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REVIEW

Peptide-Targeted Polymer Cancerostatics

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Summary

A tumor-targeting drug delivery system consists of a tumor recognition moiety and a directly linked cytotoxic agent or an agent attached to a water-soluble synthetic polymer carrier through a suitable linker. Conjugation of a drug with a polymer carrier can change its solubility, toxicity, biodistribution, blood clearance and therapeutic specificity. Increased therapeutic specificity of a polymer drug can be achieved by the attachment of a targeting moiety (e.g. a lectin, protein, antibody, or peptide) that specifically interacts with receptors on the target cells. A large number of tumor-specific peptides were described in recent years. After a short introduction, some important examples of peptide-targeted conjugates will be described and discussed.

Key words

HPMA copolymers • Tumor targeting • Peptides • Drug delivery system

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Introduction

Hydrophilic polymers are frequently used in various biologically oriented disciplines, particularly pharmacy and medicine. The application of both synthetic and natural polymers as carriers of biologically active substances in the treatment of many serious diseases has become a new sophisticated approach in modern medicine. Polymer carriers with a covalently bound drug are often called polymer therapeutics.

With the increasing number of tumor types, the attention of scientists is focused on the development of polymer therapeutics, particularly in the field of tumor therapy. In traditional chemotherapy, the free low-molecular-weight cancerostatics are rapidly eliminated from the organism due to their relatively small molecular weight. At the higher doses required to achieve a sufficient therapeutic effect, they are also toxic to healthy organs and tissues, which is manifested by numerous unwanted side effects.

In contrast, the drug bound to the polymer carrier is transported in an inactive form in the bloodstream; it is maintained for a longer time in the circulation and after the polymer accumulates in the target tumor tissue, it may be released to achieve the desired cytotoxic effect. This feature can dramatically reduce the side effects of the drug, allowing the use of higher doses needed to achieve the maximal therapeutic effect.

However, there is still the problem of ensuring the preferential or specific transport of the drug to the tumor tissue, which can be achieved in two ways:

1. By utilizing tumor specificity, the high molecular weight polymeric cancerostatics are accumulated in solid tumors *via* the enhanced permeability and retention (EPR) effect (Maeda *et al.* 2006) (so-called passive targeting).

2. Using cell specificity, the drug effect is exclusively focused on the tumor cells due to cell receptor-specific targeting ligands that are bound to the polymer carrier (so-called active targeting).

There are many types of targeting ligands

described in literature, such as antibodies, antibody fragments, carbohydrates, lectins, cytokines, vitamins, peptides, aptamers or hormones (Pearce *et al.* 2012).

Short synthetic oligopeptides (up to approximately 20 amino acids) are an important group of molecules with specific affinity to certain cell receptors. They consist of either sequences derived from the binding sites of important natural proteins (e.g. fibronectin or laminin) (Shadidi and Sioud 2003) or peptides discovered using combinatorial procedures, such as the so-called "phage display" method (Pande *et al.* 2010).

The peptides can be synthesized relatively easily. They are less immunogenic than large proteins, and they can be covalently linked to the polymer carriers using standard synthetic procedures. Some peptides can be used not only as targeting ligands but also as effective inhibitors of angiogenesis due to their interactions with the fast growing tumor endothelium (Mitra *et al.* 2006).

Damage to the endothelium leads to the formation of blood clots and clumps of platelets in blood vessels. Consequently, this blockade prevents the flow of oxygen and nutrients to the tumor and tumor regression (Leite de Oliveira *et al.* 2011).

Development of the polymer drug carriers

The attachment of a drug to both natural and synthetic polymer was first achieved more than 50 years ago (Jatzkewitz 1955). The advantage of natural polymers lies in their easy accessibility; generally they have a narrow molecular weight distribution and a biodegradable backbone. Difficulties may arise in the separation of conjugates prepared from such polymers, which may require several consecutive purification steps. Their use is often limited by their high immunogenicity, particularly after conjugation with the drug, low reproducibility and quality of the biopolymers obtained from various batches or sources.

In contrast, synthetic polymers may be tailor-made precisely for the intended purpose; they may have various physico-chemical properties and may contain defined amount of various functional groups suitable for drug conjugation. Jatzkewitz (1955) performed one of the first studies to use a synthetic polymer as a drug carrier and used a dipeptide spacer to connect the drug (mescaline) to poly(vinylpyrrolidone). Ushak's group synthesized several water-soluble polymer-drug conjugates in the 1960s and 1970s (Panarin and Ushakov 1968).

