Colloids and Surfaces B: Biointerfaces 135 (2015) 56–72

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Strategies of polymeric nanoparticles for enhanced internalization in cancer therapy

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ARTICLE INFO

Article history:
Received 2 February 2015
Received in revised form 19 June 2015
Accepted 7 July 2015
Available online 18 July 2015

Keywords:
Targeting
Anticancer
Nanoparticles
Internalization enhancement

ABSTRACT

In order to achieve long circulation time and high drug accumulation in the tumor sites *via* the EPR effects, anticancer drugs have to be protected by non-fouling polymers such as poly(ethylene glycol) (PEG), poly(ethylene oxide) (PEO), dextran, and poly(acrylic acid) (PAA). However, the dense layer of stealth polymer also prohibits efficient uptake of anticancer drugs by target cancer cells. For cancer therapy, it is often more desirable to accomplish rapid cellular uptake after anticancer drugs arriving at the pathological site, which could on one hand maximize the therapeutic efficacy and on the other hand reduce probability of drug resistance in cells. In this review, special attention will be focused on the recent potential strategies that can enable drug-loaded polymeric nanoparticles to rapidly recognize cancer cells, leading to enhanced internalization.

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1. Introduction

Chemotherapeutic drugs generally suffer from poor pharmacokinetics and inappropriate biodistribution. Because of their low molecular weight (Mw), for instance, intravenously (*i.v.*) administered anticancer agents tend to present with short circulation time and with low concentrations in tumors and metastases. To assist *i.v.* administered anticancer agents in achieving proper circulation time and tumor concentration, and to attenuate their accumulation in potentially endangered healthy organs and tissues, nanoscale drug delivery systems such as liposomes, polymeric micelles, polymersomes, nanogels, and nanocapsules have emerged as an indispensable platform for modern cancer therapy [1–3]. Their appropriate sizes (usually between several nanometers and 200 nm) and stealthy properties enable them to extravagate through the hyperpermeable blood vessels and preferentially accumulate in the tumor *via* the enhanced permeability and retention (EPR) effect [4–6]. Since the circulation time of a carrier is

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http://dx.doi.org/10.1016/j.colsurfb.2015.07.013
0927-7765/

prolonged, its opportunity of passing through the leaky vasculature increases, and thereby its extravagation into the tumor tissue [7]. Beyond that, therapeutic polymeric nanoparticles (NPs) can accommodate multiple functions including: (1) improve the pharmaceutical and pharmacological properties of drugs, potentially without the need to alter drug molecules, (2) deliver multiple types of therapeutic drugs with potentially different physicochemical properties, (3) deliver a combination of imaging and therapeutic agents for real-time monitoring of therapeutic efficacy, (4) protect drugs (small molecules, proteins, nucleic acids or peptides) from hepatic inactivation, enzymatic degradation and rapid clearance *in vivo*, (5) reduce the incidence and intensity of side effects [8]. Hence, numerous studies focused on NPs with antineoplastic drugs encapsulated in during the past decades. And a number of drug-loaded NPs have reached clinical development and even some have been clinically approved. For instance, DOXIL, doxorubicin (Dox)-loaded PEGylated liposome, was the first FDA approved liposome nanomedicine to reach clinical approval in 1995 for AIDS related Kaposi's syndrome [9]. NK911, a micellar NP comprising PEG, Dox and poly(aspartic acid), and Genexol-PM, which was a paclitaxel-encapsulated PEG-PLA micelle formulation, both were currently in phase II development for various cancers [10–12].

Thus far however, the clinical performance of EPR-exploiting drug-loaded NPs has been relatively disappointing. They do substantially reduce the incidence and intensity of side effects, such as cardiotoxicity, bone marrow depression, alopecia and nausea, but to date, they have largely failed to really improve response rates and survival times [13,14]. The majority of drug-loaded polymeric nanoparticles possess a stealth surface made of water soluble non-fouling polymers such as poly(ethylene glycol) (PEG), poly(ethylene oxide) (PEO), dextran, and poly(acrylic acid) (PAA), which confer prolonged circulation time and enhanced accumulation in tumor sites *via* EPR effect [15,16]. However, this highly hydrophilic surface failed to create optimal uptake by cancer cells within the tumor. This problem which has been referred to by some as the "PEG dilemma" has been suggested to hinder efficient drug delivery in tumors as these NPs end up releasing their therapeutic payload into the tumor milieu rather than within cancer cells [17,18]. And ubiquitously targeting cells within a tumor is not always feasible because some drugs cannot diffuse efficiently and the random nature of the approach makes it difficult to control the process of internalization. This lack of control may induce multiple-drug resistance (MDR)-a situation where chemotherapy treatments fail patients due to resistance of cancer cells toward one or more drugs. MDR occurs because transporter proteins that are overexpressed on the surface of cancer cell can expel drugs from cells [19–21]. Expelling drugs inevitably lowers the therapeutic effect and cancer cells soon develop resistance to a variety of drugs. Consequently, as vehicles, ideal nanoparticles are obliged to target cells with high drug loading levels without drug leakage on the way, while rapidly unload drug at the intracellular site of action. In this review, special attention will be focused on the recent potential strategies that can enable drug-loaded polymeric nanoparticles to rapidly recognize cancer cells, leading to enhanced internalization.

