Tumor microenvironment and intracellular signal-activated nanomaterials for anticancer drug delivery

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Cancer-associated stimuli-responsive nanosystems have been increasingly considered for the delivery of anticancer drugs, which primarily target the tumor microenvironment and/or intracellular elements to enhance intratumoral accumulation and promote drug release at the target site. The signals facilitating drug delivery include tumor and endocytic acidities, hypoxia, enzyme overexpression, as well as high levels of intracellular glutathione, reactive oxygen species, and adenosine-5′-triphosphate. This article reviews the current techniques and ongoing developments in anticancer drug delivery using these signals. In particular, the focus is placed on design strategies and methods of formulating novel nanoscaled materials. The merits and drawbacks of recent strategies, as well as potential future developments, are discussed.

Introduction

Nanomaterial-based drug delivery systems (nano-DDSs) are highly effective in enhancing the therapeutic efficacies of anticancer drugs, as well as reducing their adverse toxicities [1,2]. Nanocarriers, such as liposomes, polymeric nanoparticles, and inorganic nanovehicles, ensure that their anticancer drug cargoes are accumulated sufficiently in tumors after systemic administration, including small-molecule chemotherapeutics and macromolecular proteins/nucleic acids [3–5]. Drug delivery exploits the unique anatomical and pathological characteristics of solid tumors with extensive vascular permeability and insufficient lymphatic drainage, which is known to lead to the enhanced permeability and retention (EPR) effect [6]. Although various nano-DDSs have been successfully applied in experimental and preclinical animal or human models, the majority are not without their limitations, such as inferior pharmacokinetics, premature drug release into the systemic circulation, unwanted and nontargeted accumulation in healthy tissues, poor tumor penetration capacity, and uncontrollable drug release at the target site. To resolve these limitations, nanoparticles have been prepared using stimuli-responsive materials to enhance their predesigned functions in response to the tumor microenvironment and/or intracellular signals, such as deshielding of polyethylene glycol (PEG), conversion of the surface charge, exposure of the cell-penetrating peptide (CPP) or tumor-targeting ligand, and control of drug release in an on-demand manner (Fig. 1) [7–13]. These physiological signals include the presence of acidity, redox potential (glutathione (GSH)), specific enzymes, reactive oxygen species (ROS), hypoxia, and adenosine-5′-triphosphate (ATP). Thus, this move towards stimuli-responsive materials can overcome crucial challenges to conventional nano-DDSs, thus enhancing the therapeutic efficacies and reducing the side effects. This review outlines the emerging methodologies that use these signals in designing nano-DDSs. The advantages and drawbacks of recent strategies, as well as potential future developments, are discussed.

pH-triggered drug release and tumor targeting

The existing acidic pH of the extracellular and intracellular environment of tumors is considered an appropriate internal trigger for the controlled release of anticancer drugs in tumor tissues and/or within the tumor endocytotic vesicles such as endosomes and lysosomes. In comparison with the pH values in the blood and healthy tissues (pH 7.4), the extracellular pH values in the tumor

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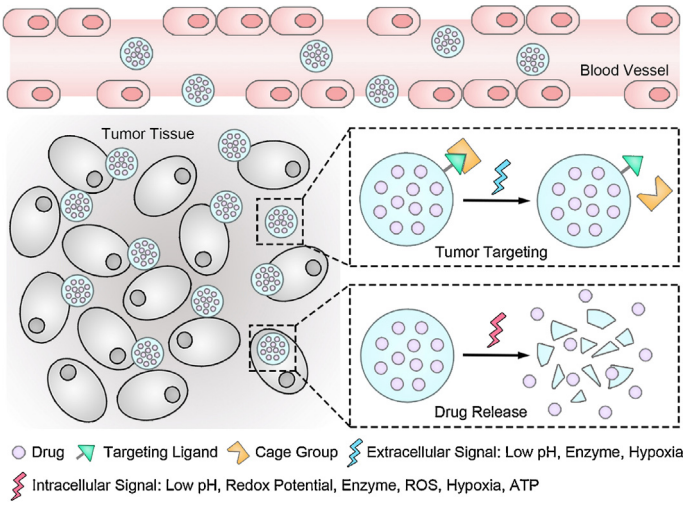

FIGURE 1

Schematic illustration of the role of tumor microenvironment and intracellular signals in tumor targeting and controlled drug release after intravenous administration. The nanocarriers can be activated to enhance accumulation in tumor and cellular uptake in response to tumor extracellular signals, including low pH, overexpressing cancer-associated enzymes, and hypoxia. The anticancer drugs can be selectively released in response to intracellular signals, including low pH, redox potential, enzymes, ROS, hypoxia, and ATP.

