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Seek & Destroy, use of targeting peptides for cancer detection and drug delivery

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ABSTRACT

Accounting for 16 million new cases and 9 million deaths annually, cancer leaves a great number of patients helpless. It is a complex disease and still a major challenge for the scientific and medical communities. The efficacy of conventional chemotherapies is often poor and patients suffer from off-target effects. Each neoplasm exhibits molecular signatures – sometimes in a patient specific manner – that may completely differ from the organ of origin, may be expressed in markedly higher amounts and/or in different location compared to the normal tissue. Although adding layers of complexity in the understanding of cancer biology, this cancer-specific signature provides an opportunity to develop targeting agents for early detection, diagnosis, and therapeutics. Chimeric antibodies, recombinant proteins or synthetic polypeptides have emerged as excellent candidates for specific homing to peripheral and central nervous system cancers. Specifically, peptide ligands benefit from their small size, easy and affordable production, high specificity, and remarkable flexibility regarding their sequence and conjugation possibilities. Coupled to imaging agents, chemotherapies and/or nanocarriers they have shown to increase the on-site delivery, thus allowing better tumor mass contouring in imaging and increased efficacy of the chemotherapies associated with reduced adverse effects. Therefore, some of the peptides alone or in combination have been tested in clinical trials to treat patients. Peptides have been well-tolerated and shown absence of toxicity. This review aims to offer a view on tumor targeting peptides that are either derived from natural peptide ligands or identified using phage display screening. We also include examples of peptides targeting the high-grade malignant tumors of the central nervous system as an example of the complex therapeutic management due to the tumor's location. Peptide vaccines are outside of the scope of this review.

1. Introduction

Personified as widespread, resistant, and adapting disease that strikes regardless of age, gender or social status, cancer embodies one of the ultimate challenges of modern medicine. Cancer is the second cause of death in the U.S. (statistics from the CDC) and expected to surpass the current No 1, cardiovascular diseases, by 2030. Cancer patients suffer from insufficient specificity and severe side effects of the conventional chemotherapies. In the new era of personalized/precision medicine, goal of the therapeutic management is to use the tumor- and patient-specific genetic and molecular aberrations for the selection of specific targeted therapies for each patient.[1–3] Inherent to this individualistic assessment of using genomic and molecular profiling of cancer, appropriate clinical management requires molecular probes capable of homing specifically to the primary or metastatic tumor mass.[4] The past decade has seen the emergence of numerous targeting agents providing the proof of concept of anticancer effects by targeted delivery. Aside of immunoglobulins, peptides or peptidomimetics have been developed. The cancer-targeting antibodies have exhibited excellent performance as vehicles to deliver radionuclides for imaging and cytotoxic agents for chemotherapies. Tested in the clinic and approved by the FDA, they unfortunately have also shown their limitations. For instance, the *fragment crystallizable* region of the antibody has a trend to non-specifically bind to the reticuloendothelial system thus causing notable toxicity towards tissues such as liver, spleen, and bone marrow.[5,6] In addition, due to their high molecular weight (up to 160 kDa), they poorly diffuse into the tumor mass or do not reach the brain in case of central nervous system neoplasms, leading to the necessity of the transient opening of the blood-brain-barrier.[7,8] Therapeutic antibodies while very specific and effective are rather difficult and particularly expensive to produce in mass scale. In the light

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of these shortcomings, targeting peptides can be considered as an alternative vehicle for the delivery of diagnostic agents and/or anti-cancer drugs. Compared to antibodies, targeting peptides benefit from non-immunogenicity, fast blood clearance, better intra-tumoral diffusion due to their lower molecular weight, and excellent tolerability by patients. The short half-life of the peptides that may in some cases reduce the accumulation at the target is often considered as one of their limitations. Prolonged half-life of the peptides can be obtained by preventing the degradation by blood proteases through *i)* presence of a cycle, formed by for instance disulfide bonds between two cysteines, *ii)* blocking of the C- and N- terminus, *iii)* replacement of eukaryotic amino acids by their D-counterparts or *iv)* use of unnatural amino acids incompatible with endogenous proteases. However, in the case of peptide-coated quantum dots the prolonged half-life in circulation achieved via the polyethylene glycol (PEG) -coating that eliminated 95% of the non-specific uptake by the liver and spleen, did not increase the homing to the tumor tissue,[9] suggesting that the receptor-mediated homing is very fast. Moreover, peptides are generally easy and relatively inexpensive to synthesize and allow myriad of possibilities for conjugation to imaging agents, therapeutic drugs, and nanodevices for targeted delivery. Thus, targeting peptides provide promising complementary tools for the modern personalized/precision medicine.