2. Ligand-targeted NPs for enhanced internalization

The addition of targeting ligands, which was installed on the surface of nanoparticles, can play a vital role in the ultimate location of the nanoparticle. For example, nanoparticles can be selectively recognized by specific tumor cells if their surfaces contain moieties such as antibodies, aptamers, proteins, peptides, folate, carbohydrate and other emerging targeting molecules. These moieties can be directed to cancer cell surface receptors, such as transferrin receptors, that are known to be increased in number on a wide range of cancer cells [22]. These targeting ligands enable nanoparticles to bind to cell-surface receptors and enter cells *via* the receptor-mediated endocytic route. Recent work comparing non-targeted and targeted nanoparticles (lipid-based [23] or polymer-based [24]) has demonstrated that the primary role of the targeting ligands is to enrich cellular uptake into cancer cells rather than to increase the accumulation in the tumor. In the following section we mainly focus on recent efforts in the development of ligand-based targeted NPs (Table 1).

2.1. Monoclonal antibody based targeting molecules

Among all the targeting molecules, monoclonal antibodies (mAbs) have been most commonly used in the development of targeted NPs owing to their high specificity and affinity to the target and so far about 30 of them have been approved for clinical use [44–48]. For example, trastuzumab and rituximab, which are mAbs currently in the clinic, have been conjugated to poly(lactic acid) (PLA) NPs leading to nanoconjugates that demonstrate a 6-fold increase in the rate of particle uptake compared with similar particles lacking mAb targeting molecules [49,50]. A nanoparticle consisting of a mucic acid polymer conjugate of camptothecin (CPT), MAP-CPT, and containing herceptin antibody was investigated in bearing HER2 overexpressing BT-474 human breast cancer cells. Cellular uptake of nanoparticles was enhanced by 70% compared to nontargeted version by the incorporation of a single Herceptin antibody targeting agent per nanoparticle [51,52]. Gold nanoparticles (AuNPs) were conjugated with cetuximab (C225) and then labeled with In-111, which created EGFR-targeted AuNPs. *In vitro* studies showed that the uptake of C225-conjugated AuNPs in high EGFR-expression A549 cells was 14.9-fold higher than that of PEGylated AuNPs; moreover, uptake was also higher at 3.8-fold when MCF7 cells with lower EGFR-expression were used. *In vivo* A549 tumor xenograft mouse model MicroSPECT/CT imaging and a biodistribution study provided evidence of enhanced internalization of the C225-conjugated AuNPs into the tumor cells *via* antibody-mediated endocytosis. But a large portion of PEGylated AuNPs remained in the tumor interstitium [53]. Despite the intense effort undertaken for their development, mAbs-conjugated NPs still encounter many challenges and limitations. First, mAbs-conjugated NPs have a large size, which curbs intratumoral distribution due to interstitial tumor pressure and limits their intracellular and intratissue penetration especially in solid tumors. Second, they require extensive optimization through molecular engineering technologies, and create engineering difficulty in NPs scale-up and manufacturing. Third, they potentially lead to increased immunogenicity – the ability to evoke an immune response – and liver and spleen uptake of the nanocarrier [54–56]. For these reasons Abs can be fragmented and only the antigen-binding fragments are used. It is true that for better treatment, the faster speed of penetration in solid tumors of antibody fragments over intact antibodies is a remarkably superiority [45]. Additionally, although antibody fragments including antigen-binding fragments (Fab), dimers of antigen-binding fragments ($F(ab)_2$), single-chain fragment variables (scFv) and other engineered fragments (Fig. 1) are less stable than whole antibodies, they are considered safer when injected systemically due to reduced non-specific binding [55]. Two antibody fragment targeting liposomal systems have progressed to clinical trials. MCC-465 is an immunoliposome-encapsulated doxorubicin (Dox), with a surface decorated with both PEG and dimers of antigen-binding fragments ($F(ab)_2$) for immune shielding and targeting respectively. The $F(ab)_2$ used in this NPs is a fragment of the human mAb, GAH, which positively reacts to >90% of cancerous stomach tissues, but negatively to all normal tissues [57]. MCC-456 exhibits significant antitumor response against GAH-positive xenografts leading