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# Ligand-targeted theranostic nanomedicines against cancer

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ABSTRACT

Nanomedicines have significant potential for cancer treatment. Although the majority of nanomedicines currently tested in clinical trials utilize simple, biocompatible liposome-based nanocarriers, their widespread use is limited by non-specificity and low target site concentration and thus, do not provide a substantial clinical advantage over conventional, systemic chemotherapy. In the past 20 years, we have identified specific receptors expressed on the surfaces of tumor endothelial and perivascular cells, tumor cells, the extracellular matrix and stromal cells using combinatorial peptide libraries displayed on bacteriophage. These studies corroborate the notion that unique receptor proteins such as IL-11Rα, GRP78, EphA5, among others, are differentially overexpressed in tumors and present opportunities to deliver tumor-specific therapeutic drugs. By using peptides that bind to tumor-specific cell-surface receptors, therapeutic agents such as apoptotic peptides, suicide genes, imaging dyes or chemotherapeutics can be precisely and systemically delivered to reduce tumor growth *in vivo*, without harming healthy cells. Given the clinical applicability of peptide-based therapeutics, targeted delivery of nanocarriers loaded with therapeutic cargos seems plausible. We propose a modular design of a functionalized protocell in which a tumor-targeting moiety, such as a peptide or recombinant human antibody single chain variable fragment (scFv), is conjugated to a lipid bilayer surrounding a silica-based nanocarrier core containing a protected therapeutic cargo. The functionalized protocell can be tailored to a specific cancer subtype and treatment regimen by exchanging the tumor-targeting moiety and/or therapeutic cargo or used in combination to create unique, theranostic agents. In this review, we summarize the identification of tumor-specific receptors through combinatorial phage display technology and the use of antibody display selection to identify recombinant human scFvs against these tumor-specific receptors. We compare the characteristics of different types of simple and complex nanocarriers, and discuss potential types of therapeutic cargos and conjugation strategies. The modular design of functionalized protocells may improve the efficacy and safety of nanomedicines for future cancer therapy.



## 1. Introduction

Limitations of conventional cancer drug efficacy include insolubility, systemic toxicity and drug resistance compounded by debilitating side effects such as nausea, fatigue, neuropathy, and organ failure. An effective solution to circumvent these limitations is to deliver cancer drugs within biocompatible nanocarriers. Simple nanocarriers span diverse materials such as magnetic or colloidal metals, carbon-based structures,

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silica, liposomes or polymeric formulations. These materials differ in size, shape, loading capacity, payload release, stability, retention and clearance from the body, which impose further restrictions on their efficacy as cancer therapeutics. For example, nanocarrier size is a critical determining parameter since particle sizes <5 nm are cleared in the urine [1] although particles up to 50 nm have been detected as well, and nanoparticles >100 nm are cleared by the mononuclear phagocyte system (MPS), respectively. Ideally, an optimally loaded nanocarrier would be stable in the circulation to protect and deliver its therapeutic cargo to the target site, have good penetrance and retention within the target site so that measured cargo release occurs within a therapeutic window, and ultimately be organically cleared to prevent toxicity from long-term accumulation [2]. By combining features from simple nanocarriers, complex nanocarriers have improved biocharacteristics so that delivery of cancer therapeutics is clinically efficacious.

Although nanocarrier technology has improved, their lack of target specificity limits their widespread use. In solid tumors however, large fenestrations at endothelial cell borders and numerous, loose pericyte attachments are characteristic of rapidly growing tumor blood vessels that allow nanocarriers to passively exit the circulation within tumors and accumulate non-specifically [3–5]. This phenomenon is referred to as the enhanced permeability and retention (EPR) effect [6,7]. Nevertheless, the EPR effect does not significantly increase payload concentrations at the target site and in fact, increased circulation times dissipate accumulation [8]. So, how could nanocarrier targeting and retention be improved for efficacious tumor treatment?

Since 1996, we and others have used, modified and adapted *in vivo* and *in vitro* phage display to identify ligand-receptor or scFv–epitope pairs as a means to specifically deliver a covalently linked apoptotic peptide, chemotherapeutic drug, reporter or suicide gene or imaging agents directly to tumors by intravenous administration [9–30]. Unlike other targeting moieties, peptides identified by *in vivo* phage display bind only to physiologically accessible receptors and, depending on the selection constraints, can enrich for targeting moieties that are internalized into cells subsequent to ligand binding. Thus, functional selection of targeting peptides embedded within the experimental design circumvents issues such as the EPR effect and non-specific uptake and obviates the need to reassess internalization of tumor-targeted therapeutics during downstream drug development. Additionally, depending on receptor location, *i.e.*, tumor *vs.* tumor endothelial cells, internalization of nanomedicines will minimize or maximize, respectively, their distribution within the tumor *via* the bystander effect [31]. Off-target effects are minimized by using targeted liposomes loaded with doxorubicin to treat neuroblastoma [32–35]. Targeting liposomal doxorubicin to cultured human breast cancer or pancreatic adenocarcinoma cells is improved by inserting different targeting peptides purified as fusion proteins of the bacteriophage pVIII major coat proteins [36]. Consequently, one could envision a modular design of a targeted, stable complex nanocarrier consisting of a peptide ligand or monoclonal antibody targeting moiety conjugated to the lipid bilayer coated mesoporous silica nanocarrier, termed a functionalized protocell, which can specifically deliver a protected therapeutic cargo intravenously or locally by peritumor injection or inhalation. The term protocell (also known as a protobiont) is utilized in evolutionary biology to describe a self-organized spherical collection of lipids proposed as a stepping-stone to the creation of life. In the context of nanomedicine (and throughout this review) we use the term protocell to refer to a cell-like nanocarrier composed of a high surface area mesoporous silica nanoparticle core enveloped within a supported lipid bilayer [37–39]. In this construct, the core can be loaded with high concentrations of disparate cargos. The lipid bilayer serves to seal and protect the cargo and provides a biocompatible interface that can be conjugated with polymers to enhance stability and peptides or antibodies to direct specific targeting and intracellular trafficking.

