REVIEW

Targeted nanoparticles – a promising opportunity in cancer therapy – Review

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Nanoparticles as drug delivery vehicles pose an exciting and promising future for cancer treatment, and offer particular benefits not only for cancer treatment, but also for overcoming of multidrug resistance in cancer tissues. Targeted delivery of anti-neoplastic drugs by nanoparticles promises enhanced drug efficacy, selectivity and reduced systemic toxicity. Nanoparticle systems have unique properties that allow for both passive and active targeting of tumors. Active targeting of nanoparticles, that usually involve surface proteins known to be upregulated in cancer cells, increases accumulation in a tumor. Targeting molecules include antibodies or their fragments, aptamers, or small molecules. This review describes a comprehensive overview of different targeting of nanodrugs.

Keywords: nanoparticles; active targeting; enhanced permeability and retention effect

1. Introduction

Nanotechnology includes development, production and utilization of materials, equipment and systems on the nm-length scale, i.e. at the level of molecules and of supramolecular structures. It may be applied apart from in biotechnology and medicine. The main applications of nanotechnology in medicine (nanomedicine) are materials and devices for diagnostics and for drug delivery. The history of nanoparticles starts in 1950s with a polymer-drug conjugate that was designed by Jatzkewitz, followed by Bangham, who discovered the liposomes in mid-1960s. In 1972, Scheffel and colleagues first reported albumin based nanoparticles, which formed the basis of albumin-bound paclitaxel (Abraxane) [1].

Concerns connected with the drug delivery, such as troublesome solubility and biological availability, short time of circulation in blood vessels and/or inconvenient biodistribution to the target organ may occur. Nanoparticle-mediated targeted delivery of drugs might significantly reduce the dosage of the drugs, increase its specificity and bioavailability, overcome chemoresistance and reduce side effects of therapy. Mainly in the cancer therapy, targeted delivery in a localized way is one of the most important challenges. Nanovectors for drug delivery typically contain a core material or matrix, a therapeutic payload, and in some cases surface modifications. The key features of anticancer nanoparticles are mainly nanoparticle size, surface properties (e.g. hydrophobicity) and targeting ligands. Generally, 200 nm is considered as upper limit for nanoparticle size, while the minimal diameter should be about 10 nm. The development of a broad range of nanoparticles with the ability to tune size, composition, and functionality has provided a significant resource for nanomedicine. Overview of core materials and matrix shows Table 1.

Although nanoparticles avoid renal clearance, they tend to accumulate in the mononuclear

phagocyte system (MPS). Surface conjugation with polyethylene glycol (PEG) and other polymers improves particle circulation by reducing uptake into the MPS. Certainly, nanoparticle property requirements also depend on tumor characteristics including a cancer type, stage of disease or location. Delivering multiple agents *in vivo* is complicated because of their independent pharmacokinetics, biodistribution and clearance. A delivery system should transport a drug with high efficiency to target cells, with minimal toxicity and immune response. Drug toxicity can be reduced by encapsulating the free drug (e.g. liposomes) or by locally activating a pro-drug.

The main challenges for drug delivery are protecting it from degradation in circulation, avoiding degradation by enzymes in endosomes of the target cell and escaping from endosomes to reach the target compartment. Nanoparticle delivery systems can consolidate these properties into one vehicle and increase the likelihood that targeted tumor cells receive both agents at a ratiometric dose. There have been several reports of codelivering multiple anticancer agents using nanocarriers, some are reaching clinical trials and certain nanodrugs are even FDA approved [2]. This review describes a comprehensive overview of different targeting of nanodrugs.

2. Nanoparticle targeting

Nanoparticle systems have unique properties that allow for both passive and active targeting of tumors. Because of up regulation of pro-angiogenic signaling, most solid tumors are hypervascular. However, the new vessels have abnormal architecture and are highly permeable. The tumor mass also has poor lymphatic drainage, allowing for accumulation of macromolecules greater than approximately 40 kDa within its microenvironment. Nanoparticles exploit this feature, which is called the enhanced permeability and retention (EPR) effect, to target solid tumors. The ideal size range to benefit from the EPR effect is between 10 to 200 nm. Particles that are too small will be cleared by kidney, preventing accumulation into the tumor site, and particles that are too large will not adequately penetrate the tumor vasculature and interstitial space [3]. Antibody drug conjugates or liposomes with a pegylated surface have comparatively long half-times (3 – 4 days). Increasing elimination is expected to increase tumor accumulation via the EPR effect. However, increased tumor accumulation does not necessarily imply improved efficacy because transport, uptake, drug release, and delivery to the appropriate cellular compartment are all downstream of extravasation by the EPR effect [4]. Particle surface modifications can

Particle type	Composition/structure	Properties
Polymer	Copolymers, hydrogels, chitosan, PLGA, glycerol etc.	Some biodegradable
Dendrimer	Poly(amidoamine)	Low polydispersity, biocompatible
Lipid	Liposomes, micelles	Can carry hydrophobic drugs, biocompatible, biodegradable
Gold	Spheres, rods, or shells	Biocompatible
Silica	Spheres, shells, or mesoporous	Biocompatible
Carbon-based	Carbon nanotubes, buckyballs, or graphene	Biocompatible

Table 1: Summary of nanoparticles platforms for drug delivery.

be incorporated to improve cell targeting and internalization while bypassing certain forms of multidrug resistance.

