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Tumor extracellular acidity-activated nanoparticles as drug delivery systems for enhanced cancer therapy

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ABSTRACT

pH-responsive nanoparticles (NPs) are currently under intense development as drug delivery systems for cancer therapy. Among various pH-responsiveness, NPs that are designed to target slightly acidic extracellular pH environment (pH_e) of solid tumors provide a new paradigm of tumor targeted drug delivery. Compared to conventional specific surface targeting approaches, the pH_e-targeting strategy is considered to be more general due to the common occurrence of acidic microenvironment in solid tumors. This review mainly focuses on the design and applications of pH_e-activated NPs, with special emphasis on pH_e-activated surface charge reversal NPs, for drug and siRNA delivery to tumors. The novel development of NPs described here offers great potential for achieving better therapeutic effects in cancer treatment.

1. Introduction

Cancer remains the leading cause of death in the world after heart and infectious diseases. After decades of intensive research and billions of dollars spent, we have remarkably increased our knowledge of the causes and progression of cancer, which has led to the development of cancer treatment strategies. Chemotherapy is one of the major treatment modalities along with surgery (Gonzalez-Angulo et al., 2007). However, although research efforts to improve chemotherapy for cancer treatment over the past years have led to significant improvement in patient survival, a large number of deaths are still caused by cancer every year because current chemotherapeutic drugs inevitably face the following challenges: 1) lack of selectivity and targeting ability, leading to severe side-effects to patients, 2) ineffective in killing drug resistant tumor cells, 3) ineffective in therapy of metastatic tumors (Brindle, 2008; Cho et al., 2008; Kievit and Zhang, 2011; Mehlen and Puisieux, 2006; Szakacs et al., 2006). It is therefore obviously desirable to improve chemotherapy to be able to kill cancer cells more efficiently.

Over the past decades, great efforts have been committed to developing safe and effective nanoparticle (NP)-based drug carriers for selective delivery of cytotoxic drugs to tumors (Allen and Cullis, 2004). NP-based drug delivery systems are thought to improve bioavailability and selectivity of anticancer drugs through alteration of their pharmacokinetics and biodistribution profiles (Peer et al., 2007). Until now, various nanocarriers including liposomes, polymeric carriers, dendrimers, inorganic NPs and others have been investigated as drug carriers and several classes are being evaluated in clinical trials or used in clinical applications (Kamaly et al., 2012; K.S. Lee et al., 2008; Liong et al., 2008; Mintzer and Grinstaff, 2011; Nishiyama and Kataoka, 2003; Rana et al., 2012; Shi et al., 2011; Torchilin, 2005). NP-based drug delivery system offers many advantages over free drugs: 1) protecting the drugs from degradation and increasing their solubility, 2) preventing drugs from interacting with the biological environment, 3) prolonging drug circulation in blood and enhancing drug accumulation in tumor tissue, and 4) improving intracellular penetration (Peer et al., 2007).

Up to today, there are nearly 250 nanocarrier-based drug delivery products in various stages of preclinical and clinical development (Kamaly et al., 2012). Some of them have been approved for clinical applications (Jain and Stylianopoulos, 2010). Among these approved NPs, Doxil, Abraxane and Genexol-PM were developed for cancer therapy. Doxil a PEG-liposome containing the anticancer drug doxorubicin, was originally approved for the treatment of AIDS-related Kaposi's sarcoma and is now approved for use in ovarian cancer and multiple myeloma. Abraxane is an albumin-bound delivery vehicle of paclitaxel and was approved in 2008 for breast cancer cases unresponsive to other chemotherapies. Genexol-PM is a paclitaxel-loaded polymeric micelle and was approved for breast cancer treatment in South Korea in 2007. It is currently in Phase II clinical trials in the USA.