2. About targeting peptides

Modern molecular biology has dramatically facilitated the discovery of hundreds of cancer targets. Playing key roles in cellular functions and intercellular communication, peptide ligands are basically composed of a rosary-like assembly of amino acids connected by amide bonds containing usually less than one hundred monomers. Their low molecular weight allows a rapid clearance from the blood and non-specific binding sites, and their high specificity results in active concentration as low as nano-molar range. Interestingly, peptide ligands can be considered highly flexible regarding their chemical composition. Indeed, modifications such as cyclisation, unnatural amino acids or their combinations linked with chemical linkers can be easily achieved. However, such modifications must be carefully considered as they might result in a great diminution or total loss of affinity towards the target.

The targeting peptide sequence can be determined *via* different techniques. These include the development of derivatives inspired by the natural protein sequences *e.g.* vascular endothelial growth factor, VEGF[10] and somatostatin (SST)[11] or screening of peptide libraries composed of billions of short random amino acid sequences ultimately displayed on viral particles.[12,13] This phage display technique was first reported in 1985 using genetically engineered filamentous DNA-containing bacterial viruses (phage) that were modified to express foreign amino acid sequences as part of their protein coat.[14] A decade later the first *in vivo* screening of peptides selectively homing to brain and lungs was performed.[15] Since then the icosahedral T7 phage system has been introduced to display peptides as a fusion of its capsid protein.[16] Within a library each phage clone displays one unique peptide in multiple copies and current libraries can account more than 10^9 different peptides in total. The multiple display increases the avidity of binding and compensates for the possible low affinity of the peptides. The library is then introduced to targeting molecules embodied by isolated single proteins/receptors, cell cultures or extracts for the *in vitro* selections. *Ex vivo* selection can be performed on cell suspensions derived from organs or tumors of interest and *in vivo* selections are performed on live animals with administration of phage libraries via intravenous, intracardiac or intraperitoneal injections. A wash-off clears the unbound phage, leaving only the ones exhibiting binding affinity to target(s) subsequently rescued and amplified.[17]

This review covers a selection of peptides, recently discovered or modified/enhanced versions of previously identified ones, summarized in the Table 1. Most of them are pre-clinically validated targeting moieties with some already transferred into the clinics.

3. Targeting peptides derived from natural ligands

The usage of a targeting ligand is generally motivated by the overexpression of tumor-specific receptors. The accumulation of targeting/homing peptide within tumors correlates with the receptor expression allowing the discrimination of the abnormal from the normal tissue. Therefore, peptides conjugated to imaging moieties as diverse as fluorescent dyes, radionuclides or iron-oxide particles are respectively used for the optical, positron emission tomography or single photon emission computed tomography (PET or SPECT) as well as magnetic resonance imaging (MRI). An ideal targeting peptide should accumulate in the target but not in the normal tissues and in case of imaging applications be cleared fast from the circulation to minimize the background and enhance the specific signal to noise ratio.[18] In case of drug delivery, the accumulation of the peptide-drug conjugate at the target will increase the efficacy and decrease the side effects.

Moreover, the recent emergence of theragnostic tools suitable for use both in imaging and therapy transcends the borders between the two disciplines.[19] The following is a non-exhaustive review of the peptides derived from natural ligands mainly used for imaging of peripheral cancers such as breast and prostate cancer and melanomas.

3.1. Somatostatin (SST) derivatives

Many solid cancers are frequently associated with aberrant overexpression of the G protein-coupled receptors (GPCR) activated by peptide ligands, including the somatostatin receptor (SSTR) family. The SSTR family comprises five receptors (SSTR1 to 5) widely distributed in the central nervous system, pituitary gland, and many peripheral organs. Binding of the natural ligand somatostatin peptide (SST) to the receptors leads to inhibition of proliferation and/or induction of apoptosis in cancer cells.[11]

SSTR2 and 5 are specifically overexpressed in breast cancer, thus allowing their use as anti-cancer targets. SST exhibits high affinity towards the receptors but has a remarkably short half-life of only 1 to 3 min in plasma.[11] To overcome this challenge, SST analogue, a cyclic SSTR agonist octapeptide called octreotide (SMS 201–995; $_{D}$FCF$_{D}$WKTCT), which contains D-amino acids in the SST backbone and selectively binds SSTR2 and 5, was developed. Compared to the SST, octreotide has significantly increased plasma half-life up to 113 min.[20] Another example of natural ligand modifications is the incorporation of fatty acyl moieties in a process called lipophilization that may increase both the peptide stability and biological activity without causing conformational changes. For example, addition of 12, 14 or 16 carbons to another SST analogue RC-160 ($_{D}$FCY$_{D}$WKVCW) with short half-life in serum resulted in better stability and a 10-fold increase in potency over the RC-160 itself.[21]

The SSTR 2 and 5 were also used to visualize primary prostate neoplastic lesions and bone metastasis in PET/CT imaging of 20 patients.[22] However, expression of SSTR 2 and 5 in the tumor tissue of the majority of the included patients was too low for the receptor-mediated delivery of therapies. Therefore, the authors suggest that SSTR subtypes 1 and 4 seem to be more prostate specific and thus should be considered for further investigations.[22]

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