Active targeting, i.e. surface modifications of nanoparticles, is a way to decrease uptake in normal tissue and increase accumulation in a tumor. Strategies for active targeting of tumors usually involve targeting surface membrane proteins that are upregulated in cancer cells. Targeting molecules are typically antibodies or their fragments, aptamers, or small molecules [4]. Nanoparticles coupled with surface ligands or antibodies can localize to tissue expressing the associated receptors or antigens and improve delivery efficacy [2]. Certain ligand receptor interactions will facilitate receptor-mediated endocytosis, which can further enhance payload delivery. A surface ligand or antibody coupling can achieve densities high enough to interact efficiently with target sites, and these techniques lend themselves well to cancer therapies.

Monoclonal antibodies usually IgG isotype are widely used for targeting. Antigen binding sites represent only a small part of the overall size of antibodies. F(ab´)2 fragments retain both antigen binding sites of the antibody coupled by disulfide linkages. Many tumors up-regulate growth factor receptors, such as HER2/neu in certain breast cancers, which can be targeted with anti-HER2/neu surface antibodies [5].

Aptamers are folded single strand oligonucleotides, 25 – 100 nucleotides in length that bind to molecular targets. EpCAM-fluoropyrimidine RNA aptamer-modified doxorubicin loaded PLGA-b-PEG nanoparticles that bond specifically to the extracellular domain of epithelial-cell adhesion molecules were tested *in vitro* and *in vivo* on non-small lung cancer models with positive results- aptamer-conjugated nanoparticles have increased cytotoxicity and more diminished volume of xenografts compared to non-targered nanoparticles [6].

Small molecules for targeting include peptides, growth factors, carbohydrates, and receptor ligands see Table 2 and Figure 1. Example of small protein targeting is the use one of HER2/neu ligands (AHNP) for targeting of poly(lactide-coglycolide) nanoparticles with docetaxel tested *in vitro* on HER2/neu+ breast cancer cells [7].

Specific examples of small molecules include folic acid, transferrin, and the RGD peptides. Folic acid (FA) is essential for amino acid synthesis and hence for cell survival and proliferation. The human folate receptor (FR), glycosylphosphatidylinositol-anchored membrane protein of 38 kDa, has high affinity for the FA, and is currently considered an essential component in the cellular accumulation of FA used in chemotherapy. FR expression is very low or not detectable in most normal cells and tissues, but it is upregulated in ovarian, breast, brain, lung, and colorectal cancers [8]. Through a process of endocytosis ligand-bound receptor is internalized and released from the receptor through intravesicular reduction in pH. Ligand--free receptor is then recycled to the cell surface [9]. Interestingly, covalent conjugation of small molecules, proteins, and even liposomes to the g-carboxyl moiety of folic acid does not alter its ability to bind the folate receptor and undergo endocytosis by receptor bearing cells. FR-mediated liposomal delivery has been shown to enhance the antitumor efficacy of doxorubicin both in vitro and in vivo, and to overcome P-glycoprotein-mediated multi-drug resistance [10].

Transferrin (Tf) is a single-chain iron-transporting glycoprotein that supplies iron into cells via receptor-mediated endocytosis. The transferrin receptor (TfR) is expressed at low levels in most normal tissues but it is overexpressed in many tumor types. Significant for its application in molecular targeting, the binding of Tf to TfR on the external surface of tumor cells is ten- to hundred-times more effective than in normal cells. This feature has been exploited for drug delivery, most often by labeling the surface of the drug carrier with Tf, which is recognized by, and actively transported into, tumor cells [11]. Therefore, Tf-modified liposomes, nanoparticles and dendrimers have been widely investigated in recent years. Ferritin protein also self-assembles naturally into a hollow nanocage called apoferritin useful for encapsulation of any molecule of interest. Apoferritin can be modified with recognition ligands to achieve tumor-specific targeting. However, these extra surface modifications can

avoid renal clearance and ensure EPR effect but also destroy the intrinsic tumor-specific binding of natural ferritin and disturb its in vivo performance and biocompatibility because of the altered surface physicochemical properties of ferritin [12].