In 1975, Helmut Ringsdorf designed his model of a pharmacologically active polymer. The model consisted of a biodegradable or biologically stable polymer that is also a carrier for at least three types of functional units. The polymer is not only the backbone of the system, its function is also to slow down the excretion of the drug from the body and simultaneously increase the probability of drug capture at the damaged site in the organism. The first unit, the so-called "solubilizer", increases the solubility of the system; the second unit enables attachment of a low-molecular-weight drug to the polymer chain. The drug is attached to the main chain *via* a spacer that is either stable under physiological conditions or it may be cleaved by hydrolysis or enzymatic process. The third unit, a transport system, is responsible for the transportation of the polymer into the target cells through the specific or nonspecific sorption of the polymer in the treated region in the organism.

Kopecek *et al.* (1991) further specified the structure of Ringsdorf's polymer drug model. He designed a water-soluble polymer carrier attached to low-molecular-weight drugs *via* enzymatically cleavable oligopeptide spacers and targeting moieties that are responsible for the delivery of the whole system to the target cells. The peptide spacer is designed to be stable during the transport in the bloodstream; it is only cleaved after the system is internalized in the target cell.

Currently, low-molecular-weight drug-polymer conjugates (Chytil *et al.* 2015), polymer-protein conjugates (Pechar *et al.* 2011), polymer micelles with covalently bound drug (Chytil *et al.* 2012) and polyelectrolyte complexes for gene delivery (De Smedt *et al.* 2000) are all commonly called polymer therapeutics (Satchi-Fainaro *et al.* 2006).

All of these polymer systems consist of at least three parts: a water-soluble polymer, a spacer and a drug. The drug can be released from the polymer carrier *via* various mechanisms, depending on the type of the spacer between the drug and the polymer. The structure of the spacer is selected according to the desired site of action; the spacer for extracellular release must be designed differently from the spacer intended for intracellular activation.

The significant development of polymeric therapeutic agents occurred over the last 50 years. Numerous types of water-soluble polymer drug carriers, including homopolymers, copolymers of vinylpyrrolidone (Yoshioka *et al.* 2004), copolymers of acrylic and methacrylic acid (Chytil *et al.* 2010, Etrych *et al.* 2011, Nan *et al.* 2005), copolymers of styrene and maleic acid

(Tsukigawa *et al.* 2015), poly(L-glutamic acid) (Li *et al.* 2013), poly(L-aspartic acid) (Zunino *et al.* 1982), poly(L-lysine) (Deng *et al.* 2007), polyethylene glycol (PEG) (Greenwald *et al.* 2000), polyoxazolines (Viegas *et al.* 2011), polysaccharides (dextrans, Ochi *et al.* 2005), cyclodextrins (Okamatsu *et al.* 2013) and proteins (albumin, Bolling *et al.* 2006), are described in the literature.

Currently, several anticancer polymer-drug conjugates are approved for clinical use, and some are in various stages of clinical trials (Duncan 2009, Canal *et al.* 2011). The copolymer styrene-maleic acid with neocarzinostatin (SMANCS) is currently the only clinically approved polymer-drug conjugate bearing a low-molecular-weight cytostatic for local administration. The second FDA-approved polymer-drug conjugate is the PEG-protein conjugate PEG-L-asparginase (Oncaspar), which is used to treat acute lymphoblastic leukemia and administered parenterally. Other polymer conjugates, including PEG-camptothecin (Garrett *et al.* 2013), poly(L-glutamic acid)-paclitaxel (Galic *et al.* 2011) and copolymers based on poly(N-2-hydroxypropyl(methacrylamide) with camptothecin (Bissett *et al.* 2004) or paclitaxel (Terwogt *et al.* 2001) are being tested in clinical trials. Surprisingly, the number of the clinical applications is still very low considering the large number of research papers in the field.

In addition to the delivery of low-molecular-weight drugs, synthetic polymers are also used for the modification of biologically active proteins. PEG-asparaginase is clinically approved for the treatment of acute lymphoblastic leukemia (Aldoss *et al.* 2016), and PEG-adenosine deaminase (Balasubramaniam *et al.* 2014) is indicated for certain human immunodeficiencies and for inborn adenosine deaminase deficiency.