In spite of these achievements, NP-based drug delivery systems still suffer from limitations. One major limitation is their uncontrolled drug release behavior after being injected into animal or human body, which always leads to insufficient drug accumulation in tumor sites. In order to address this challenge, various smart NP formulations have been developed (Fleige et al., 2012; Gullotti and Yeo, 2009; E.S. Lee et al., 2008). Such NPs can make responses to either external or internal stimuli to change their chemical or physical properties to improve drug

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delivery efficiency. Typical stimuli exploited for triggered drug release include pH, redox reaction, enzyme, temperature and light et al. (Bae and Kataoka, 2009; Bae et al., 2007; Chau et al., 2006; Dvir et al., 2010; Fleige et al., 2012; Gullotti and Yeo, 2009; Kim et al., 2009; Lee et al., 2005b, 2007; Min et al., 2010; Pan et al., 2006; Schmaljohann, 2006; Tang et al., 2009; Tong et al., 2012; Wang et al., 2011; Xu et al., 2009; Zhu et al., 2012). Of these stimuli, pH-responsiveness is one of the most frequently used, as pH is an internal stimulus and the pH values vary in different tissues and cellular compartments. For example, the tumor extracellular environment is more acidic ($pH_e \approx 6.8$) than blood (pH ≈ 7.4), and the pH values of endosome and lysosome are even lower (ca. 5.0–5.5) (E.S. Lee et al., 2008). Previously, most of the pH-responsive studies were focused on the more acidic endosome and lysosome pH condition, whereas less attention has been paid to the slightly acidic tumor extracellular environment. Several years ago, Bae group developed a kind of polymeric micelle that could destabilize at tumor pH_e to accelerate drug release (Lee et al., 2003a,b). From then on, different kinds of carriers that could respond to tumor pH_e were designed and used as drug delivery carriers. Compared to the conventional passive and active targeting approaches, tumor pH_e targeting strategy is considered to be more general due to the universality of acidic environments in various tumors. In terms of this, the review here will highlight the recent progress of tumor pH_e targeting nanotechnology especially in the field of drug delivery for cancer therapy.

2. Passive and active tumor targeting

Initially, before talking about tumor pH_e targeting, we would like to briefly introduce the concept and applications of passive and active tumor targeting strategies. Passive targeting is widely utilized in oncology applications, since tumors facilitate the accumulation of NPs *via* leaky vessels through the well-known "enhanced permeability and retention" (EPR) effect (Danhier et al., 2010; Maeda, 2012; Maeda et al., 2012; Matsumura and Maeda, 1986). Tumor tissue is highly heterogeneous and is perfused by abnormal and leaky microvasculature. The rapid and defective angiogenesis (formation of new blood vessels from existing ones) leads to large gaps between endothelial cells in tumor blood vessels. These large gaps facilitate selective extravasation of nanoparticulate material into tumor from surrounding vessels. Furthermore, the impaired lymphatic drainage of solid tumor tissue retains the accumulated NPs and allows them to release drugs in the vicinity of the tumor cells (Yuan et al., 1995). Generally speaking, NP-based carriers with diameters less than 200 nm are effective for passive targeted drug delivery to solid tumors (Hobbs et al., 1998; Torchilin, 2005).

Although passive targeting approach forms the basis of clinical cancer therapy, they suffer from several limitations. For instance, tumor tissue is proven to be highly heterogeneous, which causes the permeability of vessels may not be the same even in a single tumor mass. Not to mention that some certain tumors do not exhibit EPR effect (Maeda et al., 2012; Peer et al., 2007). Moreover, tumor has negative interstitium pressure gradient, which can substantially limit the convection of NPs from the intravascular to the extravascular space within tumors (Jain, 2001; Wong et al., 2011). One way to address these challenges is to program the NPs with active binding ability to specific cell surface after extravasation. It is known that solid tumors often overexpress specific antigens or receptors on cell surfaces (Daniels et al., 2005; Parker et al., 2005; Scholler et al., 1999), which help in transmitting signals that are essential for the growth of tumor cells from the surrounding environment. The expression of specific receptors on the surface of tumor cells enables their recognition by ligand-modified drug loaded NPs for active tumor targeting. This strategy relies on enhanced interactions between the ligands (antibodies, peptide mimics, or nucleic acids) on the carrier and receptors on the tumor cells (Patri et al., 2005; Rihova, 1998). For example, human epidermal growth factor receptor-2 (HER-2) (Kirpotin et al., 2006; Yang et al., 2007), folic acid receptor (Choi et al., 2005, 2010; Majoros et al., 2006), and vasoactive intestinal peptide receptors (VIP-R) (Dagar et al., 2003) have all been investigated as biomarkers for nanocarriers to target breast tumors. However, these approaches have only achieved limited success in clinical trials, which might be due to the significant heterogeneity of solid tumor cell types and cell surface markers (Chaidarun et al., 1994; Scholler et al., 1999). Additionally, the presence of antigens and the expression of receptors on the surfaces of these tumor cells are transient and dynamic.