The modular design of functionalized protocells will permit the targeting moiety to be exchanged depending on the tumor of interest. For instance, the targeting moiety can be a peptide or antibody-like moiety such as a single chain variable fragment (scFv) that binds to overexpressed receptor proteins such as interleukin-11 receptor alpha (IL-11Rα) [23,40–42], or the 78-kDa glucose-regulated protein (GRP78) [43–47] in prostate or breast tumors. EphA5 would be an appropriate surface receptor to target in non-small cell lung tumors due to its high expression [18,29]. scFvs that exhibit distinct receptor affinities or bind to different epitopes can be used as the binding moiety to elicit a specific therapeutic effect. For example, scFvs can be used to inhibit or modulate receptor function or act synergistically with the delivered therapeutic cargo [48]. Alternatively, binding of the functionalized protocell can elicit receptor internalization for cargo release within the cell. Table 1 lists targeting peptide ligands that have been identified by *in vivo* and/or *in vitro* phage display, whereby binding to their target receptor elicits receptor-mediated internalization.

Other examples of targeting peptides include tumor-targeting peptides derived from luteinizing hormone/chorionic gonadotropin conjugated to membrane-disrupting lytic peptides to effectively inhibit human breast and prostate xenograft tumor growth and metastases [49–52]. In addition to peptides or antibodies, aptamers, short, single stranded RNA or DNA oligonucleotides, have been developed for targeted cancer therapy to treat a variety of tumors in clinical trials by delivering intercalated chemotherapeutics or conjugated directly to nanocarriers containing therapeutic cargos (reviewed in [53,54]). Combinations of aptamers containing intercalated doxorubicin or a NF-κB decoy were effectively used *in vitro* to inhibit growth of cultured pancreatic tumor cells by inhibiting nuclear translocation of NF-κB [55].

Similar to chemotherapeutic drugs, targeted therapies are designed to inhibit tumor growth via a dynamic, progressive process. This ensures that toxic cellular byproducts are within physiological limits that can be effectively cleared. Due to the leakiness of tumor blood vessels, there is no doubt that targeted nanocarriers will accumulate in tumors partly due to the EPR effect. Nevertheless, once passive accumulation of targeted nanoparticles occurs, specific binding to tumor-specific receptors, internalization and retention in cells within the tumor microenvironment will ensure effective cargo release and higher, localized therapeutic indices with decreased systemic, collateral damage. Targeted delivery of functionalized protocells may also circumvent problems associated with “binding site inhibition” as this model does not take into account variability in receptor concentrations or turnover at the tumor site [56]. For instance, unless locally administered, intravenous infusion of targeted nanomedicines will be diluted in the circulation so that target site accumulation occurs over time. Unlike passive accumulation, targeted therapies, by definition, can be administered at lower doses due to their increased, effective concentration at the target site as confirmed both experimentally and by modeling and simulation [37,38,57]. Furthermore, functionalized protocells have a high cargo loading capacity, so that saturating receptor concentrations are avoided. Finally, the concentration of the targeting moiety can be modulated by varying the composition of functional groups available for conjugation in the protocell lipid bilayer. Given these considerations, selective targeting by functionalized protocells can successfully circumvent binding site inhibition.

Below, we will discuss in detail the advantages of the protocell over other types of nanocarriers. In a similar fashion, a variety of payload cargos or payload combinations will be discussed including non-invasive imaging agents and/or therapeutics, alone or in combination depending on the application. Thus, we envision the modular design of functionalized protocells may be tailored for a particular tumor type or tumor subtype whose therapeutic payload can be personalized to accommodate a prescribed clinical treatment plan. The objective of this review is to 1) describe how targeting peptides and scFvs are selected using *in vivo* and *in vitro* phage or antibody display and examine their clinical utility, 2) compare a variety of simple and complex nanocarriers and types of therapeutic cargos and 3) review various conjugation strategies to functionalize nanocarriers and optimize therapeutic efficacy. Ultimately, optimization and personalization of