Passive versus active tumor targeting of polymer therapeutics

Passive targeting of polymeric drug conjugates to solid tumors is based on the so-called enhanced permeability and retention (EPR) effect. This phenomenon is used to describe the enhanced non-specific uptake of macromolecules from the bloodstream to the solid tumor and their accumulation in the tumor tissue due to the increased permeability of the tumor endothelium combined with the impaired or even absent lymphatic drainage in tumors. This phenomenon can be utilized in the design of the polymer cancerostatics, as the accumulation of the polymers in the tumor can be increased by increasing the molecular weight of the polymer (up to several hundred kDa) (Maeda *et al.* 2006).

Passive targeting also reduces the toxic side effects of chemotherapy towards the healthy tissues. Unfortunately, it can only be utilized for targeting to well-vascularized tumors. Consequently, the efficiency of the EPR effect is reduced in smaller tumors (Lu *et al.* 2002). These malignancies are also more difficult to detect and remove by surgery. Therefore, actively targeted polymer therapeutics become a more suitable strategy for their therapy. In leukemias, active targeting is the only option.

Active targeting is based on presumption that the target cells express specific membrane receptors (antigens) that can be recognized by suitable targeting ligands to enable the delivery of the actively targeted polymer conjugate to the cells. The discovery of the appropriate targeting ligands and their attachment to the polymer-drug conjugates is the cornerstone of active targeting.

In this review, we will focus on the selection and application of peptides as ligands for active targeting of polymer cancerostatics.

Peptide ligands for active targeting

Peptide ligands for active targeting can be distinguished according to the method by which they were selected:

1. Specific peptide sequences derived from ECM proteins, and
2. Peptides obtained by combinatorial methods (e.g. phage display libraries, synthetic peptide libraries).

Alternatively, they can be distinguished according to the type of cells to which they are directed:

1. Tumor cells, including metastases, and
2. Endothelial cells of the tumor endothelium.

Specific peptide sequences derived from ECM proteins

Peptide sequences can be derived from the binding sites of important natural proteins (e.g. fibronectin, fibrinogen, laminin, vitronectin, or thrombospondin) (Balaoing *et al.* 2015). More information is provided in the chapter entitled “Targeting the tumor endothelium”.

Peptide sequences used for targeting can also be derived from the antigen binding region of antibodies.

Selection of the targeting peptides using combinatorial methods

Substances with a specific affinity against an antigen or receptor can be identified using simple but very efficient combinatorial methods, such as synthetic peptide libraries or the phage display method. The latter was first described by Smith (1985). The method is based on the fact that combinations of all possible peptide sequences of certain length are prepared by recombinant technology and displayed on the surface of the filamentous bacteriophage fd. For example, the library of all possible tripeptides (consisting of 20 natural amino acids) contains 203 different sequences. Obviously, the longer the sequence, the more combinations must be taken into account. For longer sequences (with more than approximately 15 amino acids), the number of viral particles in the experiment can be lower than the number of possible peptide sequences. This strategy would result in an incomplete peptide library that might miss the peptides that bind to the desired antigen, as each phage carries only one peptide sequence. The phages are then multiplied in *E. coli*, resulting in a collection of bacteriophages with a single random peptide sequence of a given length attached to every phage particle known as a phage display library.