3. pH_e targeting: pH_e-activated drug release or ligand re-emergence

With the increase of knowledge in tumor biology, researchers have found that tumors develop unique microenvironment in comparison to normal tissues. One characteristic feature is that the extracellular pH of most tumors is more acidic than normal tissues. The value is generally believed to be in the range from 6.5 to 7.2 (Cardone et al., 2005), due to the increased glycolysis and plasma membrane proton-pump activity of tumor cells. These make tumor cells produce more lactic acid than normal cells, and further leach out the acid to the extracellular milieu. This persistent high lactate production by tumors in the presence of oxygen, termed the Warburg effect (Fig. 1) (Heiden et al., 2009) provides a growth advantage for tumor cells *in vivo*. In addition, insufficient blood supply and poor lymphatic drainage, characteristics of most tumors, also contribute to the acidity of the tumor microenvironment.

Based on these discoveries, new targeting strategies using pH_e as stimuli have recently been utilized to improve targeting efficiency of nanocarriers. Two generally used methods are triggered drug release from NPs in the tumor vicinity and facilitated cellular uptake of NPs upon arrival at target tumor sites. The mechanism of these strategies is mainly based on the hypothesis that these NPs can maintain stealth during blood circulation to passively accumulate at tumor site, and then the NPs are activated by pH_e to either release their encapsulated cargos or transform to a more cell-interactive form for enhanced tumor cell internalization. Fig. 2 depicts three different forms of pH_e-activated drug delivery NPs.

3.1. pH_e-activated drug release at tumor site

A typical example of pH_e-triggered drug delivery system is L-histidine-based pH-sensitive polymeric micelle developed by Bae group (Kim et al., 2009; E.S. Lee et al., 2008; Lee et al., 2003a,b, 2005a,b). They find that poly(L-histidine) (polyHis) is a promising biomaterial for the construction of pH_e-responsive nanocarriers. The imidazole ring of polyHis has a pK_b ~ 6.5 and shows reversible hydrophobic to hydrophilic transition in accordance with its ionization/deionization states. In order to obtain pH_e-responsive nanocarriers, Bae and co-workers developed mixed micelles by blending polyHis-*b*-poly(ethylene glycol) (PEG) diblock copolymer with poly(L-lactic acid) (PLLA)-*b*-PEG at specific weight ratio. The mixed micelles were stable at pH above 7.4, while gradually destabilized below pH 7.0 due to the ionization of the polyHis block in the micelle core. *In vitro* cell killing studies revealed that the DOX-loaded micelles enhanced killing effect to MCF-7 cells at tumor acidic pH due to that more DOX were released under this condition. *In vivo* studies with intravenous administration of DOX-loaded NPs at a dose of 10 mg DOX per kilogram of mouse weight showed a significant tumor growth inhibition of MCF-7 xenografts in comparison with free DOX group.

They also tested the accumulation of DOX-loaded pH-sensitive micelles in MDA-MB-231 breast tumor xenografts and compared the results with pH-insensitive PLLA-*b*-PEG micelles. The results indicated that pH-sensitive DOX-loaded micelles accumulated much more in tumor tissue than DOX-loaded pH-insensitive micelles. This was attributed to the fact that when the DOX-loaded micelles were administered into mice *via* intravenous administration, the PEGylated NPs showed a prolonged blood circulation time and accumulated at tumor site *via* EPR effect. But after accumulation in the tumor tissue and exposure of the micelles to pH_e, the micelles dissociated quickly due to the