The RGD (Arg-Gly-Asp) peptide is a component of the extracellular matrix protein fibronectin and promotes cell adhesion and regulates migration, growth, and proliferation. RGD is known to serve as a recognition motif in multiple ligands for several different integrins. Integrin-mediated cell attachment and internalization

Name	Targeting ligands	Receptor
Antibodies	Herceptin (Trastuzumab)	Her2/neu (Breast, gastric, lung cancer)
	Rituxan (Rituximab)	CD20 (B-cell non-Hodgkin lymphoma and leukemia)
	CD19 antibody	CD19 (B-cell non-Hodgkin lymphoma and leukemia)
Aptamers	Pegaptanib	VEGF receptor
	A10 aptamer (Apt)	Prostate-specific membrane antigen (PSMA)
Peptides	RGD	Integrin receptors
	ATWLPPR (VEGF peptide)	VEGF receptor
	Vasoactive intestinal peptide (VAP)	VAP receptor
	Lyp-1	p32 receptors (p32/gC1qR)
Proteins	Transferrin	Transferrin receptor
	Luteinizing hormone releasing hormone (LHRH)	LHRH receptor
Small molecules	Folic acid	Folate receptor
	Galactose	Asialoglycoprotein receptor (ASGPR)
	Biotin	Biotin receptor
	Mannose	MRC1 mannose receptor

Table 2: Commonly used targeting ligands.

are exploited by a variety of bacteria and viruses for cell entry. It is also suggested that the RGD-containing peptide can be internalized into cells by integrin-mediated endocytosis. Recently, integrin-mediated carriers have been investigated as gene vehicles to enhance gene transfection and as vehicles to delivery anticancer agents. The upregulation of integrins is promoted by angiogenic factors in several cancer types is known [4,13].

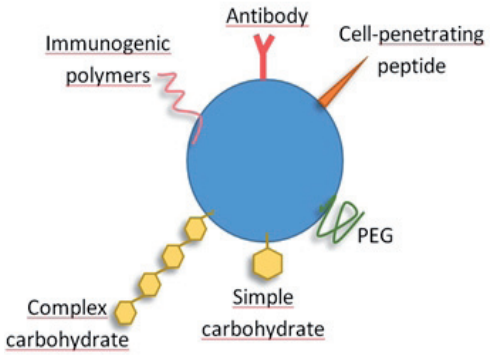

Figure 1: Some examples of nanocarrier functionalization.

3. Nanoparticles in clinical practice

Despite progress in basic and preclinical cancer nanomedicine research, the only important challenge is clinical translation. However, most of the many different nanoparticles developed for cancer therapy have not past clinical trials. There are currently only six FDA-approved nanomedicines: Adcetris (Brentuximab vedotin) and Kadcyla (Trastuzumab emtansine) are antibody-drug conjugates, Doxil (liposomal doxorubicin), DaunoXome (liposomal daunorubicin), Marqibo (liposomal vincristin), and Abraxane (human serum albumin non-specifically bound to paclitaxel). Brentuximab targets CD30, protein expressed by B cells, including B-cell lymphomas, Hodgkin lymphoma, some leukemias, and also melanoma cancer stem cells. Trastuzumab targets the human epidermal growth factor receptor 2 (HER2) overexpressed in HER2 positive breast cancer and also some other cancers (non-small lung, gastrin, ovarian, uterine). Monomethyl auristan E (Brentuximab vedotin) and mertansine (Trastuzumab emtansine) are too toxic to be used alone and hence coupling to a targeting antibody significantly reduces their side effects.

4. Future directions and conclusions

There are several challenges for targeted nanodrug delivery systems to overcome. Still most of these drug systems undergo in vitro and in vivo testing. Therefore their relevancy to the real patients has to be evaluated extensively. Each nanodrug platform is distinctive and needs to be assessed experimentally as a new system, which is strenuous. Stability of nanoparticles, size uniformity, a controlled drug release rate, sterile preparations in a large scale and the manufacturing cost have to be addressed in order to make them available to the practice. But with recent scientific advances, in the next ten years it is expected to see a large number of targeted drug delivery systems based on nanoparticles in the market [14].

There are three major challenges with using nanoparticles as in vivo diagnostics and therapeutics: high background retention in the RES, lack of complete elimination from the body and arriving at hydrodynamic diameter small enough for rapid equilibration between the intravascular and extravascular spaces. Solving all of these problems, while maintaining high specificity for desired targets, is extremely difficult. Since tumor cells generally overexpress various kinds of receptors on the cell membrane, receptor-mediated delivery of bioactive agents is an ideal way to maximize antitumor activity and minimize the side effect of anticancer drugs. Nanoparticles are an ideal candidate for these purposes because the target ligand can easily be decorated on the large surface area of nanoparticles [15].

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Conflicts of Interest

The authors declare no conflict of interest.

The authors declare they have no potential conflicts of interests concerning drugs, products,services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform reguirements“ for biomedical papers.

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