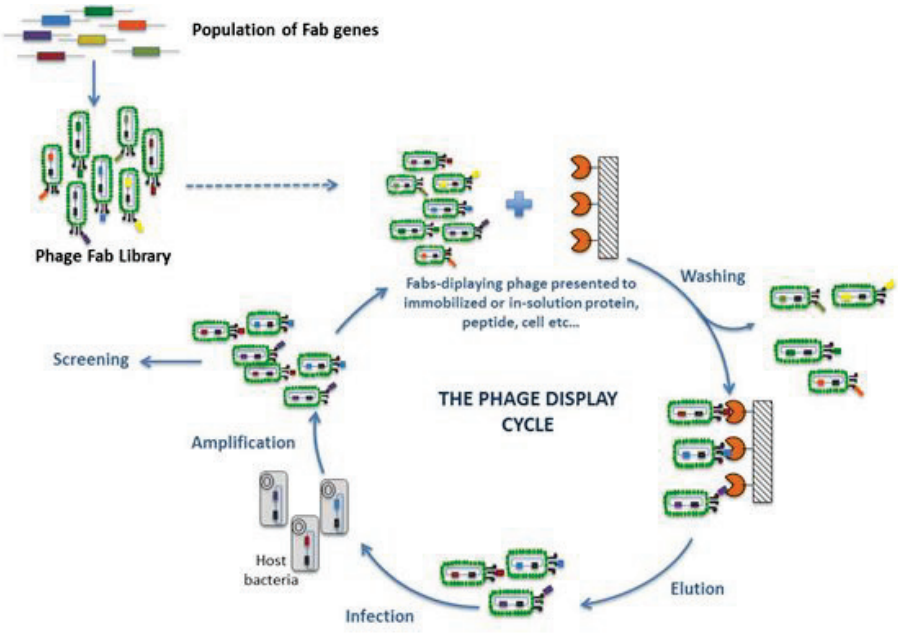

Fig. 1. The phage display method (http://fjb.pt/phage-display/).

Then, the phage library is applied to a column with the immobilized antigen of interest (Fig. 1). The phages bearing the peptides with certain affinity towards the antigen are captured on the column and the others are washed out. The antigen-bound phages are then selectively released from the column, multiplied in *E. coli* and purified on the column again. After 2-4 rounds of biopanning, the peptide sequence(s) with the highest affinity towards the antigen are selected and identified (Pande *et al.* 2010). Oligopeptides that specifically bind to the vasculature of various organs were identified after a direct i.v. injection of the phage display library into experimental animals *in vivo*. Ruoslahti and co-workers discovered that the RGD sequence specifically binds to $\alpha v\beta_5$ integrins (Pasqualini and Ruoslahti 1996).

The phage display method enabled the identification of numerous peptides targeting either to the tumor endothelium or directly to various types of tumor cells. The primary structure of some antibody binding sites was also verified using this method (Pande *et al.* 2010).

Many sequences that are already in various phases of clinical trials were identified using phage display (Table 1). Some of the peptides serve as targeting ligands of cytostatics (e.g. doxorubicin), (Pola *et al.* 2007) whereas others are used as tumor diagnostics in combination with radionuclides (Hruby *et al.* 2010), and may be eventually used to target viral (Parker *et al.* 2005) and non-viral (Huang *et al.* 2012) vectors for gene delivery.

Table 1. Examples of peptides identified using phage display.

Peptide sequence	Cell target
Antibody-derived	
YXXEDLRRR	SUP-B8 human B-cell lymphoma,
XXPVDHGL	Human myeloma, M-protein,
FXDXRL, XIHYIF	E-Selectin
IELLQAR	
To tumor cells	
CTLVPHTRCGGGK	Prostate-specific antigen
TSPLNIHNGQKL	Flat head and neck cancer cells
LTVXPWX	Breast carcinoma cells
To tumor endothelium	
CDCRGDCFC (RGD-4C)	Integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$
CGSLVRC, CGLSDSC,	Tumor vasculature
RRKRRR	VEGF

Targeting to the tumor cells

This type of targeting can be further divided to direct or indirect targeting (Minko *et al.* 2004). In the first case, the polymer conjugate is targeted directly to the tumor cells expressing the specific receptors that interact with the targeting ligands bound to the conjugate. First, the conjugate is bound to the receptor; then, it is internalized into the cell *via* the receptor-mediated endocytosis, which is significantly faster than fluid phase endocytosis. In other words, active tumor targeting is an attempt to selectively localize the biologically active substance in the tumor cells.

In contrast, indirect targeting usually refers to a specific anti-tumor response of the activated immune system against the tumor cells without the use of any chemotherapeutics. The targeting ligand is linked with a specific antigen that enables the malignant cells to be recognized by the immune system. This approach is sometimes called active specific immunotherapy (Khandare and Minko 2006).

Targeting the tumor endothelium

A new paradigm in cancer therapy was formulated by Folkman *et al.* (1971). It is based on the fact that the growing tumor tissue needs a continuous supply of nutrients that is provided by the newly formed tumor vasculature. If the formation of the new blood vessels – angiogenesis – is stopped, the tumor growth (including the formation of metastases) is discontinued and the starving tumor dies.

It was shown that a tumor larger than 1 mm in diameter or a tumor consisting of more than 108 cells needs its own vasculature to grow (Hanahan and Folkman 1996). This finding led to design and preparation of a new class of therapeutics targeted against the tumor endothelial cells forming the inner surface of the tumor blood vessels (Satchi-Fainaro *et al.* 2006).

