC1 receptors with high affinity and retained their anti-proliferative activity. The VIP-E conjugates were internalized by cancer cells expressing the VPAC1 receptor and were subsequently metabolized by proteolytic enzymes, leading to the release of ellipticine and cellular cytotoxicity. The VIP-E derivatives exhibited significant cytotoxicity toward breast cancer cells and lung cancer cells *in vitro* (83,84). Subsequently, Moody *et al* (85) extended these studies and synthesized VIP-camptothecin (CPT) conjugates that contain a novel carbamate linker with a built-in nucleophile associated releasing group, L2. The obtained conjugate (A-NL-K)-VIP-L2-CPT is metabolized by cytochrome P450 enzymes, releases CPT and exhibits cytotoxic effects on breast cancer cells.

Peptide-receptor radionuclide therapy (PRRT). As mentioned previously, VIP receptor-positive tumors can be targeted with radiolabeled VIP and its analogs (52,61). It is theoretically possible to treat such tumors selectively with therapeutic nuclide-labeled VIP analogs. This peptide-receptor radionuclide therapy is based on the presence of high levels of VIP receptors in tumors and on their ability to form ligand-receptor complexes, which allows for the internalization and accumulation of the radiopharmaceuticals inside the tumors. Although there are many studies regarding somatostatin receptor-based radiotherapy (51,86), there are currently no reports on VIP receptor radiotherapy for human tumors. One reason may be the lack of appropriate radiolabeled ligands. A second reason is certainly the inadequate tumor-to-non-tumor ratio for radiotherapy. In this case, VIP receptor-based PRRT could be

highly radiotoxic to surrounding normal tissues, especially to radiosensitive tissues such as immune, lung, kidney and liver cells (51). Therefore, the development of new peptide analogs with increased binding affinity and specificity may lead to a higher accumulation of radioactivity in tumors and improve the efficacy of peptide receptor radionuclide therapy.

9. Prospective and Conclusion

VIP receptors are very important in the biology of many malignancies and are overexpressed in many tumors; thus, VIP receptors can be targeted by receptor-specific molecules. The generation of radiolabeled peptides has opened new avenues for the molecular imaging and therapy of tumors. Due to their low molecular weight, high-affinity, and good tissue/cell penetration, they are becoming ideal candidates for molecular imaging techniques and therapeutic interventions. To date, radiolabeled peptides specific for VIP receptors have evolved from the initial native VIP peptide into peptide analogs or peptide antagonists with improved pharmacokinetic profiles. Although a series of preclinical studies on VIP receptor-based imaging and therapy using radionuclide-labeled VIP and its analogs, antagonists and cytotoxic peptide conjugates have shown promising results, the therapeutic potential of VIP receptor-based radionuclide therapy must be refined and optimized.

Under the circumstances, it is necessary to develop novel peptide analogs with improved binding affinity, specificity and stability to result in a higher accumulation of radioactivity inside tumor cells. In a recent study, our research group identified a novel dodecapeptide that could specifically bind to the VPAC1 receptor with high affinity using a phage display peptide library. The results imply that the peptide may serve as a potential molecular imaging probe and therapeutic agent (87). Increasing the therapeutic window, which can be achieved by reducing the radiation toxicity to normal organs, could significantly enhance the therapeutic effects through increased injected radioactivity. Since the kidney has been one of the dose-limiting organs in some clinical studies, several standard procedures to reduce renal uptake have been developed and followed (88). Most importantly, the combination of radionuclide imaging and therapy with other diagnosis and treatment modalities, such as CT, MRI and chemotherapy, may greatly increase the efficiency of early diagnosis and treatment for the heterogeneous tumors.

Recently, the most common somatostatin receptor-based agent ^{111}In-DTPA-pentetreotide (Octreoscan; Mallinckrodt) was approved by the US Food and Drug Administration for clinical somatostatin receptor imaging (89). Moreover, several ^{90}Y-labeled octreotides were tested in different phase-I and phase-II clinical trials (90), and a major breakthrough in the tumor receptor-targeted imaging and therapy field may be on the way. The clinical success of somatostatin receptor imaging and therapy will provide insight into the exploration and development of VIP receptor-based imaging and therapy. Although there are still many other drawbacks to overcome in VIP receptor-based imaging and therapy, we believe that major progress will be made in preclinical settings. The importance of VIP receptors in tumor biology and the ability to predict responses to targeted therapy and monitor drug interventions suggest that VIP receptor imaging will be critical for oncologic molecular imaging and will play a key role in cancer management in the future.

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