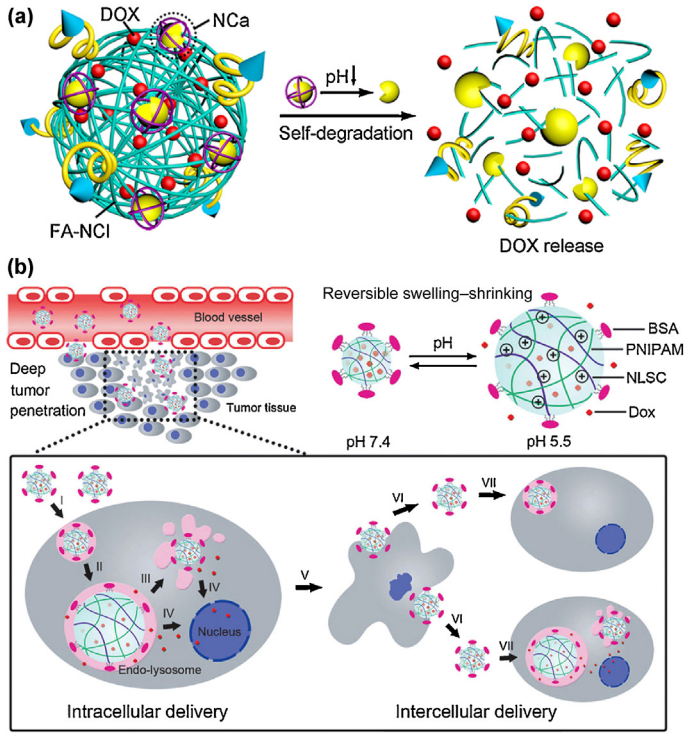

FIGURE 2

Acid-triggered drug release. (a) Cocoon-like DNA nanoclew integrated with an acid-sensitive nanocapsule encapsulating DNase I for controlled intracellular release of DOX. Reprinted with permission from Ref. [32] Copyright 2014 American Chemical Society. (b) pH-responsive reversible swelling–shrinking nanogel for deep penetration into tumors. Reprinted with permission from Ref. [36] Copyright 2014 Wiley-VCH.

have been found to range from 6.0 to 7.2 [14]. Furthermore, after endocytosis, rapid acidification is initiated via a proton influx. The intracellular pH in the subcellular organelles decreases to 5.0–6.0 in the endosomes and 4.0–5.0 in the lysosomes [15,16].

pH-sensitive DDSs have been developed by incorporating pH-labile chemical bonds into the polymer components or the polymer/carrier–drug conjugates, such as acetal [17–22], hydrazone [23–26], cis-acotinyl [27], orthoester [28,29], β-carboxylic acid amide [30], and glycerol ester groups [31,32]. These bonds are stable under neutral or alkaline conditions, but they tend to be hydrolyzed at acidic pH, thus enabling the release of the encapsulated drugs by disrupting the nanocarrier or the conjugated drugs through the degradation of the polymer/carrier–drug linkage. Kataoka et al. reported an intelligent small interfering RNA (siRNA)–polymer conjugate containing an endosomal acidic pH-cleavable maleic acid amide (MAA) for enhanced delivery of siRNAs [30]. This siRNA–polymer conjugate regenerated the polycation within the endosomes to escape, which was accompanied by the release of siRNA through the acid-responsive cleavage of MAA, which then sequence-specifically and significantly inhibited the growth of cancer cells. Recently, Gu and Mo et al. reported a novel cocoon-like DNA nanoclew that was integrated with an acid-sensitive nanocapsule containing DNase I for the controlled intracellular release of doxorubicin (DOX). This nanocapsule was synthesized by a long-chain single-stranded DNA containing numerous repeated GC-pair sequences for high DOX-loading ability (Fig. 2a) [32,33]. At the endo-lysosomal junction, DNase I was activated by the acid-responsive shedding of the polymeric shell, such that the nanoclew immediately self-degraded and rapidly released DOX for enhanced cytotoxicity against cancer cells.

Another approach involves the incorporation of protonatable groups including amino and carboxyl groups into the polymer to modulate the release of the encapsulated drug by inducing a structural change in the nanocarrier in response to the variation in pH. Once these groups become protonated below the acid dissociation constant (pK_a), the nanocarrier is destabilized by the charge repulsion between the polymers [34–37] or the change in the amphoteric properties of the components [38–40]. To enable deep penetration into tumor, Mo and Zhang et al. recently developed a virus-like pH-responsive nanogel with a cross-linked polyelectrolyte core containing *N*-lysinal-*N*′-succinyl chitosan (NLSC) (Fig. 2b) [36]. The nanogel was capable of reversibly swelling/shrinking depending on the protonation degree of the amine and carboxylic acid in the zwitterionic NLSC at different pH values. When the strong protonation of the amine at acidic pH led to charge repulsion between the NLSC chains, the volumetric expansion facilitated the rapid release of the encapsulated DOX into the tumor cells, thus inducing cell death. After escaping from the dead cells, the contractive nanogel could repeatedly infect the neighboring tumor cells, thus permitting the drug to penetrate deep into the solid tumor.

Carriers have been ruptured by acid-triggered gas generation as a means of designing pH-responsive drug release systems [41–43]. Sung et al. used poly(D,L-lactic-*co*-glycolic acid) (PLGA)-based hollow spheres to encapsulate both DOX and sodium bicarbonate ($NaHCO_3$) [41]. As a gas-forming agent, $NaHCO_3$ reacted with the proton in the endosomes and lysosomes to rapidly produce carbon dioxide bubbles, causing the sphere shell to burst, followed by the rapid release of DOX. Multidrug resistance (MDR) in cancer was significantly overcome by the prompt release of DOX, which significantly enhanced the concentration of DOX inside the cancer cells [42].