The process of angiogenesis is accompanied by the increased expression of various growth factors and their corresponding cell receptors, as well as proteases and adhesion molecules (Carmeliet and Jain 2000), which all become new targets for antiangiogenic therapy. Tumor angiogenesis differs from the physiological angiogenesis with regard to the structure of the newly formed blood vessels, their permeability, blood flow and other properties.

Various cytostatic agents can be directed to vascular endothelial cells of the tumor (Pola *et al.* 2007). The advantage of targeting these cells is their direct accessibility from the bloodstream without need for drug extravasation. As a consequence of dying cells, blood clots and clumps of platelets within the tumor further obstruct the supply of nutrients for the tumor and subsequently inhibit tumor growth. In parallel, the formation of new blood vessels is stopped due to the inhibition of the cell receptors – integrins – responsible for the adhesion of the cells to the extracellular matrix. Another advantage of endothelial targeting is that the endothelial cells are genetically stable compared to cancer cells and thus they cannot become resistant to the cytotoxic agents. The high number of the angiogenic inhibitors that are currently being tested in clinical trials documents the importance of this strategy (Ellis *et al.* 2002).

This therapeutics can be divided into two groups. The first one includes drugs that target the already formed tumor blood vessels; the second group inhibits the formation of the tumor vasculature and simultaneously promotes the regression of existing vessels (Satchi-Fainaro *et al.* 2006). Endogenous angiogenesis inhibitors (Endostatin and Angiostatin) or vascular endothelial growth factor (VEGF) antagonists (Avastin) belong to the latter group. The cellular receptor inhibitors are often peptide sequences derived from proteins of the extracellular matrix (ECM). During angiogenesis, the endothelial stem cells are bound to ECM *via* specific receptors, integrins (Fig. 2). Integrins are responsible for cell-cell interactions and the interactions between cells and ECM proteins. The interaction between the integrins and ECM is mediated by specific peptide sequences that are derived from the ECM proteins.

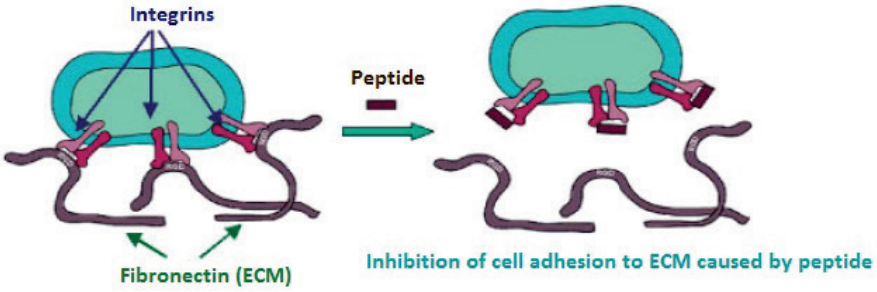

Fig. 2. The schematic of angiogenesis inhibition caused by binding of the peptide to cell surface integrins.

It was shown that $\alpha v\beta_5$ and $\alpha v\beta_3$ integrins significantly contribute to the formation of the tumor vasculature (Hynes 2002). These integrins are expressed on proliferating endothelial cells; they are mostly expressed during angiogenesis, whereas the non-dividing cells express almost no αv integrins. Their inhibition blocks angiogenesis, which leads to tumor regression. The $\alpha v\beta_3$ antagonists are being tested as potential antitumor drugs in various stages of clinical trials. Tumor-related angiogenesis can be stopped by blocking this integrin with a low-molecular-weight antagonist (Nisato *et al.* 2003).

Some therapeutics combines the effects of both groups, i.e. inhibition of angiogenesis and vascular therapy, to destroy the new tumor blood vessels. Combretastatins change the shape of the tumor endothelial cells, which results in thrombosis of the tumor blood vessels and tumor cell destruction (Zhang *et al.* 2011).

The pros and cons of vascular endothelial cell targeting versus tumor cell targeting are summarized in Table 2. Vascular targeting has several advantages. The endothelial cells are directly accessible from the bloodstream, in contrast to the tumor cells. Every endothelial cell is responsible for nurturing almost 100 tumor cells. Therefore, it is more efficient to kill the endothelial cells than the tumor cells. Moreover, the tumor cells are genetically unstable; their membrane receptors change over time (Alessi *et al.* 2004).

Table 2. Comparison of the two types of tumor targeting.

	Endothelial cells (EC)	**Tumor cells (TC)**
Genetic information	Stable	Unstable Development of resistance
Action	1 EC nurtures 50-100 TC Cytotoxic	All TC Cytotoxic
Accessibility	Directly from bloodstream	Extravasation necessary
Targets	Specific receptors	Few tumor cell-specific antigens Rapidly dividing cells
Side effects	Rare or none	Yes
Duration of therapy	Long	Short
Regression	Slow	Fast
Result of therapy	Stabilized disease Avascular stage Tumor regression	Elimination of the tumor cells

The combination of the two types of the active targeting described above seems to be a more efficient therapeutic approach than targeting of both cell types separately.

Peptide-based targeting ligands

In recent years, many potential targeting ligands of various structures that were expected to successfully and reliably deliver therapeutics to tumor cells were designed, synthesized and evaluated, including unsaturated higher fatty acids (Lau and Archer 2010), small organic molecules (Satchi-Fainaro *et al.* 2004), polymers and other substances. Antibodies, antibody fragments and peptides derived from antibody binding sites exhibited very good tumor-targeting specificity

(Zangemeister-Wittke 2005, Chytil *et al.* 2010).

The spatial accessibility of the targeting ligand and the corresponding cell receptor plays an important role in their interaction. This accessibility can be substantially affected by the length and structure of the spacer between the targeting unit and the polymer carrier. In this review, we will mostly focus on peptide-based targeting ligands.

In recent years, many peptides with potential therapeutic activity were discovered. The disadvantages of using natural peptides for direct therapeutic applications are their short half-life in the blood circulation, fast proteolytic degradation *in vivo* (Khandare and Minko 2006) and capture in kidneys (Adessi and Soto 2002). To increase their resistance to proteolysis, the peptides intended for therapeutic applications are usually chemically modified. The most common methods are incorporation of D-amino acids, cyclization or modification of the peptide termini.

Some peptides were also reported as carriers of cytostatics (Brokx *et al.* 2002) or as constituents of polymer drug carriers (Van Domeselaar *et al.* 2003). Other peptides exhibited good targeting either to various malignant cells or to tumor endothelial cells. Short peptides (up to 20 amino acids) can be prepared by solid phase synthesis, which is relatively easy and inexpensive. Recombinant DNA technology is more convenient for the preparation of longer sequences.

Peptides are relatively stable compounds; they can be relatively easily chemically modified. They can be covalently attached to a polymer carrier using standard chemical methods. The conjugation of the peptides to polymers improves their stability in the organism. Due to the selective binding of peptides to cell receptors, the peptides are ideal targeting ligands for various therapeutics (e.g. cancerostatics, oligonucleotides, toxins or radionuclides). In contrast to polymer-antibody conjugates, polymer-peptide conjugates are almost invisible to the immune system. Consequently, they exhibit minimal side effects (Shadidi and Sioud 2003) and they better penetrate the tumor tissue and the cell membranes. The peptides derived from antibody binding sites maintain their receptor binding affinity and specificity. Moreover, the corresponding polymer-peptide conjugates usually possess better defined structures.

The intracellular fate of the conjugate is influenced by numerous factors, including the type of the polymer carrier, the drug, the targeting ligand, the type of the cells being tested and the current phase of the cell cycle.

Examples of peptide-targeted polymer cancerostatics

There are still only a few papers in literature that describe oligopeptides as targeting moieties that are covalently bound to polymer carriers of biologically active molecules.

Polymer conjugates based on HPMA copolymers bearing RGD4C peptides labeled with radionuclides ^{99}Tc and ^{90}Yb were described, characterized and their biodistribution in mice was reported (Mitra *et al.* 2006). The corresponding conjugate with the RGE4C peptide was prepared and used as a negative control. The polymer conjugate with RGD4C inhibited cell adhesion *in vitro* by blocking of the $\alpha v\beta_3$ integrin; the control polymer exhibited no activity. Similarly, the *in vivo* accumulation of the RGD4C-targeted polymer in the tumor after 24 h was 1,000 times higher than the negative control. The targeted polymer was still detected in the tumor at 72 h after injection.

Another paper reported on the targeting of polymer conjugates with either RGD4C or c(RGDfK) containing ^{111}In as a radioactive label (Mitra *et al.* 2006). Both types of polymer conjugates similarly bound to $\alpha v\beta_3$ integrin, thus blocking cell adhesion. Both polymers were detected in the tumor, even 192 h after injection in the mice.

The nonapeptide CPLHQRPMC, which has high affinity for PC3MM2 human prostate tumor cells, was identified using the phage display method. The polymer conjugate bearing doxorubicin was targeted using this peptide. The targeted conjugate exhibited significantly higher antiproliferative activity against PC3MM2 cells *in vitro* (Pola *et al.* 2007) compared with the non-targeted conjugate.

In addition, some polymer conjugates targeted with cyclic and linear RGD tripeptides were prepared and tested. The conjugate with cyclic RGD showed higher efficiency of targeting to endothelial cells compared with the conjugates with the linear form of RGD (Pola *et al.* 2009).

The targeting of RGD to $\alpha v\beta_3$ integrin and the CNGRC peptide to the membrane-bound enzyme aminopeptidase N was compared to passive targeting using the EPR effect in Kunjachan's study (Kunjachan *et al.* 2014). There was no significant difference between the actively targeted conjugates. They were both visible in the tumor endothelium soon after i.v. administration; however, their long term accumulation was reduced

compared with the passively targeted conjugate.

The peptide sequence GE-7 (NPVVGYIGERPQYRDL) derived from the EGF receptor was attached to a fluorescently labeled polymer carrier (Studenovsky *et al.* 2012). The measurements of binding activity showed that the polymer conjugate binds more efficiently to EGF receptor-rich cells (FaDu and MCF-7 cells) than to cells with low EGF receptor expression (SW620 and B16F10 cells).

Reactive HPMA-based copolymers targeted to tumor endothelium with YESIKVAVS (Stevenson *et al.* 2007) or SIGYPLP (Parker *et al.* 2005) peptides were successfully used for the surface modification of both viral and synthetic vectors for gene delivery.

Another publication showed that an HPMA copolymer containing the cancerostatic drug doxorubicin that was attached *via* the enzymatically cleavable tetrapeptide spacer GFLG and peptide sequence WHYPWFQNWAMA (Nan *et al.* 2005) selected by phage display method was used as a targeting ligand to selectively bind to squamous head and neck cancer cells. Internalization of the targeted polymer conjugate into the tumor cells followed by lysosomal uptake of the polymer and doxorubicin release was confirmed by confocal microscopy.

Another publication showed that a polymer conjugate used for gene delivery was composed of poly(ethyleneimine) as a carrier and the peptide DMPGTVLP as a targeting moiety linked *via* disulfide bridges. The peptide sequence was identified by the phage display method. This conjugate was used for gene delivery to breast adenocarcinoma cells (MCF-7). Hepatocellular carcinoma cells (HepG2) were used as a negative control. The transfection efficacy was approximately 2.6- to 4-fold higher for the DMPGTVLP-poly(ethyleneimine) conjugate compared with the negative control (Mokhtarzadeh *et al.* 2015).

Zhong *et al.* (2015) prepared a polymer conjugate consisting of an HPMA copolymer carrier, the H1 peptide as a model drug and the R8 nuclear localization sequence (NLS) peptide as a targeting moiety. It consists of eight arginines and PKKKRKV. A bi-forked structure consisting of the R8 NLS and the H1 peptide is attached to the polymer carrier *via* an enzymatically degradable GFLG spacer. The R8 NLS peptide refers to a cell penetrating peptide and a nuclear localization moiety. The model drug H1 peptide is derived from c-Myc; inhibition of c-Myc signaling pathway is one of the possible strategies of antitumor therapy. This conjugate exhibits prolonged blood circulation and a tumor homing ability (Zhong *et al.* 2015).

Conclusions

Targeted polymeric drugs represent wide range of possibilities for efficient tumor elimination. Currently, many antineoplastic macromolecular therapeutics are being developed that take advantage of either the EPR effect (passive targeting) or active targeting using various targeting ligands. These ligands target the polymer therapeutics either directly to the cancer cells or to the tumor endothelium. Synthetic oligopeptides are very important targeting moieties with the advantage of the relatively easy preparation of shorter amino acid sequences (up to ca 30 AA) using solid phase peptide synthesis. A large number of various tumor-targeting peptides have already been described in the literature; new peptides derived from natural proteins or identified by combinatorial methods (e.g. phage display) are being discovered every year. The diverse array of these peptides offers an excellent opportunity for improving the outcome of current neoplastic treatments.

Conflict of Interest

There is no conflict of interest.

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